RESEARCH ARTICLE

Comparative Age-Related Studies on Lingual Histochemistry of Crossbred Sheep and Non-descript Goats of Jammu Region with Reference to Grazing and Browsing Adaptations

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Abstract

The present study evaluated the histochemical distribution of major biochemical components in the tongue of crossbred sheep and non-descript goats across different age groups. Various lingual regions, including the tip, body, torus linguae, and root, were examined for neutral mucopolysaccharides, acidic mucopolysaccharides, glycogen, proteins, and lipids. Neutral mucopolysaccharides showed strong to intense PAS-positive reactions in the keratin layer of both species across all regions. The lamina epithelialis exhibited moderate PAS positivity, while the basement membrane showed strong reactions, comparatively more intense in goats. Most lingual tissues were negative for acidic mucopolysaccharides; however, lingual glands displayed strong Alcian Blue positivity, highlighting their role in mucin secretion and lubrication. Glycogen was absent in epithelial and connective tissue layers but was weak to moderately present in intrinsic muscle bundles and strongly expressed in lingual glands. Protein reactions were strong in the keratin layer and intrinsic muscles, while the lamina epithelialis showed age-related variation. Lipids were weak to moderate in the epithelium and basement membrane, moderate to strong in muscle bundles, and strong in lingual glands and blood vessels. Overall, both species exhibited similar histochemical distribution patterns, indicating comparable structural organization and functional adaptations of the tongue.

Key words: Age-related variation, Goat, Jammu, Lingual histochemistry, Sheep.

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Introduction

Sheep and goat being well conformed and stress resilient animals thrive better in the hilly and other inaccessible areas where it is difficult for other livestock to cope the adversities of terrain and harsh environment, and warrants food and economic security of the poor communities (Khan *et al.,*2013). The state of Jammu and Kashmir being a hilly and temperate state is well-suited for rearing of sheep and goat owing to its favourable agro-climatic and topographical conditions and richness in natural fodder resources in the form of pastures, orchards, aquatic vegetation etc (Tomar and Sharma, 2002). Sheep and goat rearing as secondary occupation has played a salient role in boosting the economy of these rural people.

The tongue of mammals is a movable musculo-membranes organ contributing significantly to food appreciation; it varies in form and size that is greatly influenced by feeding habits. The tongue of sheep and goat has free apex, body and attached root (Kadhim, 2016). The morphological appearance, prevalence, orientation, and structure of lingual papillae are modified in accordance with the nutritional requirements, the types of nutritional particles accessible, and the various environmental conditions (Farrag *et al.*, 2021). In herbivores, the tongue functions as the most important prehensile organ for grasping food items (Meier *et al.,* 2016). It is capable of both strong and precise movements, as in prehension, lapping, grooming, and manipulation of food within the mouth on the one hand and speech articulation on the other, while in other animals, the tongue assists in the heat loss by panting, such as the dog (Dyce *et al*., 2010). Considering all the above facts, the present study has been planned to elucidate the probable histochemical differences of the tongue (major prehensile organ of the small ruminants) of crossbred sheep and non-descript goats at various ages in Jammu region of the UT of Jammu & Kashmir.

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Materials and Methods

Thirty-six (36) heads of freshly slaughtered apparently healthy crossbred sheep & non-descript goats were collected from the slaughter houses located in and around Jammu city and immediately brought to the laboratory of Division of Veterinary Anatomy, Faculty of Veterinary Science & Animal Husbandry, SKUAST-J, R.S. Pura, Jammu (India). The tongues of crossbred sheep and non-descript goats were divided into three age groups (young: below 1 year; Adult: 2-3 years and Senile: 4 years and above) as per the dentition of the goats and availability of the samples. Six (6) samples from each age group of sheep and goats were collected for this study. After recording the gross parameters, the tongues were preserved in 10% neutral buffered formalin solution (Luna, 1968). The tissue pieces from the tip, body, torus linguae and root were processed for paraffin block preparation by alcohol-benzene schedule (Luna, 1968). Tissue sections of 5-6 mm were obtained from these blocks on clean glass slides with the help of rotary microtome. The sections were then subjected to various histochemical methods as detailed in Table 1. The selected histochemical fields were photographed by using a microscope and image analyzer (Magnus MX2iLED).

