The Haryana Veterinarian (June, 2023) **62(1),** 118-122 ISSN 0033-4359 (P) **Research Article**

MICROANATOMICAL OBSERVATIONS ON THE FORESTOMACH OF SAMBAR DEER

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ABSTRACT

The stomach of Sambar deer is composed of four distinct compartments namely rumen, reticulum, omasum and abomasum. Small pieces of the forestomach of two male and one female Sambar deer (*Rusa unicolor*) were collected and fixed in 10% neutral buffered formalin. Paraffin processing was done and were subjected to routine and special histological staining technique. The wall of forestomach showed four layers namely tunica mucosa, submucosa, muscularis and serosa. All the ruminal, reticular papilla and omasal lamina was lined with keratinised type of stratified squamous epithelium. The reticular epithelium was found thicker at the tip of the papilla and thinner at the base of the papilla. The omasal lamina was branched off from the basal layer and composed of only two layers namely tunica mucosa and submucosa. The lamina propria was composed of loose connective tissue. In the reticular lamina propria, the papilla invaginated into it forming the epithelial pegs. Muscularis mucosa was not found in rumen. Muscularis mucosa layer varied in thickness at various section of reticulum. Muscularis mucosa layer ran parallel to the length of the omasal lamina. Tunica submucosa was observed as loose connective tissue with collagen fibres, fibroblast, blood vessels, lymphatics and nerve fibres. Tunica muscularis was composed of two layers of smooth muscles in oblique direction. Myenteric plexus was located between the two layers of smooth muscles. Tunica serosa consisted of loose connective tissue with large blood vessels.

Keywords: Microanatomy, Rumen, Reticulum, Omasum, Sambar deer

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Sambar deer (*Rusa unicolor*) is a wild ruminant, belong to the family Cervidae. It has short thick dark hair in lighter brown to white creamy color. It is the largest deer distributed in southeast Asia. They are nocturnal and browse leaves, berries, grasses, bark from young trees, fallen fruits, herbs, buds during dusk and at night time (Stafford, 1995). It consumes several toxic plants without adverse consequences due to special microflora in intestine, big size of rumen. It lives in solitary or in small groups. Stags mark their territory with the secretion of pre-antler glands on the branches of trees and bushes.

The stomach of Sambar deer is composed of four distinct compartments namely rumen, reticulum, omasum and the abomasum (Stafford, 1995). Rumen occupies most of the left side of the abdominal cavity (Getty, 2012). Stafford (1995) reported that the interior of rumen of sambar deer was divided into various compartments by ruminal pillars and possessed short poorly developed papilla at the roof of the interior of rumen and also no papillae were found at the pillars and reticulum was divided into reticular cells without any secondary or tertiary crest within the reticular cells. The papilla was present on both inside the cells and over the primary crests. He further reported that omasum in sambar deer was large, roughly kidney shaped with 46-71 omasal lamina, containing short poorly developed blunt papillae of less than 1 mm in length and with a round base of diameter 1 mm.

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Histologically the rumen, reticulum and omasum of ruminants was made up of tunica mucosa, submucosa, muscularis asnd serosa and having keratinised-type of stratified squamous epithelium (Eurell and Frappier, 2006). The macro anatomy of forestomach of Sambar deer has been carried out in details but there is lack of literature on the microanatomy. So, this study was carried out to explore the microanatomy of forestomach of Sambar deer.

MATERIALS AND METHODS

The forestomach was collected from two male and one female adult sambar deer during post-mortem examination (male sambar deer of PM No. 369, Forest Veterinarian Unit, Tirunelveli dated 12/02/2022, female sambar deer of PM No. 371, Forest Veterinarian Unit, Tirunelveli dated 19/03/2022, male sambar deer of PM No. 375, Forest Veterinarian Unit, Tirunelveli dated 02/04/2022). The sambar deer were clinically healthy with an average age of 10 years. The average weight was 198 kg. Abdominal cavity was opened to expose the viscera and identified the organs of digestive system and small pieces of forestomach of sambar deer namely rumen, reticulum and omasum was cut, washed in the normal saline and fixed in 10% neutral buffered formalin for histological studies. Then the tissues were dehydrated in the ascending grades of the alcohol, cleared in xylene and embedded in paraffin (58-60º C). Tissue sections of 4-5µm thickness were used for the Haematoxylin and Eosin Staining method to know the

