



Anatomical Studies on the Reticulum of Bakerwali Goat of Jammu Region

Jasvinder Singh Sasan*, Shalini Suri and Kamal Sarma

Division of Veterinary Anatomy, FV.Sc & A.H., SKUAST-J, R.S. Pura, J&K., INDIA

**Corresponding author: J.S. Sasan; E-mail: jssasan216@gmail.com*

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ABSTRACT

Present study was carried on six stomach samples from adult apparently healthy Bakerwali goat. Reticulum was spherical and was separated from rumen by rumino-reticular groove ventrally. Mucosa was dark brown in colour having reticular cells of variable shapes. Cells were smaller towards lesser curvature. Mean empty weight of reticulum was 12.95% of the total empty weight of the stomach whereas the capacity was 7.78% of the total volume of the stomach. Histologically, reticulum consisted of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Lamina epithelialis was lined by keratinized stratified squamous epithelium. Lamina muscularis mucosae was present only towards the tip of primary reticular crests. Tunica muscularis had inner circular and outer longitudinal layers of smooth muscle. Number of crests per field, length and width of primary reticular crests were significantly ($p < 0.05$) higher at lesser curvature. Histochemically, stratum corneum showed strong PAS reaction, stratum granulosum showed strong reaction for Alcian Blue (pH 2.5). Distribution of glycogen was moderate in the layers of lamina epithelialis mucosae except stratum corneum. Lamina epithelialis mucosae and tunica muscularis showed strong reaction for basic proteins. Stratum corneum showed moderate reaction for lipids. Floor of reticular groove at the level of reticulo-omasal opening presented papillae resembling the claws of bird. Entire reticular groove was lined by stratified squamous keratinized epithelium. Lamina muscularis mucosae was incomplete and seen mainly in the lips of reticular groove. Floor of groove had transverse smooth muscle fibers whereas lips contained thick longitudinal smooth muscle fibers.

HIGHLIGHTS

- The mucosa of the reticulum was dark brown and formed of honeycomb cells which were smaller near lesser curvature
- Histologically, lamina muscularis mucosae was present only at the tip of primary reticular crests
- Lamina muscularis mucosae was incomplete and seen mainly in the lips of reticular groove.

Keywords: Anatomy, Bakerwali goat, histology, reticulum, reticular groove

Union territory of Jammu & Kashmir is blessed with environment which is appropriate for the rearing of small ruminants. When compared to other farm animals, goat rearing has advantages as goats do not require expensive buildings and equipments. Goats have high dry matter and fiber digestibility and thus can subsist on poor woody vegetation which no other animal will consume. Hence, the people below poverty line are also able to rear the goat. Hence, goat is popularly known as 'Poor Man's Cow'.

According to the ICAR/NBAGR report (2008), there were roughly twenty well-defined breeds of goats in India, along with non-descripts, of which "Bakerwali" is mostly raised by Bakerwal tribes in Jammu & Kashmir. This goat

is one of the most meat-producing goats in the country, not just in Jammu & Kashmir. Bakerwali goat is noted for its long-distance migration (Gupta and Bakshi, 2009). The word 'Bakerwali' comes from the Hindi or Urdu Indic language phrases (bakri or bakara) for goat or sheep, and wal for "one who looks for". Bakerwali goats are white, brown, or black goats with white markings and exquisite silky hair from the Northern Himalayan region (about 10-

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12 cm long). Bakerwali goats are horned and have lengthy hair. Ears might have long, medium, or little flaps. The tail is short and hairy, and the legs are thick (Gupta and Bakshi, 2009).

The digestive system of ruminants is unique in a way that it allows them to use energy from fibrous plant material better than other herbivores. Ruminant forestomach includes rumen, reticulum and omasum. Forestomach have a unique sorting function which ensures that large ingesta particles are regurgitated and remasticated (Fritz *et al.*, 2009). This mechanism facilitates a high digestive efficiency (Clauss *et al.*, 2009).

Literature is available on the histology of forestomach of cattle, buffalo and sheep (Scala *et al.*, 2011), gross morphology and biometry of forestomach of Black buck (Kumari *et al.*, 2013), morphology of stomach of Swamp buffalo (Nurliani *et al.*, 2015), macroscopic anatomy of reticulum in wild ruminant species (Clauss *et al.*, 2009). However, meager information is available on the anatomy of reticulum Bakerwali goat of Jammu region. Hence, the present study was planned and executed.

