Light and scanning electron microscopic studies on the rumen of goat (*Capra hircus*)

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**Abstract**

The dorsal and ventral walls of rumen of goat studded with papillae of varying shapes and dimensions were lined by stratified squamous keratinized epithelium having varying number of rows in strata basale, spinosum, granulosum and corneum. Iron granules were observed in superficial layers of stratum corneum. The propria submucosa having loose irregular connective tissue was comprised of collagen, reticular and elastic fibres. Tunica muscularis was constituted by a thick inner circular and a thinner outer longitudinal layer of smooth muscles. Nerve bundles representing submucosal and myenteric plexus were also observed. The structural details of longitudinal and coronary pillars were similar to those of walls except minute variations. Scanning electron microscopy revealed more characteristic features of shapes of papillae. The higher magnification revealed depressions and meshwork like structures.

**Key words**: Rumen, goat, histology, scanning electron-microscopy.

**Introduction**

Rumen has capability to digest cellulose and hemicelluloses to produce volatile fatty acids by microbial fermentation which is main source of energy in ruminants. The ruminal papillae increase surface area and led to increase absorption upto ten times. The concentration of volatile fatty acids, ammonia, pH, and osmotic pressure in rumen along with internal factors such as glucose and insulin concentration affected the size and number of papillae (Sakata *et al*., 1980). The literature is available on histomorphology of rumen and omasum of sheep (Poonia *et al*., 2011; 2012). There are meager reports on structural details of papillae especially scanning electron microscopy and hence, the present study was envisaged to explore the histology and scanning electron microscopy of the rumen in goats.

**Material and Methods**

The present study was conducted on 10 young goats of local mixed breed of either sex. The tissues were collected from dorsal and ventral walls of the rumen along with longitudinal and coronary pillars and fixed in 10 % neutral buffered formalin solution for 72 hours and processed for routine paraffin technique of light microscopy. The paraffin sections of 5-6 μ were stained with routine Harris’ hematoxylin and eosin stain for demonstration of morphostructural characteristics, Gomori’s method for reticulum, Weigert’s method for elastic fibres, Mallory’s method for iron, Ayoub-Shklar method for keratin and pre-keratin, McManus’ PAS method for glycogen, PAS-Alcian blue method for mucosubstances (pH 2.5), Alcian blue method for mucosubstances (Luna, 1968) and Crossman’s trichrome stain for collagen fibres (Crossman, 1937).

Fresh tissues collected from different sites of rumen were washed thoroughly in chilled 0.1 M phosphate buffer (pH 7.4) solution. The tissues were again washed twice with 0.1 M phosphate buffer after fixation in 2% glutaraldehyde solution for 6-8 hours and rest of the procedure was carried out at EM-Laboratory, AIIMS, New Delhi. The processed tissue samples were viewed under LEO 435-VP scanning electron microscope.

**Results and Discussion**

**Dorsal wall of rumen**

The mucosal surface of dorsal wall of the rumen was uneven due to the presence of varying sized papillae which were elongated and cylindrical in shape (Fig 1). In between these papillae, a few small papillae were also present. The free surface also showed similar
type of papillae cut in different profiles. These papillae generally tapered towards the tip which presented a few projections in some of the papillae. The rumen papillae in sheep were generally conical or tongue shaped (Wardrop, 1961; Poonia et al., 2011). The ruminal mucosa of the cow presented three types of papillae viz. elongated filiform, short conical and short fungiform, whereas those of the buffalo were comprised of only two types viz. long foliaceous and blunt conical (Bastain and Menon, 1963). The growth of papillae of the rumen wall varied in different rumen sacs due to varying localization of metabolic processes in the rumen at different stages of growth (Vrakin and Davydova, 1975). These papillae provided a larger surface area for the biochemical activities such as absorption of volatile fatty acids, water and electrolytes (Singh et al., 1983). These authors reported that volatile fatty acids were major end products of fermentation in the rumen and thus potentially important source of energy for ruminants.

The dorsal wall of the rumen was lined by stratified squamous keratinized epithelium having strata basale, spinosum, granulosum and corneum (Fig 1).

Fig 1: Photomicrograph of papillae of the dorsal wall of the rumen showing stratified squamous keratinized epithelium with different strata. H. & E. X 400

The epithelium was comprised of 4-6 layers from the stratum basale to stratum granulosum and 3-4 flat layers in stratum corneum. The stratum basale was well differentiated and had simple cuboidal to low columnar epithelial cells. Cytoplasm of these cells was less eosinophilic. The nuclei were round to oval in shape and were more basophilic. Multiple small foldings of the basal layer of the epithelium forming papillary bodies or pegs were observed as reported earlier in lambs (Wardrop, 1961; Poonia et al., 2011), in calves (Tamate et al., 1962a-b, 1964), buffalo (Sengar and Singh, 1970; Taluja and Saigal, 1987), in buffalo calves (Singh et al., 1982) and adult goats (Chungath et al., 1985). The formation of the papillary bodies would reduce the distance between the mucosal surface and the absorptive site, and also it led to increase the absorptive area to a greater extent which would be accounted for by an increase in the external surface of the epithelium.

