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Original Article

Histomorphometrical and histochemical studies on the omasum of adult Bakerwali goat, non-descript goats and non-descript sheep of Jammu region: A comparative adaptational perspective

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Abstract

Background: The omasum absorb water, electrolytes, volatile fatty acids by regulating the flow of digesta from the reticulum to the abomasum. In the Jammu region, small ruminants are raised under various agro-climatic conditions. Sheep mainly graze, while goats primarily browse. Such differences in feeding habits influences anatomy of digestive tract, especially the omasum. Studying omasum structure helps explain differences in digestive efficiency, nutrient utilization, and environmental adaptation among these species. **Aims:** The present study aimed to compare anatomy of omasum in Bakerwali goats, non-descript goats and non-descript sheep and help develop breed-specific management strategies. **Methods:** Gross morphological, biometrical, histomorphological, micrometrical and histochemical studies were undertaken. Samples were fixed and processed for standard histological procedures and stained with various stains for histological and micrometrical observations. **Results:** Omasum was laterally compressed oval to bean-shaped sac in all the three species. Internally, omasum was characterized by omasal laminae. The 4th order laminae were prominent in adult non-descript sheep. Lateral surface of the 1st order laminae presented ridges running parallel to the free concave border in adult non-descript sheep. Total empty weight, capacity and number of omasal laminae was significantly highest in non-descript sheep. Histologically, omasal laminae were thickest in non-descript sheep and thinnest in non-descript goats. **Conclusion:** The present research extends the depth of the knowledge regarding anatomy of omasum. The anatomical differences in the omasum between browsers and grazers can be correlated to their digestive physiology.

Key words: Bakerwali goat, Histology, Lamina epithelialis, Omasum

Introduction

Union territory of Jammu & Kashmir is generally provided with an environment and socio-cultural system that is conducive to small ruminant production. When compared to other farm animals, goats have the advantage of not requiring expensive structures or equipment. Furthermore, goats have a high digestion of dry matter and fibre, and can eat inferior woody vegetation that no other animal would eat. As a result, the goat is known as the ‘Poor Man’s Cow’. Similarly, sheep farming is popular among India’s economically disadvantaged people and is seen as a “mortgage lifter” for poor farmers (Poonia *et al*., 2012).

On the basis of foraging behavior, ruminants can be classed as grazers, browsers, or intermediate grazers (Gordon, 2003). Sheep prefer to graze, but goats prefer to browse (Mosavat and Chamani, 2013). Browsers eat high-nutrient twigs, shrubs, leaves and fruits which are generally less fibrous, richer in protein and soluble nutrients, while grazers eat lower-quality grasses which are high in fiber (cellulose and lignin), low in protein. Fadel Elseed *et al*. (2002) also reported high nutritive value of browse species compared with grasses. Grazers and browsers thus have evolved different feeding strategies and digestive anatomies to efficiently extract nutrients from the types of plants they eat. Their structural differences, particularly in their digestive systems play a major role in enhancing nutrient absorption efficiency based on their diets. The structural differences in the digestive system allow grazers to efficiently extract nutrients from low-quality, fibrous grasses, while browsers are specialized for rapid digestion and detoxification of high-quality, toxin-rich plant material. These differences are crucial for maximizing nutrient absorption efficiency and overall survival in their respective ecological niches.

The diversity of plant morphology and chemistry has resulted in diverse anatomical adaptations in herbivores and omasum is one such organ greatly influenced by this diversity. Omasum is spherical with a short tunnel connecting it to the reticulum. It is known as the “many piles” or “butcher’s bible” because of the numerous folds or leaves that mimic book pages (Pasquini *et al*., 2003). These folds improve the surface area available for absorbing nutrients from feed and water. The omasum is where water is absorbed.

The present study hypothesized that there were significant anatomical changes in the structure of the omasum between sheep and goats that reflect their differing feeding practices and dietary preferences. This may guide us to a better understanding of the organ, thereby correlating with the digestive physiology of small ruminants. By better understanding how the digestive system of the ruminant works, livestock producers can better understand how to care for and feed ruminant animals. Developing a good understanding of ruminant digestive anatomy and function can help livestock producers better plan appropriate nutritional programs and properly manage ruminant animals in various production systems.

Materials and Methods

Place of work

Division of Veterinary Anatomy, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J), R.S. Pura, Jammu, Jammu and Kashmir, India.

Collection of samples

The stomach samples from apparently healthy adult Bakerwali goat, non-descript goats and non-descript sheep were collected from the slaughter houses in and around Jammu city. The age of the animals was estimated by examining the dentition (Solaiman, 2010). Six (06) samples from each breed were collected. Immediately after collection, the stomach was cleaned with running tap water and brought to the laboratory for recording the gross morphological and biometrical studies.

Gross morphological studies

Immediately after the slaughter of animals, the stomach was separated by cutting at the atrium ventriculi and pylorus in the cranial and caudal directions, respectively. Omasum was excised for evacuation at the point of lesser curvature. After thorough cleaning and removal of omental coverings, omasum was photographed to document gross morphology. Following gross morphological and biometrical analyses, specimens were kept in 10% Neutral Buffered Formalin for tissue processing.

Biometrical studies

The biometry of stomach of adult Bakerwali goat, non-descript goat and non-descript sheep was recorded (Bello *et al*., 2020) and it includes the following parameters:

1. Total empty weight of stomach (gm) with help of Monopan balance
2. Total capacity of stomach (ml) was calculated by filling stomach with water and measuring with help of measuring cylinder

The biometrical observations of the omasum included (Fig. 1):

1. Weight (gm) of empty omasum with help of Monopan balance
2. Capacity (ml) of omasum was calculated by filling omasum with water and measuring with help of measuring cylinder
3. Length (cm) of omasum was measured between its two extremities, i.e., reticular and abomasal end using non-stretchable thread and scale along its greater and lesser curvature
4. Height (cm) was taken as the distance between greater and lesser curvature by using non-stretchable thread and scale
5. Width (cm) was taken as the distance between cranial and caudal end of omasum by using non-stretchable thread and scale
6. Total Number and arrangement of omasal laminae
7. Number of omasal laminae of different orders
8. Height (cm), length (cm) and thickness (cm) of omasal laminae of different orders were recorded by using non-stretchable thread, scale and Vernier Calliper

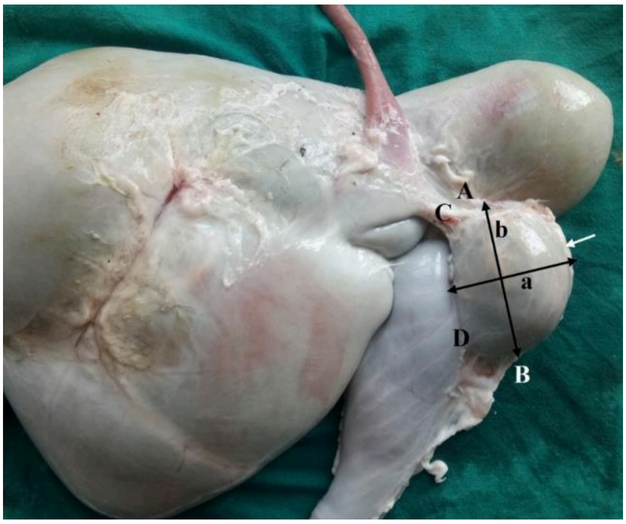

Fig. 1: Photograph of stomach of adult non-descript goat showing different biometrical parameters of omasum. a: Height of omasum, b: Width of omasum, AB: Length of omasum along greater curvature (white arrow), and CD: Length of omasum along lesser curvature