MATERIALS AND METHODS

For the study, six stomach samples from adult apparently healthy Bakerwali goat were collected from slaughter houses in and around Jammu region. Immediately after collection, the stomach was cleaned with running water and brought to the laboratory for recording the gross morphological and biometrical studies. Following parameters were recorded:

1. Total empty weight (gm) of stomach and weight (gm) of reticulum was recorded with help of Monopan balance.
2. Capacity (ml) of entire stomach and reticulum was carried out by filling with water and measuring with help of measuring cylinder.
3. Circumference (cm) of reticulum as longitudinal and transverse circumference from the middle of the reticulum,
4. Dorso-ventral height (cm) and cranio-caudal width (cm) of reticulum
5. Length (cm) of honey-comb cells was taken by measuring maximum distance between two angles

of large sized cells at floor (greater curvature) of reticulum as well as towards reticular groove (lesser curvature)

6. Width (cm) of honey-comb cells was taken by measuring maximum distance between two crests of large sized cell at floor (greater curvature) of reticulum as well as towards reticular groove (lesser curvature)

After recording the gross and biometrical parameters, tissue samples were collected from two sites, i.e. lesser curvature (close to reticular groove) and greater curvature (floor of reticulum) and preserved in 10% Neutral Buffered Formalin (NBF) solution (Luna, 1968). The tissue samples were processed and tissue sections of 5 μ thickness were obtained. The sections were stained with Haematoxylin & Eosin for routine histomorphology, Von Gieson & Verhoeff's and Gomori stains for connective tissue fibers, Ayoub-Shklar method for keratin (Luna, 1968). For histochemistry, Best Carmine, Bromophenol Blue, PAS-AB and Sudan Black B methods were used for demonstration of glycogen, basic proteins, neutral & acidic mucopolysaccharides and lipids, respectively. The micrometrical observations were taken i.e. thickness (μ) of lamina epithelia, lamina-propria submucosa, tunica muscularis and tunica serosa of reticulum, length (μ) and width (μ) of primary reticular crest. All the data was subjected to standard statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Gross morphology and biometry of reticulum

Reticulum was most cranially located compartment of goat stomach as also observed in Surti goat (Mahendrakumar, 2017) and non-descript goats (Sasan *et al.*, 2022). It was somewhat spherical in outline (Fig. 1) and located in between diaphragm and rumen. The reticulum of marsh deer was of pyriform shape (Machado and Oliveira, 2008). It consisted of parietal/left and visceral/right surface both of which were convex. Dorsally, it continued with the cranial sac of rumen whereas ventrally a distinct rumino-reticular groove was seen separating reticulum from rumen. Similar observation was made by Mahendrakumar (2017) in Surti goat and Sasan *et al.* (2022) in non-descript goats. Interiorly, reticulum communicated with

oesophagus and rumen through reticular groove and with omasum via reticulo-omasal orifice.

The mucosa of the reticulum of Bakerwali goat was dark brown in colour and formed permanent crests known as *cristae reticuli* which intersect to form 4-6 sided cells known as *cellulae reticuli* (Fig. 2). In non-descript goats, the mucosa was pale brown in colour (Sasan *et al.*, 2022). The cells were further subdivided by secondary and occasionally tertiary crests. The *cellulae reticuli* were variable in shape (Fig. 2). However, in non-descript goats, the cells were more hexagonal in outline (Sasan *et al.*, 2022). Colville and Bassert (2008) stated that 4-6 sided structures of the honeycomb served to increase the surface area of the reticulum and thus increased the absorptive surface. Hofmann and Schnorr (1982) suggested that the honeycomb pattern of reticulum helps in contracting the organ. Reece (2005) observed that the crests were papillated in sheep but smooth in cattle. These cells were smaller near lesser curvature as compared to the cells towards greater curvature (Fig. 2). Similar observation was made by Nurliani *et al.* (2015) in swamp buffalo and Mahendrakumar (2017) in Surti goat. In adult Bakerwali goat, few *cellulae reticuli* towards greater curvature presented only the secondary crests whereas *cellulae reticuli* towards lesser curvature were devoid of both secondary and tertiary crests (Fig. 2). In non-descript goats, the cells towards greater curvature were subdivided by both secondary as well as tertiary crests (Sasan *et al.*, 2022). Both the crests and floor of cells were studded with small conical papillae as also observed by Konig and Liebich (2009) in ruminants.

Various biometrical parameters of reticulum are presented in Table 1. The mean empty weight of reticulum (126.03 ± 9.15 gm) was 12.95 ± 0.76 % of the total empty weight of the stomach (969.75 ± 23.17 gm). It was almost similar to the findings of Mahendrakumar (2017) in Surti goat (121.5 ± 5.29 gm) but greater than the findings of Sasan *et al.* (2022) in non-descript goats (88.52 ± 2.04 gm). The capacity of reticulum (766.67 ± 78.17 ml) was 7.78 ± 0.59 % of the total volume of the stomach (9965.00 ± 1016.75 ml). The longitudinal circumference (29.87 ± 1.47 cm) was greater than transverse circumference (27.43 ± 0.8 cm) which were in approximation with the findings of Mahendrakumar (2017) in Surti goat (29.08 ± 1.27 cm and 25.95 ± 1.86 cm, respectively) and Sasan *et al.*, (2022) (29.88 ± 0.28 cm and 23.70 ± 0.89 cm, respectively). In

black buck, the longitudinal diameter was 17.7 ± 0.72 cm and transverse diameter was 14.4 ± 0.72 cm (Kumari *et al.*, 2013).