Table 1: Different histochemical stains used in this study

Purpose	Stain	Source
Glycogen	McManus Method	Luna (1968)
Proteins	Bromophenol Blue	Pearse (1972)
Neutral mucopolysaccharides	PAS method	Luna (1968)
Acidic mucopolysaccharides	Alcian Blue Method (at pH 1.0)	Singh and Sulochana (1997)
Lipids	Sudan Black B	Luna (1968)

Results and Discussion

Neutral Muco-Polysaccharides

In both crossbred sheep and non-descript goats, the keratin layer at the tip, body, torus linguae and root of the tongue exhibited a strong to intense PAS-positive reaction across all the age groups (Fig. 1). The similarity between sheep and goats suggests a comparable degree of keratinization across both species. These neutral mucins (likely glycoproteins and certain glycolipids) are integral to the cornified epithelium, contributing to the physical barrier and potentially maintaining a hydration layer against mechanical wear from roughage (El-Bakary and Abumandour, 2017). Similar pattern of reaction towards neutral muco-polysaccharides was also reported in Muntjac deer (Zheng and Kobayashi, 2006). However, in senile sheep the keratin layer consistently showed strong (Fig. 2) rather than strong to intense reactivity, whereas goats retained strong to intense reactivity at all ages. The lamina epithelialis demonstrated a moderate PAS reaction in all regions and age groups of both species, indicating a similar distribution of neutral mucopolysaccharides within this layer (Fig. 1, 2).

The basement membrane in both species (sheep and goats) showed a strong PAS-positive reaction, but goats exhibited a comparatively more intense reaction across all levels of the tongue and age groups (Fig. 1), indicating a thicker or more concentrated polysaccharide component in the goat's basal lamina, perhaps a structural adaptation related to epithelial-connective tissue anchorage strength. In contrast, the lamina propria and submucosa were PAS-negative in all regions of the tongue in both species, irrespective of age, indicating a lack of neutral mucopolysaccharides in these connective tissue layers (Fig. 3).

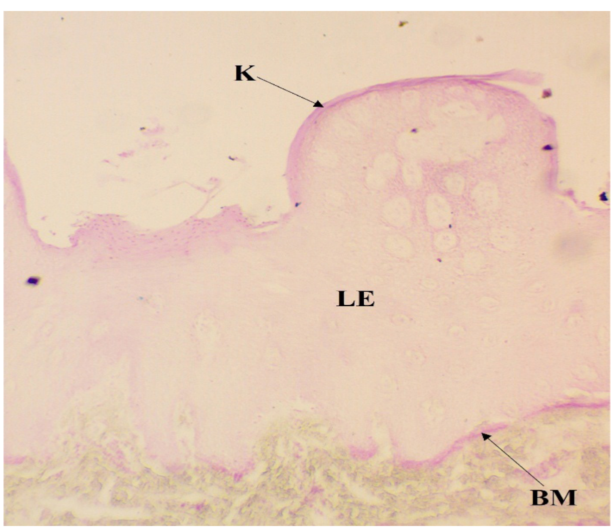

Fig. 1: Tongue of adult non-descript goat at the level of tip. Keratin layer (K) and basement membrane (BM) showing strong to intense reaction for neutral mucopolysaccharides. Lamina epithelialis (LE) showing moderate reaction. x100 PAS stain