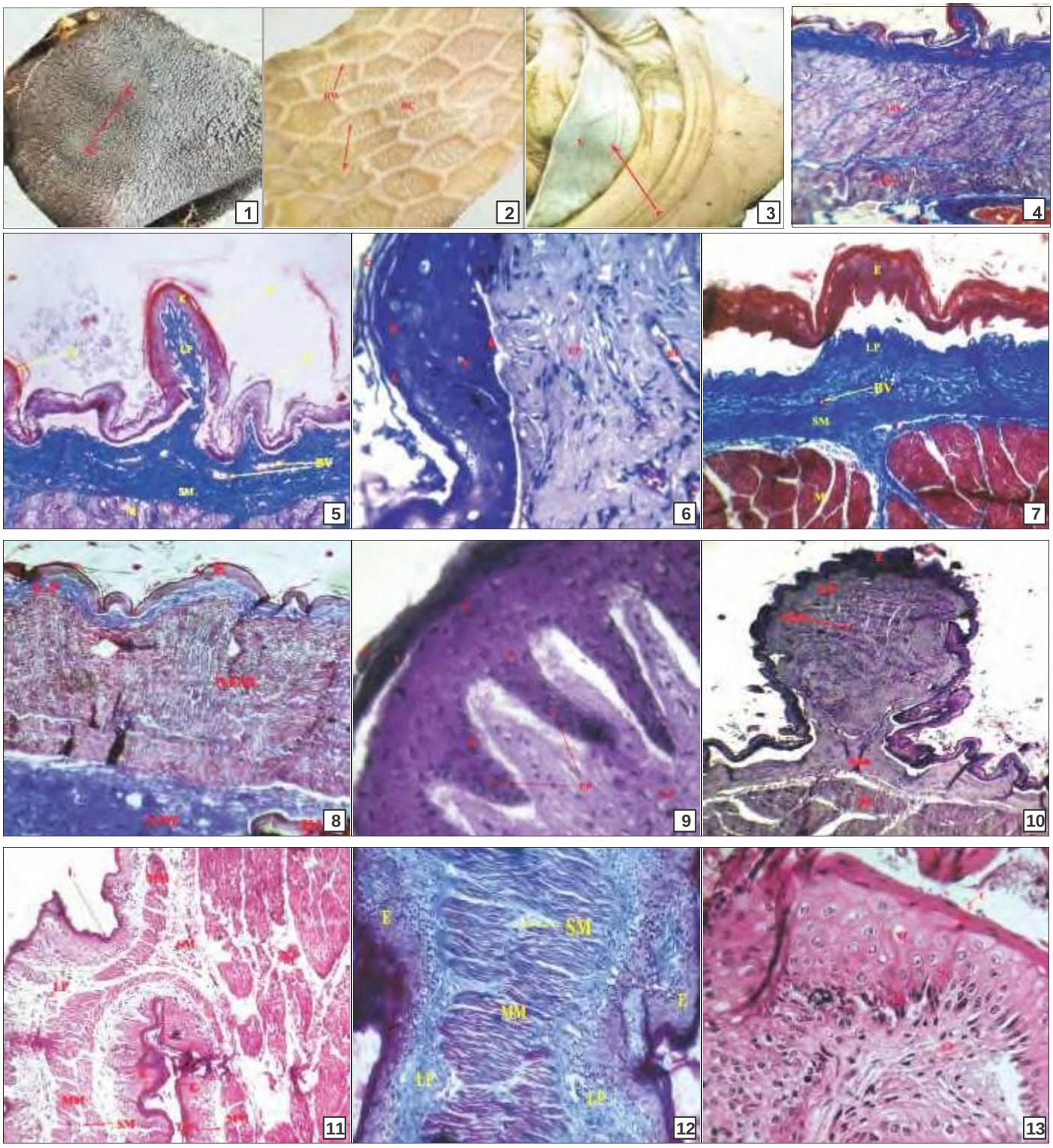
general histological architecture of the tissue and Masson Trichrome Staining method for collegen fibers (Luna, 1968).