Table 1: Showing various biometrical parameters of reticulum of Bakerwali goat of Jammu region

Sl. No.	Parameter	Mean \pm SE
1	Empty weight (gm) of reticulum	126.03 ± 9.15
2	Capacity (ml) of reticulum	766.67 ± 78.17
3	Longitudinal circumference (cm)	29.87 ± 1.47
4	Transverse circumference (cm)	27.43 ± 0.80
5	Dorso-ventral height (cm) of reticulum	13.32 ± 0.58
6	Cranio-caudal width (cm)	12.05 ± 0.63
7	Length (cm) of honeycomb cells towards greater curvature	2.13 ± 0.06
8	Width (cm) of honeycomb cells towards greater curvature	1.53 ± 0.04
9	Length (cm) of honeycomb cells towards lesser curvature	1.33 ± 0.06
10	Width (cm) of honeycomb cells towards lesser curvature	0.97 ± 0.03
11	Length (cm) of reticular groove	8.04 ± 0.33
12	Width (cm) of reticular groove towards cardia	2.03 ± 0.05
13	Width (cm) of reticular groove in middle	2.81 ± 0.27
14	Width (cm) of reticular groove towards reticulum	2.66 ± 0.14

The dorso-ventral height of reticulum (13.32 ± 0.58 cm) was greater than its cranio-caudal width (12.05 ± 0.63 cm). In non-descript goats, the same was 13.77 ± 0.61 cm and 10.78 ± 0.34 cm, respectively (Sasan *et al.*, 2022). Perez and Vazques (2012) recorded slightly lower values in Brown Brocket deer (reticulum height as 12.0 ± 1.41 cm and cranio-caudal length as 8.38 ± 1.49 cm). Perez *et al.* (2015) calculated the height and cranio-caudal length of reticulum of deer as 19.2 ± 0.8 cm and 11.6 ± 1.2 cm, respectively. The same was 12.2 ± 2.4 cm and 12.0 ± 0.5 cm, respectively in Dorcas Gazelle (Jerbi *et al.*, 2016). Length (2.13 ± 0.06 cm) and width (1.53 ± 0.04 cm) of honeycomb cells towards the greater curvature of reticulum was greater than the length (1.33 ± 0.06 cm) and width (0.97 ± 0.03) of cells present towards lesser curvature of reticulum. In Surti goat, length and width of reticular cells was 19.17 ± 0.67 mm and 13.97 ± 0.48 mm, respectively

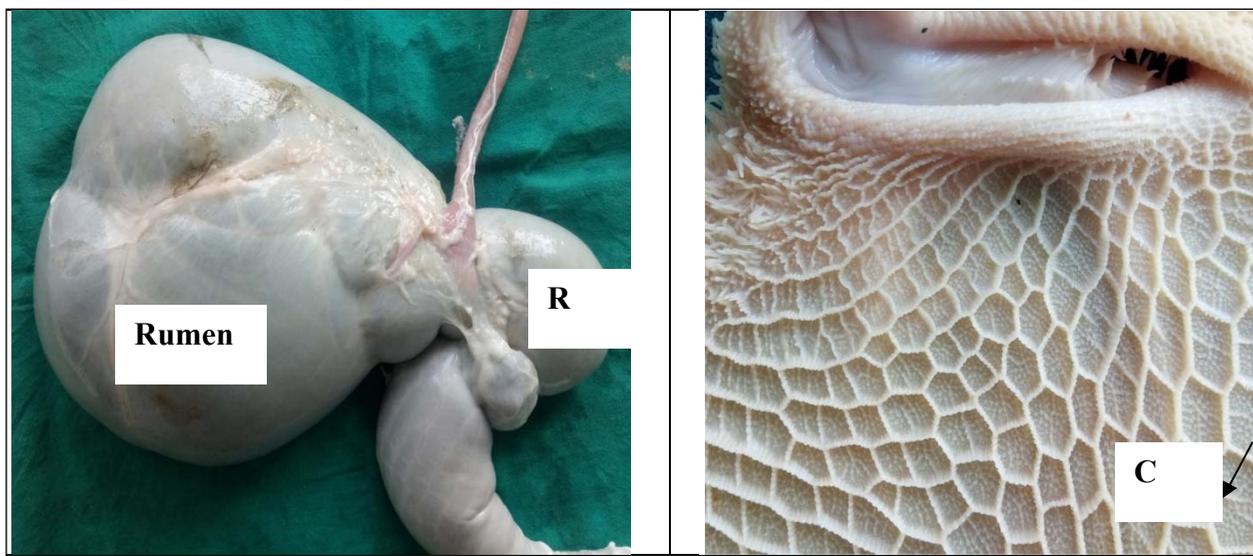


Fig. 1: Photograph of reticulum (R) of adult Bakerwali goat showing visceral surface

Fig. 2: Photograph showing interior of reticulum having reticular cells (C) of variable shapes. Few cells towards greater curvature presented secondary crests (arrow)