The cells of stratum spinosum and stratum granulosum were mixed with each other and could not be differentiated from each other. The cytoplasm was finely granular and eosinophilic in these layers. The round to oval nuclei had fine chromatin material being distributed irregularly throughout the nucleoplasm. One or two nucleoli were centric or eccentric in position. Some of the cells of the stratum granulosum towards the stratum corneum contained fine keratohyaline granules. Their large sized round to oval nuclei presented vacuolated appearance however, centric or eccentric nucleoli were very much prominent. The cells of stratum corneum possessed elongated darkly stained nuclei and had more eosinophilic cytoplasm. The superficial cells were highly keratinized. The cornified epithelium offered physical protection against potentially sharp fibres being consumed by an animal (Samuelson, 2007). Present observations were in consonance with the findings of Singh et al. (1982) in buffalo calves and Taluja and Saigal (1987) in buffalo.

Iron granules were demonstrated in the superficial layers of stratum corneum by Mallory’s method (Fig 2).

Fig 2: Photomicrograph showing the presence of iron pigment (blue colour) in the superficial layer of stratum corneum in the dorsal wall papillae of rumen. Mallory’s method X 100

The iron has been related to the degree of microbial activity in sheep (Sinclair and Kunkel, 1959), pigmentation in cattle (Brownlee and Elliot, 1961) and to high energy ration in sheep (Nockels et al., 1966). However, Tiwari and Jamdar (1969) in buffalo calves and Taluja and Saigal (1989b) in buffaloes reported the presence of iron in both pigmented and non-pigmented epithelium.

The loose, irregular connective tissue of the propria submucosa formed the core of the papillae because of absence of lamina muscularis mucosae as reported earlier by Wardrop (1961) in lambs, Sengar and Singh (1970), Tiwari and Jamdar (1970) and Taluja
and Saigal (1988) in buffaloes, Chungath et al. (1985) in goats, Franco et al. (1992) and Poonia et al. (2011) in sheep. At the base of the papillae, the connective tissue was comparatively denser and also had fine blood capillaries. The superficial connective tissue was comprised of collagen, reticular and elastic fibres. The collagen fibres present in the subepithelial part also extended into the core of the papillae along with a few reticular fibres. The deeper part of the propria submucosa also had loose irregular connective tissue, fine blood vessels and connective tissue cells. Collagen fibres were irregularly placed along with a few reticular and elastic fibres. At places transversely cut nerve bundles were also observed which represented the presence of submucosal plexus.

The tunica muscularis was comprised of a thick layer of inner circular and a thinner outer longitudinal layer of smooth muscles. At places, nerve bundles representing myenteric plexus were also visible between the muscle layers. Few collagen and fine reticular fibres were present in between the muscle fasciculi. The tunica serosa was comprised of an outer flat mesothelial cell layer and an inner loose irregular connective tissue having fine blood capillaries and fatty tissue. Isolated collagen and very few elastic fibres were present in the subserosa.

**Ventral wall of rumen**

The mucosa of the ventral wall was folded due to the presence of large number of densely populated large and a few smaller papillae. Towards the base of the large papillae, smaller pointed or blunt projections like secondary papillae were also present. The cell layers of stratum spinosum could be well differentiated from those of stratum granulosum due to their pricky cell appearance. Stratum granulosum cells were flattened and had flat elongated nuclei. Some of these cells towards the stratum corneum appeared to have pyknotic nuclei. The stratum corneum was well developed and at places showed desquamation due to wear and tear.

The collagen fibres were denser in the subepithelial part of the propria submucosa. These fibres were longitudinally oriented and extended vertically into the core of the papillae. Isolated elastic fibres and few reticular fibres were also present in the superficial part. Fine elastic fibres extended vertically into the core of the papillae. The collagen fibres were more in the deeper propria submucosa but loosely arranged along with a few reticular fibres. Elastic fibres could be well appreciated in the tunica intima and periphery of the small blood vessels. The rest of features were similar to those of the dorsal wall.

The transition between the large and small papillae was clearly demarcated towards ventral sac region having highly folded mucosa. The smaller papillae were widely placed whereas, the larger papillae were closely placed. Papillae were generally blunt towards their tips. The number of cell rows was comparatively more in the stratified squamous keratinized epithelium of these papillae. The stratum corneum was comparatively thinner as compared to the dorsal and ventral walls. The concentration of collagen fibres was drastically increased in the superficial part of lamina propria submucosa and in the core of the papillae. The outer longitudinal layer of tunica muscularis was comparatively lesser in thickness as compared to the inner circular layer and also thinner when compared to the outer longitudinal muscular layer of the dorsal and ventral wall of the rumen.

In the present study, PAS positive reaction along with glycogen was not observed in the ruminal epithelium as reported in buffalo (Taluja and Saigal, 1989a) and sheep (Poonia et al., 2011). However, Franco et al. (1992) in sheep reported that the PAS positive cells were scattered over the apical two thirds of the epithelium, but were lacking in the stratum basale and stratum corneum. Acidic mucopolysaccharides could not be demonstrated in stratified squamous keratinized epithelium of rumen during present study. However, Lavker et al. (1969) in Hereford cows and Taluja and Saigal (1989a) in buffalo reported that the stratum corneum contained neutral, sulfated acidic and non-sulfated acidic mucosubstances.