RESULTS AND DISCUSSION

The interior of rumen in the present study was divided into sacs by ruminal pillars. The interior of rumen was found covered with papillae and the papilla was not present in the ruminal pillar (Fig. 1). Reticulum was the cranial most part of the stomach. The reticulum was divided into reticular cells, each of which had 5-7 walls (Fig. 2). And there were between 40 and 100 papillae in each reticular cell and some papilla was also present in the wall of reticular cells. Omasum was roughly kidney shaped organ containing 40-80 laminae with numerous papilla on each lamina (Fig. 3). Similar observation was also found in sambar deer (Stafford, 1995). Micro anatomically, the wall of rumen showed four layers namely tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Fig. 4). The findings are in support of the results of Amit *et al.* (2011) in sheep and Al-Araji and Dhyaa (2018) in Gazelle. The tunica mucosa in the present study showed ruminal papilla of various dimensions and shapes. Most of the papilla was short and some were tall in shape. Tall ruminal papilla was found tongue shaped and small papilla was found filliform, conical and fungiform shaped (Fig. 5). Similar findings were also reported by Amit *et al.* (2011) in sheep, Al-Araji and Dhyaa (2018) in Gazelle and Sulthana *et al.* (2021) in sheep. All the ruminal papilla was lined with keratinised type of stratified squamous epithelium which was in concurrence with the findings of Amit *et al.* (2011) in sheep and Al-Araji and Dhyaa (2018) in Gazelle. The stratified squamous epithelium in the present study consisted of single layer of stratum basale of columnar cells and basally located oval nucleus. This was continued towards the luminal surface by stratum germinatum by rhomboid cells with oval nucleus showing many mitotic figures. This layer was observed as 2-3 layers which were in contrary to the findings of Eurell and Frappier (2006) in domestic ruminants where he quoted that the thickness of stratum spinosum layer varied from 1-10 cells. This layer was topped with 5-7 layers of stratum granulosum with cuboidal cells with centrally located large round nucleus. This finding was in opposition to the recording of Eurell and Frappier (2006) in domestic ruminants where he mentioned that the stratum granulosum was of 1-3 cell layers thick. This layer was covered by the layer of stratum lucidum with 10-12 layers with few elongated nucleated cells and many elongated non-nucleated cells. The cells of these layers were observed highly eosinophilic when compared to the cells of stratum granulosum, germinatum and basale (Fig. 6). This layer was upcovered by a layer of stratum corneum with highly keratinized flat, non-nucleated eosinophilic cells which was found separated from the remaining layers of stratified squamous epithelium at most places. Each papilla was found covered with stratified squamous epithelium and was rested on the lamina propria (Fig. 6).

The lamina propria was composed of loose connective tissue with connective tissue cells and small and large blood vessels which was responsible for the absorption of volatile fatty acids. Muscularis mucosa was not found in the present study which was as per the findings of Eurell and Frappier (2006) in domestic ruminants, Amit *et al.* (2011) in sheep and Al-Araji and Dhyaa (2018) in Gazelle. The tunica submucosa was found in continuation with lamina propria since muscularis mucosa was not observed (Fig. 7) which was supported by the findings of Eurell and Frappier (2006) in domestic ruminants, Amit *et al.* (2011) in sheep, Al-Araji and Dhyaa (2018) in Gazelle. Tunica submucosa was observed as loose connective tissue with collagen fibres, fibroblast, blood vessels, lymphatics and nerve fibres as reported by Eurell and Frappier (2006) in domestic ruminants. Tunica muscularis was composed of smooth muscles fibres with two layers of smooth muscles in oblique direction and were almost perpendicular to each other (Fig. 7). Similar findings were recorded by Sulthana *et al.* (2021) in sheep in which they observed tunica muscularis consisted of inner circular and outer longitudinal layer. But this findings was in contrary with the reports of Al-Araji and Dhyaa (2018) in Gazelle where they mentioned that the tunica muscularis of rumen showed three layers namely inner circular, middle oblique and outer longitudinal layers of smooth muscle fibers. In present observation, the inner layer was thick in comparision to the outer layer. Myenteric plexus was located between the inner and outer layer of smooth muscles which was in line with the findings of Amit *et al.* (2011) in sheep and Sulthana et al. (2021) in sheep. Tunica serosa consisted of loose connective tissue with large blood vessels which was similar to the results of Amit *et al.* (2011) in sheep and Sulthana *et al.* (2021) in sheep.

Histologically in the reticulum the tunica mucosa showed papilla of various dimensions and shapes. The shape of the papilla varies from conical to fungiform shape in different heights and thickness (Fig. 8). Similar finding was recorded by Sulthana et al. (2021) in sheep. The reticulum in the present study was observed to be lined with stratified squamous epithelium of keratinized typeas reported by Al-Araji and Dhyaa (2018) in Gazelle and Kumar and Kumar (2012) in sheep. The epithelium was found thicker at the tip of the papilla and thinner at the base of the papilla. The epithelium in the present study consisted of single layer of stratum basale with cylindrical