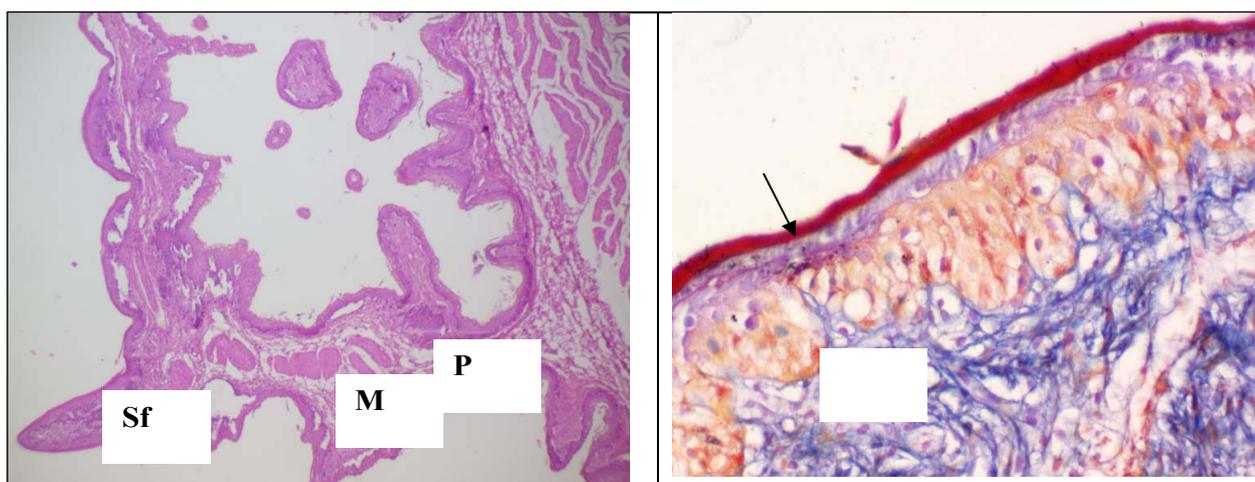


Fig. 3: Photograph of reticulum of Bakerwali goat showing primary reticular crests (P) containing smooth muscle bundles (M) whereas secondary folds (Sf) were devoid of muscle bundles. H&E stain, 40x

Fig. 4: Photograph of reticulum of non-descript goat showing keratinized epithelium (arrow). Ayoub-Shklar method, 400x

(Mahendrakumar, 2017). In non-descript goats, the length and width of reticular cells was 2.00 ± 0.05 cm and 1.42 ± 0.03 cm towards greater curvature and 1.14 ± 0.03 cm and 0.88 ± 0.03 cm towards lesser curvature, respectively (Sasan *et al.*, 2022).

Histomorphology and micrometry of reticulum

Histologically, reticulum consisted of four layers namely

tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The lamina epithelia consisted of keratinized stratified squamous epithelium (Fig. 4) having stratum basale, stratum spinosum, stratum granulosum and stratum corneum. The cells of stratum basale were columnar, stratum spinosum were polyhedral and larger than the basal cells. The stratum granulosum was one to three cells thick. The upper keratinized layer protect against abrasion

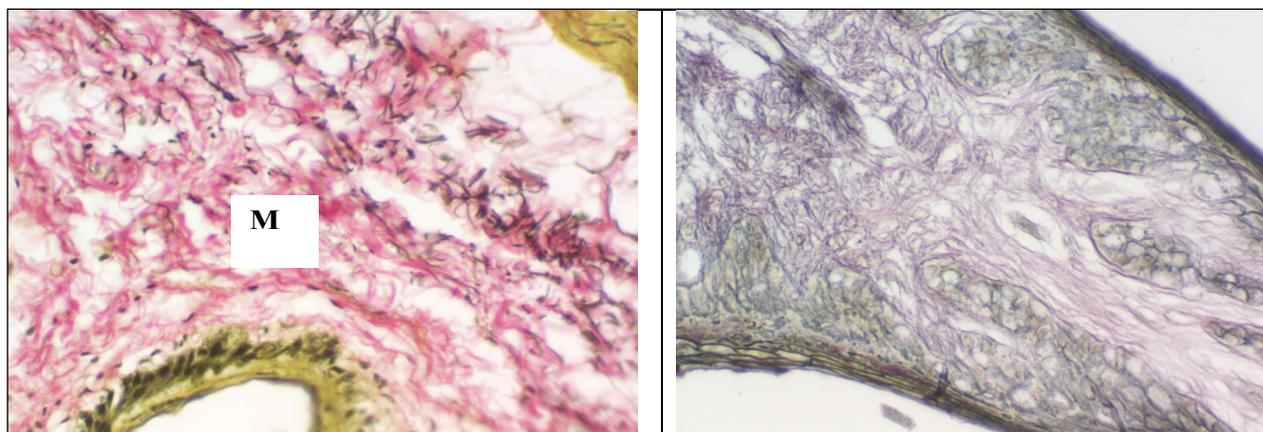


Fig. 5: Photomicrograph of reticulum of adult Bakerwali goat showing collagen fibers (pink colour) and elastic fibers (black colour) in Lamina propria submucosa (M). Von Geison Verhoeff stain, 400x

Fig. 6: Photomicrograph showing presence of reticular fibers. Gomori's stain, 400x