Histological and histochemical studies

After recording the gross and biometrical parameters, tissue samples from omasum of Bakerwali goat, non-descript goat and non-descript sheep were preserved in 10% Neutral Buffered Formalin (NBF) solution. After proper fixation, the tissue samples were washed, dehydrated, and cleared by standard tissue processing schedule (Luna, 1968). The tissue samples were embedded in paraffin wax (58-60°C). Tissue sections of

5 µm was obtained from these blocks on clean glass slides with the help of rotary microtome (Yorco, Lipshaw type). The sections were subjected to various histological and histochemical methods as detailed hereunder.

1. Hematoxylin and Eosin stain for routine histomorphology
2. Van Gieson's method for collagen fibers
3. Weigert's method for elastic fibers
4. Bielschowsky's method for nerve fibers
5. Ayoub-Shklar method for keratin
6. Periodic Acid Schiff's Method and Alcian blue (pH=2.5) for neutral and acidic mucopolysaccharides
7. Best Carmine method for glycogen
8. Bromphenol blue for basic proteins
9. Sudan Black B for lipids

Microscopy and micrometry

The stained sections were analyzed using an optical microscope (Magnus microscope, MX21i-TrLED). Digital histological photographs were captured with the help of microscopic camera (Magnus microscope, MX21i-TrLED), and MagVision software. Histomorphological observations of the omasum of different species under study were recorded.

Micrometry was performed on Hematoxylin and Eosin-stained sections by microscopic image analysis software (MagVision). The micrometrical observations included thickness of different layers of omasum (µ), width of omasal laminae (µ) at base, number of omasal laminae per field at 40x, keratin thickness (µ), size of nucleus (µ) in different layers of lamina epithelialis mucosae (Lakshmishree, 2014). All the micrometric observations were recorded in ten randomly selected fields of the stained tissue section from each species (Fig. 2).

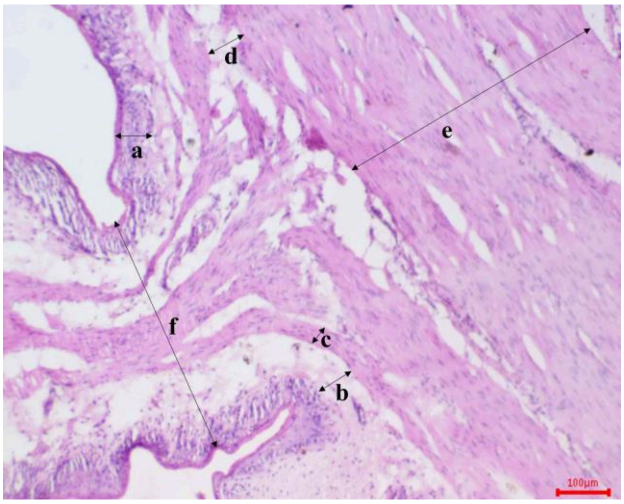

Fig. 2: Photomicrograph of omasum of adult non-descript goat showing different micrometrical parameters. a: Thickness of lamina epithelialis mucosae, b: Thickness of lamina propria, c: Thickness of lamina muscularis, d: Thickness of tunica submucosa, e: Thickness of tunica muscularis, and f: width of omasal laminae (staining method: H&E, scale bar, 100 µm, magnification: ×100)

Statistical analysis

The data recorded in the study was expressed as Mean±SE (Standard Error of Mean) and was subjected to Standard Statistical Analysis (Snedecor and Cochran, 1994). One-Way Analysis of Variance (ANOVA) was used to determine the level of significant difference in mean values.

Results

Gross morphology

In adult Bakerwali goat, non-descript goats and non-descript sheep, the omasum was laterally compressed sac and was slightly oval to bean-shaped. The interior of omasum of adult non-descript goats and non-descript sheep was light brown in color whereas it varied from brown to dark brown in Bakerwali goat. Internally, the omasum of all the three species had numerous page-like structures which were its defining characteristics called "omasal laminae" (Figs. 3a, b). These laminae had four orders i.e., the 1st, 2nd, 3rd and 4th order laminae. The 1st order laminae were the tallest while those in the 4th order were the shortest. The 4th order laminae were more prominent in adult non-descript sheep whereas in adult Bakerwali goat and non-descript goats, the 4th order laminae were either absent or were in the form of small elevations (Figs. 3a, b). These laminae protruded from the greater curvature of omasal wall. They had a greater degree of convexity at their attached border than at their free border (strongly concave). Through the reticulo-omasal orifice, the omasum communicates with the reticulum and it communicates with the abomasum through omasal-abomasal orifice. Between these two orifices, a groove present called as sulcus omasi.

The pointed or conical papillae were scattered throughout the omasal laminae and these papillae were more prominent towards the free concave border of omasal laminae. These papillae were large claw shaped towards reticulo-omasal orifice in all the three species which decrease in size towards omaso-abomasal orifice. In adult non-descript sheep, the lateral surface of the 1st order laminae presented ridges running parallel to the free concave border (Fig. 3c). These ridges were also studded with pointed papillae. In adult Bakerwali goat and non-descript goats, the lateral surface of the 1st order omasal laminae were devoid of such ridges. However, no significant difference in the shape of omasal papillae among different orders of omasal laminae was reported in any of the three species under study.

Biometry

The various biometrical parameters of omasum of adult Bakerwali goat, non-descript goats and non-descript sheep are presented in Table 1. Total empty weight and capacity of omasum was significantly highest in non-descript sheep and lowest in non-descript goats. Length of omasum along greater curvature, height and width were significantly ($P<0.05$) highest in Bakerwali goat, however, length along lesser curvature did not show any significant difference ($P>0.05$). Total number of omasal laminae were significantly (P<0.05) highest in non-descript sheep. The 1st order, 3rd order and 4th order laminae were significantly (P<0.05) highest in non-descript sheep, however, the 2nd order laminae did not show any significant difference (P>0.05). All the omasal laminae were significantly longer in Bakerwali goat except the 3rd order laminae which did not show any significant difference (P>0.05). However, the height of the 1st, 2nd and 3rd order omasal laminae was greater in non-descript sheep. The 1st order and 3rd order omasal laminae showed significant difference (P<0.05).