Figs.1-13. **(1)** The interior of rumen showing ruminal papilla (RP). **(2)** The interior of reticulum showing reticular cell (RC) and reticular wall (RW) with papilla (P). **(3)** The interior of omasum showing omasal lamina (L) with papilla (P). **(4)** Photomicrograph of rumen showing stratified squamous epithelium (E), lamina propria (LP), sub mucosa (SM), inner muscular layer (IM), outer muscular layer (OM) and serosa (S) containing blood vessel (BV). MTS x 40; **(5)** Photomicrograph of rumen showing tall tongue shaped papilla (T) and short filliform (F) and conical papilla (C) with Epithelium (E), Lamina propria (LP), Submucosa (SM), Blood vessel (BV) and tunica muscularis (M). MTS x 100; **(6)** Photomicrograph of rumen showing stratified squamous epithelium with stratum Basale (B), Stratum spinosum (S), Stratum granulosum (G), Stratum corneum (C) and Stratum lucium (L) Lamina propria (LP) Submucosa (SM) Blood vessel (BV). H&E x 400; **(7)** Photomicrograph of rumen showing papilla with epithelium (E), Lamina Propria (LP), Blood vessel (BV), Submucosa (SM) and Tunica muscularis (M). MTS x 100; **(8)** Photomicrograph of reticulum showing conical and fungiform papillae of various size with stratified squamous epithelium (E), muscularis mucosa (MM) separating lamina propria (LP) and submucosa (SM) containing blood vessel (BV). MTS x 40; **(9)** Photomicrograph of reticulum showing stratified squamous epithelium with stratum Basale (B), Stratum spinosum (S), Stratum granulosum (G), Stratum corneum (C) and Stratum lucium (L). Epithelial peg (EP), Lamina propria (LP). H & E x 400; **(10)** Photomicrograph of reticulum showing large fungiform papilla with secondary papilla covered by epithelium (E), lamina propria (LP), muscularis mucosa (MM), tunica submucosa (SM), tunica muscularis (M). H & E x 100; **(11)** Photomicrograph of omasum showing lamina (L) getting separated from the basal layer (B), epithelium (E), lamina propria (LP), muscularis mucosa (MM), tunica muscularis (M). H & E x 100; **(12)** Photomicrograph of omasum showing epithelium (E), lamina propria (LP) on both side of the lamina, muscularis mucosa (MM) with thin layer of submucosa (SM) in between them. MTS x 100; **(13)** Photomicrograph of omasum showing stratified squamous epithelium with stratum Basale (B), Stratum spinosum (S), Stratum granulosum (G), Stratum corneum (C) and Stratum lucium (L). Lamina propria (LP) MTS x 400

cells containing large ovoid nucleus with prominent nucleolus as stated by Eurell and Frappier (2006) in domestic ruminants. Stratum spinosum layer contained polyhedral cells with round to oval shaped nuclei showing the presence of mitotic figures. Similar results were also mentioned by Eurell and Frappier (2006) in domestic ruminants. This layer was 3-5 cell layer thickness. This layer was covered with 5-7 layers of stratum granulosum with cuboidal cells with centrally located large round nucleus which was in line with the reports of Eurell and Frappier (2006) in domestic ruminants. Some dark-blue coloured pointed granules were noticed in the cytoplasm of the cells of this layer, stained by Haematoxylin and Eosin stains which were believed to be keratohyalin granules as reported by Eurell and Frappier (2006) and Ghosh (2013) in domestic ruminants. This layer was covered by the layer of stratum lucidum with 7-8 layers with few elongated nucleated eosinophilic cells and many elongated non-nucleated cells. The Stratum corneum layer was thick homogenous cytoplasm, contained occasional elongated nucleated cellsas stated by Eurell and Frappier (2006) in domestic ruminants.

The epithelial layer rested on the lamina propia layer. In the lamina propria, the papilla invaginated into it forming the epithelial pegs and further branched into it to form multiple finger-like projection (Fig. 9). Muscularis mucosa layer varied in thickness at various section of reticulum. It was found as cluster of bundles of muscle fibres on large papilla at its centre of the papilla (Fig. 10). Similar observation was reported by Poonia *et al.* (2011) in sheep and Al-Araji and Dhyaa (2018) in Gazelle. But this finding is in opposition to that of Sulthana *et al.* (2021) in sheep where they mentioned that the lamina muscularis of reticulum was present in the tip of the papilla along with lamina propria. In this present study, muscularis mucosa was observed as a bundle of smooth muscle fibres of various thickness, separated the layer of lamina propria from submucosa. Smooth muscle fibres in this layer were found separated by loose connective tissue which contained few numbers of small blood vessels and these fibres were responsible for the contraction of reticular papilla during and rumination and digestion as suggested by Al-Araji and Dhyaa (2018) in Gazelle. The submucosa layer was composed of loose connective tissue with collagen fibres, large and small blood vessels and connective tissue cells as reported by Eurell and Frappier (2006) in domestic ruminants. The submucosa layer rested on tunica muscularis layer. The tunica muscularis layer was composed of two layers of smooth muscle fibres in oblique direction and almost perpendicular to each other. This finding is in contrary to the observation of Poonia *et al.* (2011) in sheep where they mentioned the presence of three smooth muscle layers in tunica muscularis namely outer longitudinal, inner circular and middle oblique layer. The tunica muscularis was covered by tunica serosa. Tunica serosa was composed of loose connective tissue with adipocytes as reported by Poonia *et al.* (2011) in sheep.