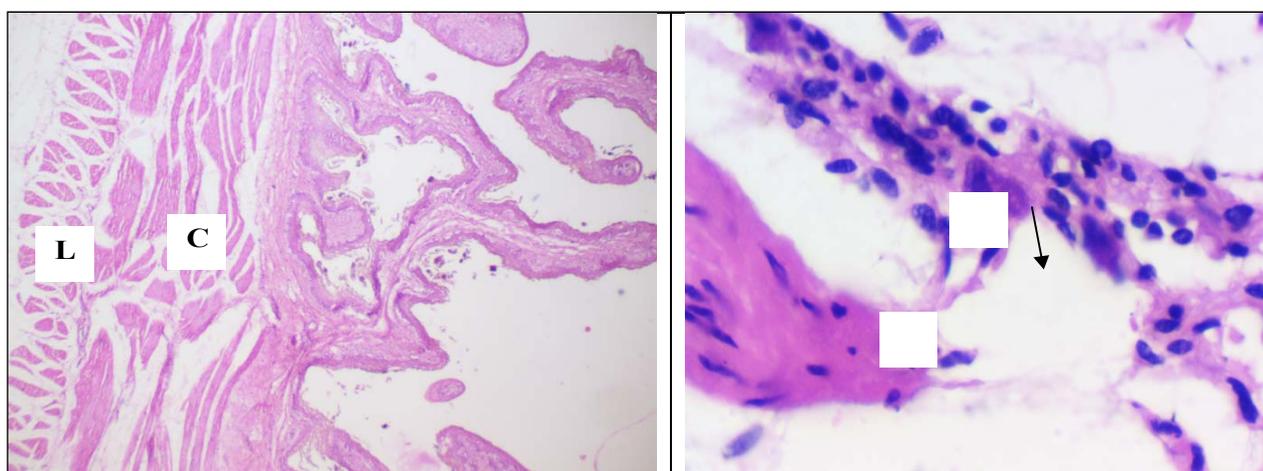


Fig. 7: Photomicrograph of reticulum of Bakerwali goat showing inner circular (C) and outer longitudinal (L) muscle layers. H&E stain, 40x

Fig. 8: Photomicrograph of reticulum of adult Bakerwali goat showing presence of parasympathetic ganglionic cells (arrow). H&E stain, 1000x

by the rough, fibrous diet as also quoted by Dyce *et al.* (2002). In yak, the mucosal epithelium of reticulum was not keratinized (Wang *et al.*, 2014). In present study, the epithelial mucosa of reticulum formed primary reticular crests with lateral border presenting prominent conical ridges. The epithelium formed epidermal pegs which were more prominent in reticular crests. Lamina propria consisted of dense irregular connective tissue as also observed by Ramkrishna and Gadre (2004) in domestic animals and contained abundant collagen fibers with few reticular and elastic fibers (Fig. 6). Smaller vessels, lymphatics and capillaries were also seen. Lamina

muscularis mucosae was present only towards the tip of primary reticular crests (Fig. 3) as reported earlier by Sultana *et al.* (2021) in sheep. Secondary reticular crests were devoid of any muscularis mucosae layer. Due to absence of muscularis mucosae layer, the lamina propria merged with tunica submucosa forming lamina propria submucosa rich in collagen and elastic fibers (Fig. 5).

Tunica muscularis consisted of two layers of smooth muscle fibers, namely inner circular and outer longitudinal layers (Fig. 7) as also reported by Malik (2014) in Gaddi sheep. Numerous coarse reticular fibers reinforced the muscle bundles of tunica muscularis. Loose connective

tissue and blood vessels were also recorded in between. Nerve plexus and parasympathetic ganglionic cells were seen between layers of tunica muscularis (Fig. 8). Outermost layer was tunica serosa which consisted of loose connective tissue, various blood vessels and nerve fibers as also reported by Mahendrakumar (2017) in Surti goat. There was mixed distribution of collagen and elastic fibers in this layer (Fig. 9).

Various micrometrical parameters of reticulum are presented in Table 2. Most of the micrometrical parameters were higher towards greater curvature as compared to lesser curvature but the difference was non-significant ($p > 0.05$).

However, number of crests per field, length and width of primary reticular crests were significantly ($p < 0.05$) higher at lesser curvature than at greater curvature. In non-descript goats, the primary crests were longer and wider at greater curvature ($1436.6 \pm 131.88 \mu$ and $638.98 \pm 56.83 \mu$, respectively) than at lesser curvature ($1256.8 \pm 87.58 \mu$ and $496.59 \pm 46.69 \mu$, respectively) (Sasan *et al.*, 2022).

Histochemistry

The histochemical distribution of neutral and acid mucopolysaccharides, basic proteins, glycogen and

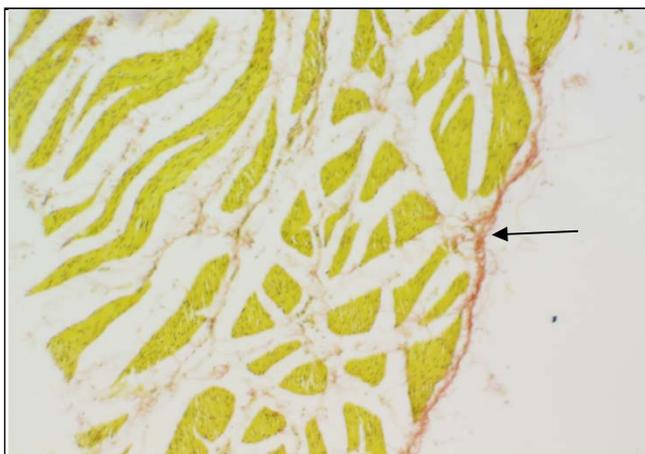


Fig. 9: Photomicrograph of reticulum of adult Bakerwali goat showing collagen fibers (pink colour) in tunica serosa (arrow). Von Geison Verhoeff stain, 100x