The 1st order omasal laminae were thicker in adult non-descript goats (0.15±0.01 cm) followed by Bakerwali goat (0.13±0.01 cm) and thinnest in non-descript sheep (0.12±0.01 cm). The 2nd order omasal laminae were thicker in Bakerwali goat and non-descript goats. The 3rd order omasal laminae were thicker in Bakerwali goat followed by non-descript goats and least in non-descript sheep.

Histomorphology

In the present study, the wall of omasum was composed of four distinct layers; tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa irrespective of the species (Fig. 4a).

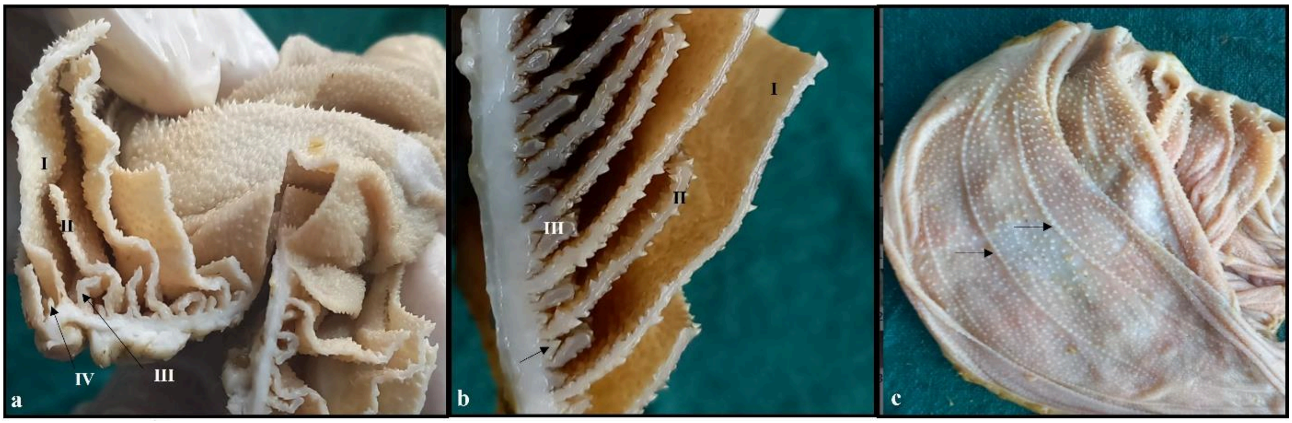

Fig. 3: (**a**) Photograph showing I, II, III and IV orders of omasal laminae in adult non-descript sheep, (**b**) Omasal laminae in adult Bakerwali goat. IVth order laminae (arrow) were less prominent as compared to non-descript sheep, and (**c**) Omasum of adult non-descript sheep. On the lateral surface of omasal laminae of non-descript sheep, papillae were present in rows on the elevated ridge like structures (arrow)

Table 1: Comparative gross biometrical parameters of omasum in adult Bakerwali goat, non-descript goat and non-descript sheep

Parameters		Bakerwali goat	Non-descript goat	Non-descript sheep
Total empty weight of stomach (gm)		969.75 ± 23.17^{a}	760.07 ± 38.69^{b}	913.30 ± 78.84^{ab}
Total capacity of stomach (ml)		9965.00 ± 1016.75^{a}	10301.05 ± 610.76^{a}	10770 ± 575.64^{a}
Total empty weight of omasum (gm)		84.15 ± 4.22^{a}	54.87 ± 6.28^{b}	92.60 ± 7.87^{a}
% Omasum weight		8.67 ± 0.36^{a}	7.17 ± 0.63^{b}	10.15 ± 0.12^{a}
Total capacity of omasum (ml)		117.50 ± 5.29^{a}	107.33 ± 9.30^{a}	143.33 ± 7.60^{b}
% Omasum capacity		1.22 ± 0.13^{a}	1.06 ± 0.09^{a}	1.33 ± 0.02^{a}
Length of omasum (cm)	Greater curvature	19.08 ± 2.03^{a}	15.03 ± 0.49^{b}	17.27 ± 0.72^{ab}
	lesser curvature	9.97 ± 0.57^{a}	8.22 ± 0.27^{a}	8.50 ± 1.08^{a}
Height (cm)		7.18 ± 0.78^{a}	5.29 ± 0.38^{b}	6.94 ± 0.22^{a}
Width (cm)		9.73 ± 0.82^{a}	7.17 ± 0.38^{b}	8.60 ± 0.49^{ab}
Number of omasal laminae		53.67 ± 2.59^{a}	57.67 ± 2.70^{a}	66.67 ± 3.04^{b}
Number of omasal laminae	1st order	7.83 ± 0.54^{a}	10.00 ± 0.63^{b}	11.33 ± 0.56^{b}
	2nd order	9.83 ± 0.75^{a}	10.00 ± 0.58^{a}	9.67 ± 0.42^{a}
	3rd order	15.50 ± 0.43^{a}	16.17 ± 1.25^{a}	19.33 ± 0.21^{b}
	4th order	20.50 ± 1.63^{a}	24.83 ± 1.40^{a}	26.33 ± 2.23^{b}
Length (cm) of omasal laminae	1st order	18.73 ± 1.21^{a}	15.08 ± 1.16^{b}	16.57 ± 0.66^{ab}
	2nd order	17.58 ± 1.22^{a}	13.58 ± 1.39^{b}	15.63 ± 0.87^{ab}
	3rd order	14.93 ± 1.80^{a}	10.73 ± 1.37^{a}	14.07 ± 0.95^{a}
	4th order	14.67 ± 0.91^{a}	9.40 ± 1.31^{b}	12.40 ± 1.21^{ab}
Height (cm) of omasal laminae	1st order	4.39 ± 0.41^{a}	3.20 ± 0.20^{b}	4.45 ± 0.50^{a}
	2nd order	2.39 ± 0.31^{a}	2.19 ± 0.17^{a}	2.63 ± 0.11^{a}
	3rd order	0.87 ± 0.08^{a}	0.83 ± 0.11^{a}	1.13 ± 0.02^{b}
Thickness (cm) of omasal laminae	1st order	0.13 ± 0.01^{ab}	0.15 ± 0.01^{a}	0.12 ± 0.01^{b}
	2nd order	0.13 ± 0.004^{a}	0.13 ± 0.01^{a}	0.10 ± 0.003^{b}
	3rd order	0.11 ± 0.002^{a}	0.102 ± 0.002^{a}	0.06 ± 0.01^{b}
Length of omasal sulci (cm)		2.60 ± 0.28^{a}	2.09 ± 0.60^{a}	3.57 ± 0.35^{b}

Mean value with different superscript ($^{a,\,b}$) within row differ significantly (P>0.05)