Histologically, the omasum in the present study was found with two parts namely basal layer and omasal lamina. Both lamina and the basal layer were lined by keratinised stratified squamous epithelium as reported by Al-Araji and Dhyaa (2018) in Gazelle and Poonia *et al.* (2012) in sheep. The lamina was branched off from the basal layer (Fig. 11). The lamina was again branched into papilla like structures and was helpful in omasal functions like crushing the food particles so that they could easily pass on to the abomasums. The basal layer was composed of four layers namely tunica mucosa, tunica submucosa, tunica muscularis, tunica serosa, while the omasal lamina was composed of only two layers namely tunica mucosa and tunica submucosa (Fig. 12).

Tunica mucosa was based by a single layer of stratum basale containing elongated columnar cells with oval nucleus containing many mitotic figures. The stratum basale was covered by stratum spinosum of rhomboid cells with round nucleus with the presence of hallow around the nucleus. This layer was 3-5 cell layers thick (Fig. 13). This finding is in contrary to the finding of Ghosh (2013) in domestic ruminants where he reported that the stratum spinosum was 1-10 cell layers thick. This layer was covered by layer of stratum granulosum of 3-4 cells thick whereas Ghosh (2013) in domestic ruminants found that the stratum spinosum was 1-3 cell layer thick. Stratum granulosum was enclosed by stratum lucidum layer by tightly packed cells with few cells containing slender elongated nucleus. This layer was covered with layer of stratum corneum. Similar finding was reported by Ghosh (2013) in domestic ruminants.

The omasal epithelium was rested on thin layer of lamina propria. The lamina propria layer was composed of loose connective tissue containing loosely arranged collagen fibres, fibrocytes, many blood vessels and lymphatic vessels as documented by Poonia *et al.* (2012) in sheep. It was observed that the smooth muscle fibres of the muscularis mucosa layer ran parallel to the length of the lamina (Fig. 13). Similar findings were reported by Poonia *et al.* (2011) in sheep. Butthis finding is in contrary to observation of Al-Araji and Dhyaa (2018) in Gazelle in which they reported that the muscularis mucosa was intermingled within the lamina propria and such intermingling was not observed in the present study. The muscularis mucosa was present on either side of the laminae (Fig. 12). Similar observation was reported by Eurell and

Frappier (2006) in domestic ruminants and Poonia et al. (2012) in sheep. But there was not any smooth muscle layer in between the muscle bundles of muscularis mucosa. But Amit *et al.* (2011) in sheep reported that the muscle bundles of muscularis mucosa of outer and inner surfaces lamina were separated from each other by type-I laminae. It was observed that the muscularis mucosa was also run parallel to the epithelium in the basal layer. Tunica submucosa was observed as thin layer composed of loose connective tissue containing collagen fibres, large blood vessels, connective tissue cells and adipocytes as reported by Eurell and Frappier (2006) in domestic ruminants. It was separated from lamina propria by muscularis mucosa. In the basal layer, tunica muscularis was observed as smooth muscle fibers in two layers with thick inner layer and thin outer layer which was similar to the findings of Poonia *et al.* (2012) in sheep. But Al-Araji and Dhyaa (2018) in Gazelle reported that both inner and outer muscular layer had same thickness. In this finding, both the inner and outer layers were observed in oblique direction. But this finding was in contrary to the findings of Eurell and Frappier (2006) in domestic ruminants and Poonia *et al.* (2012) in sheep where they mentioned that inner layer was directed circularly and outer layer directed longitudinally. Tunica muscularis and tunica serosa was not observed in the omasallamina. Loose connective tissue containing nerve elements, many blood vessels and lymphatics were observed in between the inner and outer muscular layer. Tunica muscularis was covered by tunica serosa. Tunica serosa was composed of loose connective tissue with adipocytes.

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