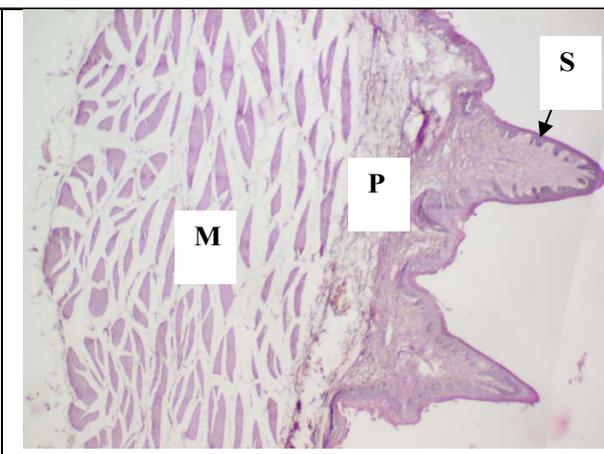


Fig. 10: Photomicrograph of reticulum of adult Bakerwali goat showing reaction of stratum corneum (S), lamina propria-submucosa (P) and tunica muscularis (M) to PAS. 40x

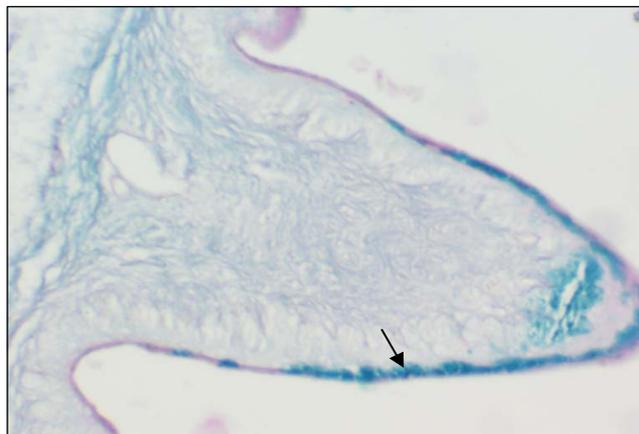


Fig. 11: Photomicrograph of reticulum of adult Bakerwali goat. Stratum granulosum (arrow) showing strong reaction for Alcian Blue. PAS-AB stain, 100x

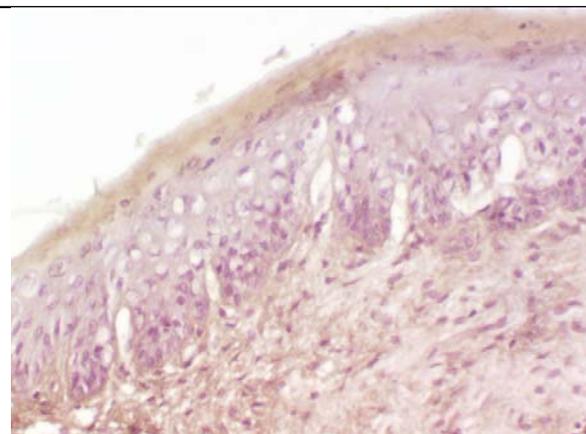


Fig. 12: Photomicrograph of reticulum of adult Bakerwali goat. Stratum corneum showed negative reaction whereas other epithelial layers showed moderate reaction for glycogen. Best Carmine stain, 400x

Table 2: Showing various micrometrical parameters of reticulum of Bakerwali goat of Jammu region

Sl. No.	Parameter (μ)	Towards lesser curvature		Towards greater curvature	
		Mean	SE	Mean	SE
1	Thickness epithelium	58.62	4.53	61.65	4.77
2	Thickness LP submucosa	226.25	25.70	277.26	47.87
3	Inner muscle layer	613.38	71.63	628.36	51.26
4	Outer muscle layer	424.03	33.56	446.30	34.67
5	Tunica muscularis	1036.13	88.75	1142.08	88.24
6	Tunica serosa	248.29	31.44	257.21	37.76
7	Primary Crest per Field	1.50 ^a	0.22	1.00 ^b	0.001
8	Length of primary crest	2683.49 ^a	155.35	2074.44 ^b	189.75
9	Width of primary crest	770.24 ^a	157.29	436.23 ^b	43.22

Mean value with same superscript within row do not differ significantly ($p>0.05$).

Table 3: Histochemical observations of reticulum of adult Bakerwali goat

Parameters	Stain	<i>Lamina epithelialis</i>					LP-S	TM	TS
		SC	SG	SS	SB	BM			
Carbohydrates	PAS	+++	-	-	-	++	++	++	-
Glycogen	Best carmine	-	++	++	++	-	-	+	-
Basic proteins	Bromphenol Blue	+++	+++	+++	+++	-	-	+++	-
Lipids	Sudan Black B	++	-	-	-	-	-	-	-

Strong: +++, Moderate: ++, Weak: +, Negative: -

SC: *Stratum corneum*, SG: *Stratum granulosum*, SS: *Stratum spinosum*, SB: *Stratum basale*, BM: *Basement membrane*, LP-S: *Lamina propria-submucosa*, TM: *Tunica muscularis*, TS: *Tunica serosa*.

sudanophilic lipids in the reticulum of Bakerwali goat has been summarized in Table 3.