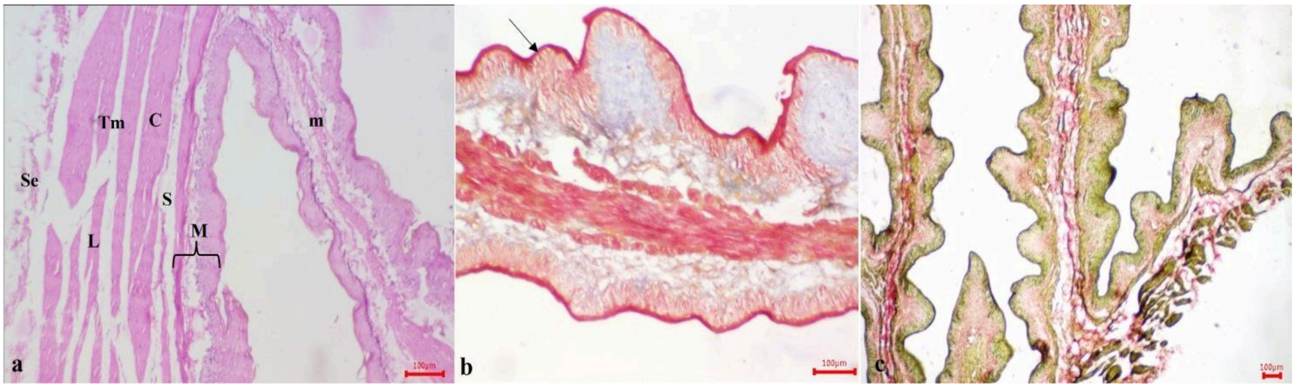

Fig. 4: (**a**) Photomicrograph of omasum of adult Bakerwali goat showing different layers. Tunica mucosa (M), tunica submucosa (S), tunica muscularis (Tm), and tunica serosa (Se). Tunica muscularis had inner circular (C) and outer longitudinal (L) layer (staining method: H&E, scale bar, 100 µm, Magnification: ×100), (**b**) Omasum of adult non-descript goat showing keratin layer (arrow) of omasal laminae (staining method: Ayoub-Shklar, scale bar, 100 µm, Magnification: ×100), and (**c**) Omasum of adult non-descript sheep showing collagen fibers (pink colour) in lamina propria. Lamina muscularis mucosae (arrow) was prominent (staining method: Von Gieson Verhoeff, scale bar, 100 µm, Magnification: ×40)

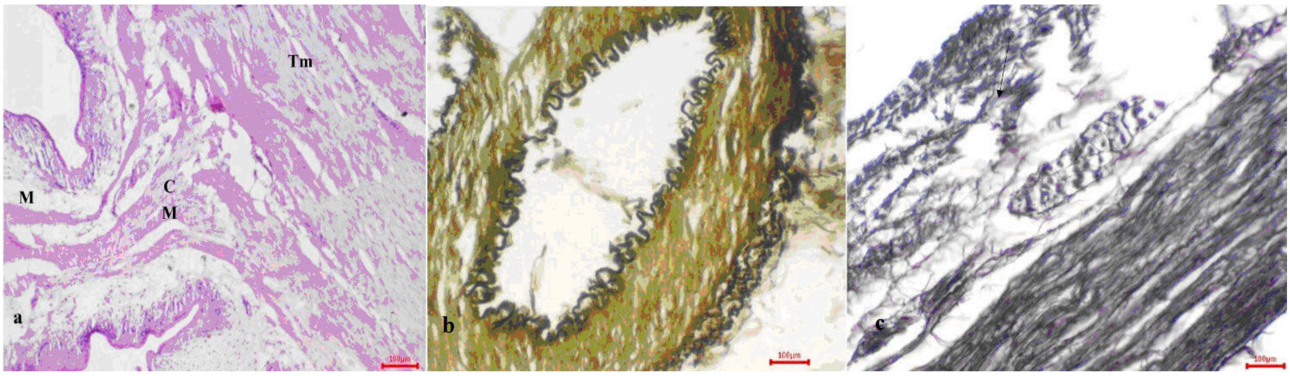

Fig. 5: (**a**) Photomicrograph of omasum of non-descript goat showing central muscular lamina (C) arising from tunica muscularis (Tm), and lamina muscularis mucosae (M) on its either side (staining method: H&E, scale bar, 100 µm, Magnification: ×100), (**b**) Omasum of adult non-descript sheep showing wavy internal elastic lamina in blood vessel (staining method: Weigert, scale bar, 100 µm, Magnification: ×400), and (**c**) Omasum of adult non-descript sheep showing nerve plexus (arrow) (staining method: Bielschowsky, scale bar, 100 µm, Magnification: ×400)

Omasal laminae

Omasum presented primary, secondary tertiary and quaternary laminae. The core of omasal laminae presented central muscular lamina which was the extension of inner circular layer of tunica muscularis flanked on both sides by lamina mucularis mucosae. The primary laminae showed the presence of inner circular muscle layer of tunica muscularis which were merged at the tip with the muscularis mucosae. However, throughout length of primary laminae there was no distinct division between inner circular muscle layer of tunica muscularis and muscularis mucosae except at the base where primary laminae showed the separation of tunica submucosa and muscularis mucosae (Fig. 4a).

Tunica mucosa

Lamina epithelialis mucosae was lined by keratinized stratified squamous (Fig. 4b). Stratum corneum had one or two cell layers which were flat. Stratum granulosum had 1-2 cell layers. The cells were elongated. Stratum spinosum was 4-5 cells thick but had less vacoulation as reported in reticulum. The cells were polyhedral in shape. However, vacuolation was more prominent in adult non-descript goats than in Bakerwali goat and non-descript sheep. Stratum basale had single columnar cell layer.

Lamina propria was made up of connective tissue fibers mainly consisting of collagen fibers. It was limited by prominent lamina muscularis mucosae externally. The lamina muscularis mucosa was present throughout the mucosal layer of each omasal fold. In omasal laminae, tunica muscularis layer was found in between lamina muscularis mucosae (Fig. 4c).

Lamina muscularis was seen running from base of laminae, extending into the core of omasal laminae and bounded by extension of inner circular muscle layer of tunica muscularis (Fig. 5a).

Tunica submucosa

Tunica submucosa was thin layer of loose connective tissue consisting of collagen, elastic and reticular fibers along with blood vessels. Elastic fibers were prominent in blood vessels mainly in tunica intima layer (Fig. 5b).