Scanning electron microscopy of the rumen revealed large leaf like ruminal papillae. The papillae of dorsal wall of rumen were tongue shaped, pointed and blunt. These papillae also showed varying patterns of their origin from the ruminal mucosa. Some papillae emerged from the mucosa by a pedicle like structure whereas, others had a broader base at the point of origin (Fig 3a).

![Fig 3a: SEM of dorsal wall showing different types of papillae. Note a tongue shaped papilla attached to the wall through a pedicle like structure. X 25](image)
The surface of all these papillae presented pit like depressions which were separated from each other by fine mucous membrane (Fig 3b). The ventral wall of the rumen had densely packed large papillae of varying shapes (Fig 4). Higher magnification of these papillae revealed meshwork like structure similar to those of the dorsal wall papillae (Fig 5). Microplicae were delicate plasmalemmal folds of lining cells found in regions subjected to abrasive abuse (Andrews, 1976). It might provided protection by reducing the surface area of contact and by holding a polyanionic acid mucopolysaccharide.

Longitudinal pillar

The longitudinal pillar of the rumen had dorsal and ventral surfaces. The epithelium on the ventral surface was stratified squamous keratinized lining leaf like large papillae (Fig 6). These papillae were generally broader towards the base and oval or blunt towards the apex as reported in the sheep (Poonia et al., 2011). The dorsal surface had papillae which were comparatively smaller with blunt tips, but were closely placed. The basal surface of the epithelium presented large sized papillary bodies which were closely placed. The stratum basale was having simple columnar epithelial cells and possessed darkly stained basophilic nuclei. The layers of stratum spinosum were more in number and distinctly visible because of their prickly appearance. The cytoplasm was eosinophilic and only a few cells possessed fine keratoxyline granules. The keratinized layer with dark flat degenerating nuclei was evident. A few cells with less eosinophilic cytoplasm and vesicular nuclei were also present in between the stratum corneum cells. The vesicular cells were having generally large flat nuclei and their cytoplasm contained fine basophilic particles like keratoxyline granules. Towards the free end of the pillar, the epithelium of both the surfaces was continuous with each other.
The pillar also had small and large papillae which were generally blunt towards the free tips (Figs 7 and 8). The surface of these papillae also had meshwork of small depressions which were comparatively lesser in depth as compared to those of the ruminal papillae.

Coronary pillar

The coronary pillar also had two surfaces. The caudal surface facing the ventral blind sac was lined by stratified squamous keratinized epithelium and had papillae of varying size and shapes. The luminal surface also presented transversely or longitudinally cut papillae. This surface was continuous with the other surface having small sized papillae towards the tip. The cells of the stratum basale were not strongly basophilic as compared to the opposite surface and possessed round to oval lightly stained nuclei (Fig 9). Rests of the layers were again fewer in number possessing lightly stained nuclei. The nuclei were very less basophilic but nucleoli were generally strongly basophilic and centric in position. Some of the cells showed two nucleoli. The cytoplasm of these cells was eosinophilic, finely granular and homogeneous. The stratum corneum was uniform and thinner as compared to the opposite surface. It was having degenerated elongated nuclei which were more prominent because of basophilic nuclei and strongly eosinophilic cytoplasm.

The cranial surface facing the ventral sac possessed very small sized papillae of low height with blunt tips having stratified squamous keratinized epithelium. The basal surface of the epithelium showed large papillary pegs which were widely placed. The stratum basale was having high cuboidal to simple columnar epithelial cells. The stratum spinosum and stratum granulosum cell layers were only few and could not be differentiated from each other. The cell layers just adjacent to stratum corneum contained more basophilic darkly stained elongated nuclei. The cytoplasm of these cells was strongly eosinophilic but did not contain keratohyaline granules.

Propria submucosa having loose irregular connective tissue was comprised of reticular, collagen and elastic fibres. Towards the ventral sac where papillae were small, the collagen fibres were regularly placed at the base of the epithelium and only few fibres
penetrated into the core of the papillae. In contrast, the collagen fibres extended up to the tip of large papillae present towards ventral blind sac. In deeper part the propria submucosa became loose but close to tunica muscularis it had an uniform layer of collagen fibres, from which a few collagen fibres along with reticular fibres extended in between the fasciculi of the muscles of tunica muscularis.

The tunica muscularis was constituted by a thicker inner circular and a thinner outer longitudinal layer of smooth muscles. A thin uniform layer of elastic fibres surrounded the outer layer of tunica muscularis. Tunica serosa was very thick having large elastic fibres surrounded the outer layer of tunica muscularis. A thin uniform layer of collagen fibres, cells, blood capillaries and nerve bundles. The tunica muscularis and serosa were well developed. Scanning electron microscopy confirmed the observations of light microscopy especially shape and size of the papillae. The higher magnification revealed a meshwork of shallow depressions being separated by small elevations.

References


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