Neutral mucopolysaccharides

Strong positive PAS reaction was seen in the stratum corneum of lamina epithelialis mucosae whereas basement membrane exhibited moderate PAS reaction. Lamina propria-submucosa and tunica muscularis also showed moderate PAS reaction (Fig. 10). Lakshmishree (2014) in sheep, goat and buffalo observed strong reaction of stratum corneum for carbohydrates. Basement membrane, lamina muscularis mucosae and tunica muscularis showed moderate reaction however Malik (2014) in Gaddi sheep observed mild PAS reaction in lamina propria-submucosa.

Acid mucopolysaccharides

The inter-cellular spaces in stratum spinosum layer were moderately positive for Alcian Blue (pH 2.5) indicating the presence of acid mucopolysaccharides. Cells of stratum granulosum showed strong reaction whereas sub-epithelial connective tissue showed moderate reaction for Alcian Blue (pH 2.5) (Fig. 11). In goats of 90-120 days of age, PAS-AB reaction was weak in epithelium and tunica serosa, moderate in tunica submucosa and absent in tunica muscularis (Pachpande, 2008) in contrary to the findings of Malik (2014) in Gaddi sheep.

Glycogen

The distribution of glycogen was moderate in the layers of lamina epithelialis mucosae except stratum corneum.

Tunica muscularis showed weak glycogen reaction by Best Carmine stain. However, lamina propria-submucosa and tunica serosa showed negative reaction for glycogen (Fig. 12). However, Lakshmishree (2014) in sheep, goat and buffalo observed moderate glycogen reaction of lamina muscularis mucosae and tunica muscularis.

Basic proteins

Lamina epithelialis mucosae and tunica muscularis

showed strong reaction for basic proteins whereas all the other layers showed negative reaction for basic proteins (Fig. 13).

Lipids

Moderate lipid reaction was observed in stratum corneum only as also observed by Malik (2014) in Gaddi sheep who observed moderate reaction in lamina epithelialis (Fig. 14).

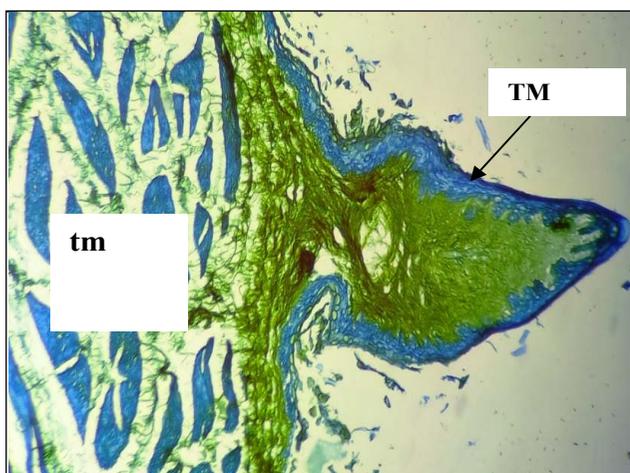


Fig. 13: Photomicrograph of reticulum of adult Bakerwali goat. Only Tunica mucosa (TM) and tunica muscularis (tm) showed strong reaction. Bromophenol Blue stain, 100x

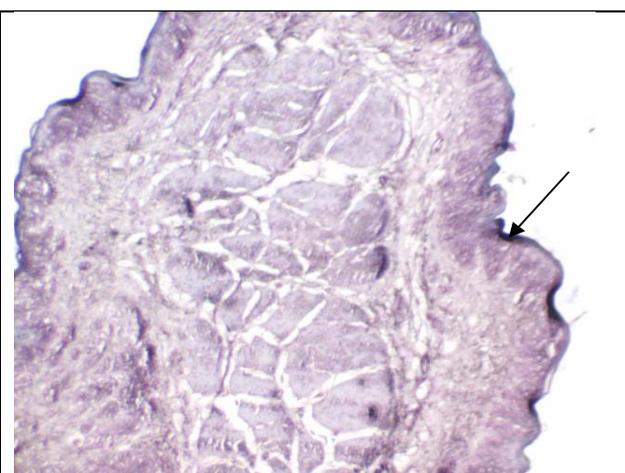


Fig. 14: Photomicrograph of reticulum of adult Bakerwali goat. Stratum corneum showed moderate reaction. Sudan Black B stain, 400x