Table 2: Comparative micrometrical parameters (μ) of omasum in adult Bakerwali goat, non-descript goat and non-descript sheep

Parameters	Bakerwali goat	Non-descript goat	Non-descript sheep
Thickness of lamina epithelialis mucosae	70.04 ± 5.80^{a}	96.19 ± 9.53^{a}	84.85 ± 9.39^{a}
Thickness of lamina propria	37.25 ± 2.26^{a}	44.45 ± 7.87^{a}	98.90 ± 12.18^{b}
Thickness of lamina muscularis mucosae	25.84 ± 1.78^{a}	18.52 ± 1.05^{b}	23.29 ± 2.67^{ab}
Thickness of tunica submucosa	64.70 ± 1.83^{a}	80.58 ± 9.23^{a}	90.74 ± 14.34^{a}
Tunica muscularis thickness	510.02 ± 47.51^{a}	618.52 ± 163.63^{a}	1352.05 ± 123.67^{b}
Tunica serosa	159.20 ± 16.24^{a}	59.41 ± 7.58^{b}	268.40 ± 28.44^{c}
Omasal laminae per field at ×40	2.50 ± 0.22^{a}	3.83 ± 0.31^{b}	2.50 ± 0.22^{a}
Width of omasal laminae	449.33 ± 81.14^{a}	265.54 ± 11.49^{b}	490.57 ± 19.22^{a}
Keratin thickness	11.91 ± 0.09^{a}	13.37 ± 0.83^{a}	13.77 ± 0.78^{a}
Nuclear size at stratum basale	6.14 ± 0.32^{a}	5.89 ± 0.24^{a}	6.05 ± 0.08^{a}
Nuclear size at stratum spinosum	7.34 ± 0.09^{a}	7.54 ± 0.11^{a}	$7.89^{b} \pm 0.08$
Nuclear size at stratum granulosum	8.06 ± 0.29^{a}	8.79 ± 0.23^{a}	8.52 ± 0.19^{a}

Mean value with different superscript ($^{a, b, c}$) within row differ significantly (P>0.05)

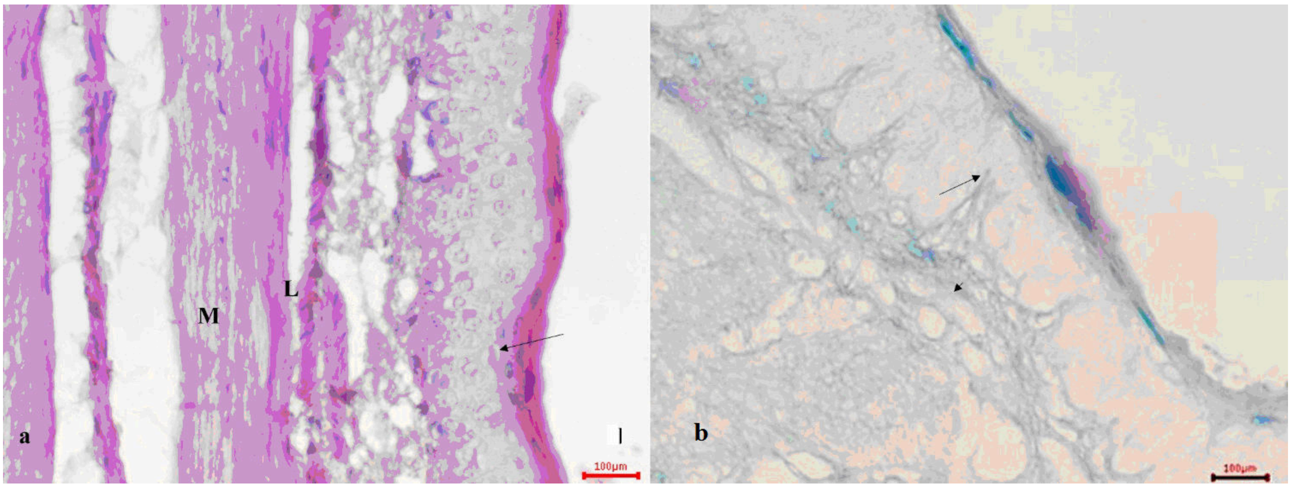

Fig. 6: (**a**) Photomicrograph of omasum of adult Bakerwali goat. Stratum corneum (arrow) showing strong reaction for PAS, lamina muscularis mucosae (L) and tunica muscularis (M) showing moderate reaction (staining method: PAS, scale bar, 100 µm, Magnification: ×400) and (**b**) Omasum of adult non-descript goat. Basement membrane (arrow head) showing moderate reaction for PAS. Stratum granulosum (arrow) showing strong reaction for Alcian Blue (staining method: PAS-AB, scale bar, 100 µm, Magnification: ×400)

Tunica muscularis

The tunica muscularis was composed of thin outer longitudinal layer and thick inner circular layer of smooth muscle (Fig. 4a). The innermost fibers of the circular layer were continued into the large omasal laminae as the intermediate muscle sheet. Most of nerve plexus and ganglion were seen in between outer longitudinal muscle fiber bundles (Fig. 5c).

Tunica serosa

Tunica serosa was made up of loose connective tissue with blood vessels were seen in all the species under study (Fig. 4a).

Micrometry

The lamina epithelialis mucosae was thickest in non-descript goat although the difference was statistically (P>0.05) non-significant (Table 2). The thickness of lamina propria was significantly highest in non-descript sheep (98.90 ± 12.18 μ) and least in Bakerwali goat (37.25 ± 2.26 μ). However, the thickness of lamina muscularis mucosae was significantly higher in Bakerwali goat (25.84 ± 1.78 μ) as compared with non-descript sheep and non-descript goat. The thickness of tunica submucosa did not differ significantly among the three species under study (P>0.05). Thickness of tunica muscularis was significantly maximum in non-descript sheep (1352.05 ± 123.67 μ) as compared with non-descript goat and Bakerwali goat. The tunica serosa was also thickest in non-descript sheep (268.40 ± 28.44 μ) whereas the thickness of keratin layer did not show any significant difference (P>0.05).

At ×40 magnification, the number of omasal laminae per field was highest in non-descript goats. The laminae were widest in non-descript sheep and narrowest in non-descript goats. The difference was statistically significant. The nuclear size showed significant difference only in stratum spinosum layer where it was greatest in non-descript sheep.

Histochemistry

The histochemical distribution of neutral and acid mucopolysaccharides, basic proteins, glycogen and sudanophilic lipids in omasum of Bakerwali goat, non-descript goats and non-descript sheep was recorded.

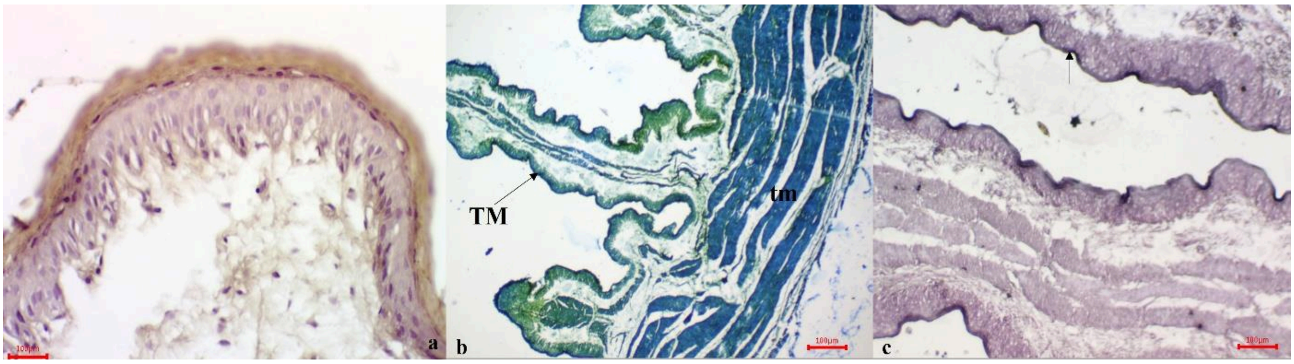

Fig. 7: (**a**) Photomicrograph of omasum of adult Bakerwali goat. Stratum corneum showed negative reaction whereas other epithelial layers showed moderate reaction for glycogen (staining method: Best Carmine, scale bar, 100 µm, Magnification: ×400), (**b**) Omasum of adult non-descript sheep. Only Tunica mucosa (TM) and tunica muscularis (tm) showed strong reaction (staining method: Bromophenol Blue, scale bar, 100 µm, Magnification: ×40), and (**c**) Omasum of adult non-descript goat. Stratum corneum showed moderate reaction (staining method: Sudan Black B, scale bar, 100 µm, Magnification: ×100)

In all the three species, strong Periodic Acid Schiff's (PAS) positive reaction was seen in the stratum corneum and moderate PAS reaction in basement membrane. Lamina muscularis and tunica muscularis also showed moderate PAS reactivity (Fig. 6a). Strong reaction for Alcian Blue (pH 2.5) was observed in the cells of stratum granulosum. The sub-epithelial connective tissue exhibited moderate reaction for Acid mucopoly-saccharides (AMPS) (pH 2.5) in all the three species under study (Fig. 6b).

Glycogen was moderately present in the epithelial layers of the lamina, except in the stratum corneum, regardless of species. However, lamina muscularis and tunica muscularis showed weak reaction for glycogen (Fig. 7a).

All the layers of lamina epithelialis, lamina muscularis and tunica muscularis showed strong reaction for basic proteins (Fig. 7b). In all the three species, only stratum corneum showed moderate reaction for lipids (Fig. 7c).

Discussion

The present study aimed to investigate the gross, biometrical, histological, and histochemical characteristics of the omasum in adult, apparently healthy Bakerwali goats, non-descript goats, and non-descript sheep that are native to the Jammu region. Significant anatomical differences were observed which can be associated with the species-specific feeding strategies and regional forage availability. Based on their dietry preferences, ruminants have been classified into three categories, namely concentrate selectors, grass and roughage eaters, and intermediate or mixed feeders (Hofmann and Stewart, 1972). These classifications have been revised as browsers, grazers, and intermediate feeders, respectively, to align with their natural foraging behavior (Clauss *et al*., 2003).

Gross morphology

In all the three species under study, the omasum was oval to bean-shaped, corroborating previous reports in Baladi goats (El-Gendy and Derbalah, 2010), Surti goat (Mahendrakumar, 2017), red Sokoto goat (Bello *et al*., 2020) and Gaddi sheep (Malik *et al*., 2018), but differing from earlier descriptions in species like Sambar deer (Stafford, 1995) and marsh deer (Machado and Oliveira, 2008), where the omasum was kidney-shaped and pyriform, respectively. Coloration of the omasal mucosa varied interspecifically, with non-descript goats and sheep exhibiting light brown mucosa, whereas Bakerwali goats displayed a darker hue, suggesting possible breed- or diet-induced pigmentation differences. Nurliani *et al*. (2015) reported that black colored mucosa of omasum of swamp buffalo. In Surti goat, the color of omasal mucosa varied from brown to greenish black.

All species exhibited omasal laminae of four orders, with the first-order laminae being the tallest and fourth-order the smallest similar to the finding of El-Gendy and Derbalah (2010) in goat. Due to page-like appearance of laminae, Pasiquini *et al*. (1989) used the termed "Butcher's bible" for omasum. Notably, the fourth-order laminae were more prominent in non-descript sheep than in goats, where they were either absent or rudimentary. These findings were in line with earlier observations indicating that goats often lack fully developed fourth-order laminae (Chungath *et al*., 1985; Lakshmishree, 2014), while sheep consistently exhibit four well-defined orders (Poonia *et al*., 2012; Malik *et al*., 2018). Such differences likely reflect dietary adaptations, as the greater complexity of laminae in grazers like sheep may enhance the efficiency of particle retention and fluid absorption from fibrous diets. Difference in number of omasal lamina orders between different breed might be due to influence of different foliage availability in different regions (Hofmann, 1973). Dyce *et al*. (2010) reported that the omasal laminae alternate in size to split the lumen into succession of thin uniform recesses. Perez and Jerbi (2012) observed omasal laminae of 4 orders in *Oryx dammah* which was a grazing ruminant of bovidae family. However, in Brown Brocket deer, which was browser ruminant, omasal laminae were of 3 orders (Perez and Vazquez, 2012). These laminae protruded from the omasal walls and greater curvature as reported by Eurell and Frappier (2006) in domestic animals, Samuelson (2007) in ruminants and Lakshmishree (2014) in goats. Their attached border was convex and free border was concave similar to the findings of Chandrasekar *et al*. (1993).

Pointed or conical papillae were scattered throughout the omasal laminae. Yamamoto *et al*. (1994) reported omasal papillae of variable shape in ruminants ranging from conical, claw-like to rounded. The cornified omasal laminae might serve as a sieve to allow small particles to pass (Bost, 1970). In all the three species, the papillae were more prominent towards their free concave border. Towards reticulo-omasal orifice, large claw shaped pointed papillae were seen in all the three species which decreased in size towards omaso-abomasal orifice similar to the observation by Jelinek (1995) in goats of 212-232 days of age and Lakshmishree (2014) in black buck, sheep and goat. In non-descript sheep, the lateral surface of the 1st order laminae presented ridges running parallel to the free concave border. These ridges were also studded with pointed papillae. Such ridges were not observed over the lateral surface of omasal laminae of Bakerwali goat and non-descript goat. Similar types of ridges were also reported by Yamamoto *et al*. (1994) in sheep and cattle.

The structural complexity of the omasum directly correlates with the physiological requirements of different feeding types. Grazers like sheep consume high-fiber, low-digestibility forages that necessitate prolonged fermentation and effective absorption. Anatomical features such as more numerous and higher-order laminae, and increased mucosal surface area contribute to improved efficiency of volatile fatty acid (VFA) and water absorption (Langer, 1988; Clauss *et al*., 2006). In contrast, browsers or intermediate feeders like goats rely on more digestible, lower-fiber diets, thereby requiring less structural complexity in the omasum.

These findings were supported by comparative data from Clauss *et al*. (2006), where grazing species like the muskox exhibited larger omasal surface area and more numerous laminae compared with browsing species like the moose. Similar patterns were observed in the present study, where non-descript sheep had a significantly larger and more developed omasum than either of the goat breeds.

Biometry

The present study revealed significant interspecies variations in the biometric parameters of the omasum among adult Bakerwali goats, non-descript goats, and non-descript sheep of the Jammu region. These differences were functionally significant and can be attributed to their respective feeding strategies-grazing versus browsing.