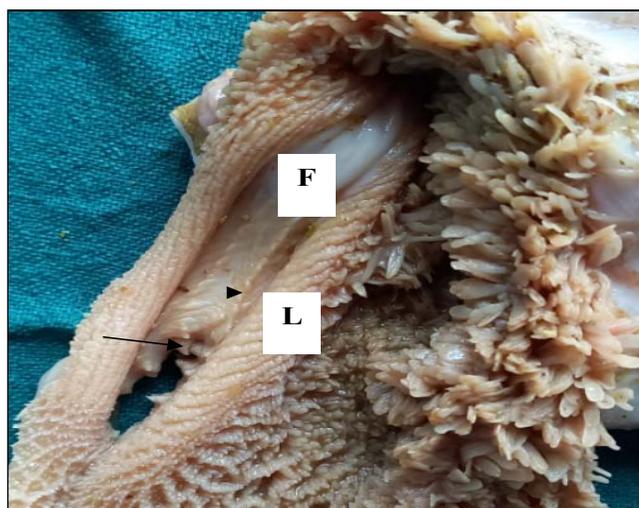


Fig. 15: Photograph showing lips (L) and floor (F) of reticular groove with presence of papillae unguiculiformes (arrow) towards reticulo-omasal opening. Floor presented longitudinal folds (arrow head).

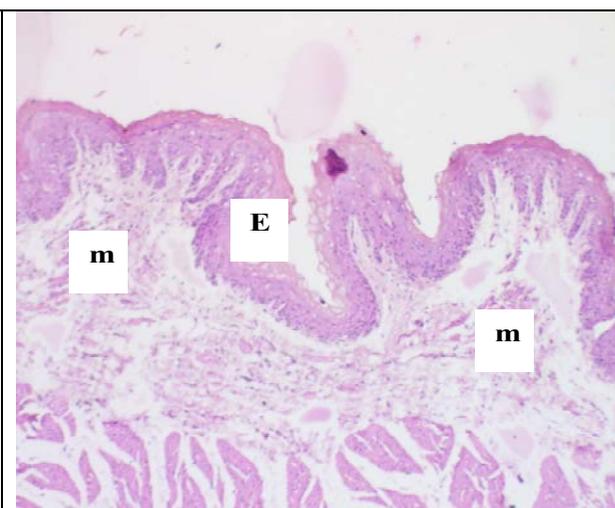


Fig. 16: Photomicrograph of lip of reticular groove showing keratinized stratified squamous epithelium (E), presence of lamina muscularis (m) in lamina propria submucosa. H & E stain, 100x

Reticular groove

Gastric groove extends from cardia to the pylorus and is divided into three segments i.e. reticular groove, omasal groove and abomasal groove (Nickel *et al.*, 1979). The reticular groove was characterized by two muscular ridges/lips which extended from the cardia to the level of reticulo-omasal opening. Both the lips were less developed towards the cardia but well developed towards the reticulo-omasal orifice with separate termination. The length of reticular groove was 8.04 ± 0.33 cm. It was about 15-20 cm long in ox and 7-10 cm long in small ruminants (Nickel *et al.*, 1979). In non-descript goats, the length was 8.70 ± 0.07 cm (Sasan *et al.*, 2022). The width was maximum in middle (2.81 ± 0.27 cm) followed by width towards reticulum (2.66 ± 0.14 cm) and least towards cardia (2.03 ± 0.05 cm). Towards the level of reticulo-omasal opening, the floor of the groove presented papillae resembling the claws of a small bird (Fig. 8). Such papillae were absent at reticulo-omasal orifice in deer (Perez *et al.*, 2015). Teixeira *et al.* (2009) suggested the role of these papillae as filter barrier and check the passage of those particles which were of inappropriate size and not suitable to be forwarded into the omasum and abomasum. The floor of the groove was pale and marked by longitudinal folds (Fig. 15).

The entire reticular groove and its lips were lined by stratified squamous keratinized epithelium. Lamina muscularis mucosae was incomplete and seen mainly in the lips of reticular groove (Fig. 16). Lamina propria blended with tunica submucosa which mainly consisted of collagen and elastic fibers. Glands were not observed in lamina propria. In the floor of groove, mainly transverse smooth muscle fibers were seen whereas lips contained thick longitudinal smooth muscle fibers whereas Pochon (2002) observed that the muscle fibers present in the lips of groove were arranged longitudinally and the floor of the groove presented outer longitudinal muscle fibers and inner layer of fibers arranged perpendicular to the long axis of the groove.

CONCLUSION

Reticulum was spherical in outline. Mucosa formed variable shaped reticular cells which were smaller towards lesser curvature. Mean empty weight of reticulum was 12.95% of the total empty weight of the stomach

whereas the capacity was 7.78% of the total volume of the stomach. Lamina epithelialis consisted of keratinized stratified squamous epithelium. Lamina muscularis mucosae was present only towards the tip of primary reticular crests. Number of crests per field, length and width of primary reticular crests were significantly higher at lesser curvature. The entire reticular groove and lips were lined by stratified squamous keratinized epithelium. Stratum corneum showed strong PAS reaction and stratum granulosum showed strong reaction for Alcian Blue (pH 2.5). Distribution of glycogen was moderate in the layers of lamina epithelialis mucosae except stratum corneum. Lamina epithelialis mucosae and tunica muscularis showed strong reaction for basic proteins. Stratum corneum showed moderate reaction for lipids. Lamina muscularis mucosae was incomplete and seen mainly in the lips of reticular groove. Floor of groove had transverse smooth muscle fibers whereas lips contained thick longitudinal smooth muscle fibers.

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