The mean empty weight and volume of the omasum were found to be significantly highest in non-descript sheep (grazers), followed by Bakerwali goats and least in non-descript goats. These findings align with the earlier observations by Clauss *et al*. (2006), who reported that the omasum was generally more developed in grazing ruminants compared with browsers. In Surti goat, the empty weight of omasum was 105.25±4.97 gm (Mahendrakumar, 2017) whereas in Gaddi sheep, the weight was 126.67±2.82 gm (Malik *et al*., 2018). The mean empty weight and volume of omasum was significantly higher in non-descript sheep which was similar to the findings of Chandrasekar *et al*. (1993) who also reported greater volume of omasum in sheep (129.3±8.4 ml) than in goats (109.8±2.9 ml).

The mean length of the omasum at the greater curvature was highest in Bakerwali goats, yet non-descript sheep exhibited greater dimensions than non-descript goats. This corresponds to earlier findings by Chandrasekar *et al*. (1993), who reported longer greater curvatures in sheep than in goats, reflecting the adaptation of the omasum to retain ingesta for a prolonged period in grazers to facilitate fermentation and absorption. In Surti goat, the greater and lesser curvatures were much longer (29.08±1.27 cm and 25.95±1.86 cm, respectively) (Mahendrakumar, 2017). In Gaddi sheep (Malik *et al*., 2018), the length of greater and lesser curvature (15.17±0.32 cm and 6.45±0.13 cm, respectively) was less than our findings in non-descript sheep of Jammu region (17.27±0.72cm and 8.50 ± 1.08 cm, respectively).

The mean height and width of the omasum were also significantly higher in Bakerwali goats, although non-descript sheep exhibited intermediate values. Despite Bakerwali goats showing larger overall dimensions, their internal structure (especially omasal lamination) was less complex, indicating that absolute size alone may not directly reflect absorptive efficiency. Instead, the internal surface area provided by omasal laminae is more critical functionally. Lakshmishree (2014) calculated height and width of omasum in goats as 6.75±0.79 cm and 6.04±0.46 cm, respectively. The same in sheep was 7.58 ± 0.92 cm and 7.04±0.48 cm, respectively.

The number of omasal laminae were significantly higher in non-descript sheep followed by non-descript goat and least in Bakerwali goat. More number of omasal laminae may be an adaptation to increase surface area of the organ to the feeding behavior of sheep which is a grazer and eat lower-quality grasses. Grasses often have thicker cell walls made primarily of slowly digesting plant fibres like cellulose (Demment and Van Soest, 1985). Thus, grass is more difficult and energy-intensive to bite and chew (Wright and Illius, 1995). Hofmann (1989) also reported that book-like structure of omasum was more differentiated in grass and roughage eaters such as cattle and sheep as compared with concentrate selectors. In Merino sheep and crossbreed goats, the number of omasal laminae ranged from 33-35 (McSweeney, 1988). The number of omasal laminae in Baladi goat was 64 (El-Gendy and Derbalah, 2010) which was similar to our findings in non-descript goats.

The 1st order, 3rd order and 4th order laminae were significantly ($P<0.05$) highest in non-descript sheep, however, the 2nd order laminae did not show any significant difference ($P>0.05$). Clauss *et al*. (2006)

reported that muskoxen (grazer) had more 3rd and 4th order than in moose which is strictly browser. This increase in number of omasal laminae in grazer might be an adaptation to increase surface area in order to re-absorb water and volatile fatty acids (Clauss *et al*., 2006). In small ruminants, the number of omasal laminae of the 1st, 2nd, 3rd and 4th order was 8, 8, 17 and 34 (Chandrasekar *et al*., 1993). In goat, the number of the 1st, 2nd and 3rd order laminae were 8.0±0.81, 8.0±1.41 and 14.7±2.22, respectively (Jelinek, 1995). Pachpande (2008) calculated number of primary, secondary and tertiary laminae in goats of 90-120 days of age which was 18.10 ± 1.09, 10.90 ± 0.34 and 11.40±0.30, respectively. In Baladi goat, the 1st, 2nd, 3rd and 4th order laminae were 8, 8, 16 and 32, respectively (El-Gendy and Derbalah, 2010). In Surti goat, the number of the 1st, 2nd, 3rd and 4th order laminae was 7.50±0.37, 7.17±0.27, 18.17±0.57 and 42.67 ± 0.70, respectively (Mahendrakumar, 2017).

The individual laminae height measurements were also indicative of feeding behavior. In non-descript sheep, the heights of the 1st, 3rd, and 4th order laminae were significantly greater than those in goats, enhancing the mucosal surface area for reabsorption. These results were consistent with previous studies in grazing ruminants such as cattle and sheep, which demonstrate well-differentiated and tall omasal laminae (Chandrasekar *et al*., 1993; El-Gendy and Derbalah, 2010).

The length of the omasal groove (sulcus omasi) was highest in non-descript sheep, followed by Bakerwali goats and least in non-descript goats. This structural feature aids in directing ingesta flow and may reflect prolonged ingesta retention time in grazers, enabling more efficient particle sorting and nutrient absorption. However, Chandrasekar *et al*. (1993) reported longer omasal groove in goats (4.0-4.3 cm) than in sheep (3.8-3.9 cm). In Gaddi sheep, length was 1.0-4.3 cm (Malik *et al*., 2018). In Baladi goat, length of sulcus omasi was 2.5-3.4 cm (El-Gendy and Derbalah, 2010). In Surti goat, the same was 4.85 ± 0.22 cm (Mahendrakumar, 2017).

The differences in omasal biometry among these species are indicative of evolutionary adaptations to diet composition and feeding strategy. Browsers such as Bakerwali and non-descript goats, which consume high-quality, easily digestible forage, exhibit less complex omasal morphology. In contrast, grazers like non-descript sheep, which consume fibrous, nutrient-dilute forage, show significant omasal development in terms of both size and lamination. This structural complexity enhances their ability to process low-quality forage efficiently through extended retention time and increased absorptive capacity.

Histomorphology

The omasum in all three species under study, i.e., Bakerwali goat, non-descript goat, and non-descript sheep was histologically composed of the typical four-layered wall structure: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa, consistent with classical ruminant morphology as reported by Eurell and Frappier (2006) and Malik *et al*. (2018).

The tunica mucosa in all species formed prominent omasal laminae, which projected into the lumen and played a critical role in the mechanical processing and absorption of ingesta. These laminae were supported by a central muscular core derived from the inner circular layer of the tunica muscularis, flanked on either side by well-defined lamina muscularis mucosae, corroborating the findings of Poonia *et al*. (2012) in sheep and Malik *et al*. (2018) in Gaddi sheep. These laminae significantly increased the mucosal surface area, facilitating the reabsorption of water, volatile fatty acids (VFAs), and electrolytes, especially in grazers like sheep. The more developed omasal laminae in grazers reflect an adaptation to fibrous diets where effective water conservation and nutrient extraction are essential (Clauss *et al*., 2006). In browsers like goats, although the same layered structure was present, omasal laminae were generally less complex, as their diet is richer in nutrients, requiring less surface area for reabsorption.

The lamina epithelialis, lining the omasal laminae, consisted of keratinized stratified squamous epithelium in all species, similar to reports by Mahendrakumar (2017), Malik *et al*. (2018). While keratinization provides mechanical protection against abrasive ingesta, the epithelium retains the ability to absorb water and electrolytes, indicating a dual role in protection and absorption (Engelhardt and Hauffe, 1975).

Lamina propria was made up of connective tissue fibers similar to the findings by Lakshmishree (2014) in goats. It was limited by prominent lamina muscularis mucosae externally. Lamina muscularis mucosa was present throughout the mucosal layer of each omasal fold. In omasal laminae, tunica muscularis layer was found in between lamina muscularis mucosae. It was thin similar to the findings of Mahesh (2008) in goats.

The tunica submucosa was a thin layer of loose connective tissue rich in collagen, elastic, and reticular fibers, along with blood vessels and nerves. Prominent elastic fibers in the vascular tunica intima were observed, indicating the dynamic nature of the vasculature, crucial for regulating blood flow during absorption. It was in accordance with the findings of Mahesh (2008) in goats and Poonia *et al*. (2012) in sheep. Lakshmishree (2014) in goats observed distinct tunica submucosa.

The tunica muscularis was composed of an inner circular and outer longitudinal muscle layer, with some interspecies variation in relative thickness. While Mahesh (2008) and Nwaogu and Ezeasor (2008) observed a thicker outer longitudinal layer in goats, other studies by Poonia *et al*. (2012) in sheep and Mahendrakumar (2017) in Surti goat reported a thicker inner circular layer, especially in sheep.

The outermost layer, the tunica serosa, was made up of loose connective tissue with embedded blood vessels, and lined externally by a mesothelial cell layer, consistent with findings by Mahesh (2008) and Mahendrakumar (2017). The serosa serves to protect and lubricate the omasum within the abdominal cavity, minimizing friction against surrounding organs.

Micrometry

The micrometric evaluation of the omasal wall revealed notable interspecies variations in layer thicknesses, reflecting functional adaptations related to the feeding behavior and dietary requirements of grazers and browsers.

Lamina epithelialis was thickest in non-descript goat but the difference was non-significant (P>0.05). In Gaddi sheep, lamina epithelialis was 48.8±2.3µm thick at greater curvature and 88.2±3.8 µm at lesser curvature (Malik *et al*., 2018). The lamina propria was significantly thickest in non-descript sheep (98.90 ± 12.18 µm), indicating an enhanced vascular and supportive connective tissue framework, vital for efficient absorption of water and volatile fatty acids (VFAs) from fibrous plant material. The values in our study were higher than those reported by Malik *et al*. (2018) in Gaddi sheep (37.5±1.9 µm at greater curvature and 40.8±2.1 µm at lesser curvature), suggesting regional or breed-specific adaptation. However, lamina muscularis mucosae was significantly thicker in Bakerwali goat as compared with non-descript sheep and non-descript goat. In goats of 90-120 days of age, the thickness of lamina muscularis mucosae was 25.52±0.69 µm in middle part and 20.09±0.40 µm in terminal part (Pachpande, 2008). Malik *et al*. (2018) in Gaddi sheep reported thicker lamina muscularis mucosae both at greater curvature (43.3±2.0 µm) and lesser curvature (33.0±1.7 µm). Tunica submucosa was thickest in non-descript sheep but the difference was non-significant statistically which were lesser than the values in goats of 90-120 days of age (158.18±4.71 µm in middle part and 105.83±2.76 µm in terminal part) (Pachpande, 2008). Thickness of tunica muscularis was significantly greater in non-descript sheep as compared with non-descript goat and Bakerwali goat. Similarly, tunica serosa was significantly thicker in non-descript sheep followed by Bakerwali goat and non-descript goat. Pachpande (2008) in goats (90-120 days of age) recorded higher values of thickness of tunica muscularis (896.87±11.48µm in middle part and 742.75±8.78 µm in terminal part) whereas tunica serosa was 108.65±2.03 µm in middle part and 88.64±6.23 µm in terminal par. The number of omasal laminae per field at ×40 was significantly highest in non-descript goat as the laminae were thinnest.

Histochemistry

The histochemical characteristics of the omasal tissues in the present study revealed distinct localization and intensity of reactions for glycogen, mucopoly-saccharides, and lipids, reflecting the specialized functional roles of different tissue layers in relation to the feeding behavior and digestive strategies of grazers and browsers.

Strong Periodic Acid Schiff's (PAS) reaction was observed in the stratum corneum of all the three species similar to the reports of Lakshmishree (2014) in sheep, goat and buffalo. In contrast, Malik *et al*. (2018) reported moderate PAS reaction in superficial layer of epithelium in Gaddi sheep.

Cells of stratum granulosum showed strong reaction for Alcian Blue (pH=2.5) and moderate reaction in subepithelial connective tissue in all the three species under study. The findings align with Yamamoto *et al*. (1993), who reported strong Alcian Blue activity in the core of omasal papillae in sheep, and Malik *et al*. (2018), who observed strong acid mucopolysaccharide presence in the lamina propria and submucosa in Gaddi sheep. Presence of neutral and acid mucopolysaccharide in lamina epithelialis perform cementing function (Brody, 1964).

In all the three species, glycogen was moderately observed in all the layers of lamina epithelialis except stratum corneum. Lamina muscularis and tunica muscularis showed weak reaction for glycogen similar to the findings of Lakshmishree (2014) in sheep, goat and buffalo. However, Pachpande (2008) in goats of 90-120 days of age observed absence of glycogen.

Only stratum corneum showed moderate reaction for lipids irrespective of species. However, Lakshmishree (2014) in sheep, goat and buffalo reported that stratum granulosum and tunica muscularis showed moderate reaction for fat. Lamina epithelialis showed mild reaction for Sudan Black B (Malik *et al*., 2018) in Gaddi sheep.

While this study provides a detailed anatomical and histological comparison of omasal structures in small ruminants, there were limitations that might influence the interpretation of the results. The animals used in this study came from specific geographic regions, and their feeding history was based on local forage availability, which may not represent all populations of the same species or breed. Lack of precise dietary records also limits the ability to directly correlate specific anatomical features with dietary fiber content or moisture levels.

In summary, the present study provided a thorough comparison of the omasum in browsers and grazers, highlighting distinct anatomical differences consistent with dietary adaptation. This study has also provided photographic evidence and acts as a useful guide for those wishing to understand the functional anatomy of the omasum in browsers and grazers.

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Conflict of interest

The authors declare no competing interests.

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Use of generative AI and AI-assisted

technologies statement

The authors used generative AI-assisted tools (online tools) to improve the clarity, grammar, and readability of the manuscript. These tools were not used to generate data.

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