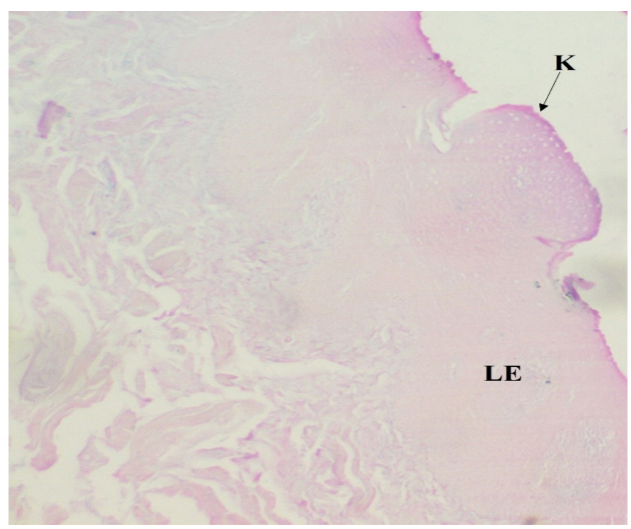

Fig. 2: Tongue of senile crossbred sheep at the level of tip. Keratin layer (K) showing strong reaction for neutral mucopolysaccharides. Lamina epithelialis (LE) showing moderate reaction. x100 PAS stain

The intrinsic muscle bundles displayed a moderate PAS reaction in young, adult, and senile animals of both species, with no notable interspecies differences (Fig. 4). The lingual glands and blood vessels of both crossbred sheep and non-descript goats showed moderate to strong PAS-positive reactions at all tongue levels. These findings corroborated with the reports of El-Bakary and Abumandour (2017) in buffalo, and Jabur and Atyia (2023) in local Iraqi goat. The similarity between sheep and goats suggests a comparable degree of keratinization and secretory function across both species.

Acidic Muco-Polysaccharides

In both crossbred sheep and non-descript goats, most lingual tissues showed a similar pattern of histochemical reaction to acidic mucopolysaccharides (Alcian Blue, pH 1.0). In all age groups (young, adult, and senile) of both species, the keratin layer at the tip, body, torus linguae, and root of the tongue consistently showed a negative reaction. Likewise, the lamina epithelialis exhibited negative to weak reactions across all regions and ages in both animals, while the basement membrane, lamina propria, submucosa, and intrinsic muscle bundles uniformly showed negative reactions in sheep and goats (Fig. 5). Such negative reactions for acidic mucopolysaccharide exhibited by these tissue components of the tongue was also reported in Iraqi sheep (Hussein and AL-Asadi, 2010), cattle (Sari *et al*., 2010), goat (Ammar, 2014), Iraqi goat (Jabbar, 2014), and Awassi rams and Billy-goats (Kadhim, 2016). The main point of contrast concerned the blood vessels: sheep showed a weak reaction for acidic mucopolysaccharides, whereas goats showed a negative reaction. In both species, however, the lingual glands demonstrated strong to intense positive reactions at all tongue levels regardless of age, making them the only structures with consistently marked Alcian Blue affinity in both sheep and goats (Fig. 6, 7, 8). This highly specialized distribution confirms that the primary function of sulphated mucins providing viscous, highly protective lubrication for bolus formation and its transit, is executed exclusively by the glandular secretions and might be helpful in aiding moisture retention and antimicrobial defense in both the species under study (Jabur and Atyia, 2023).

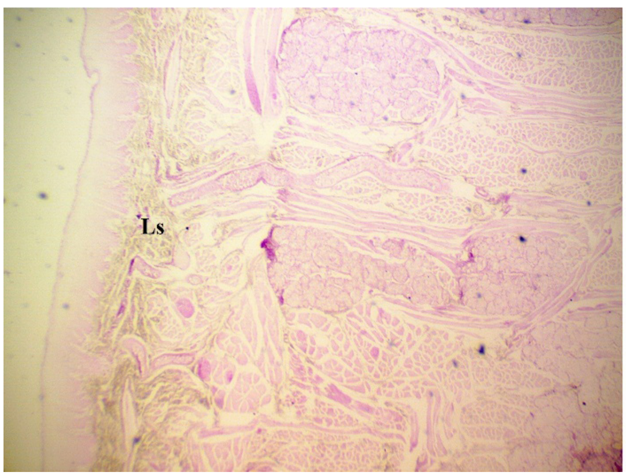

Fig. 3: Tongue of senile crossbred sheep at the level of root. Lamina propria-submucosa (Ls) showing negative reaction for neutral mucopolysaccharides. x40 PAS stain

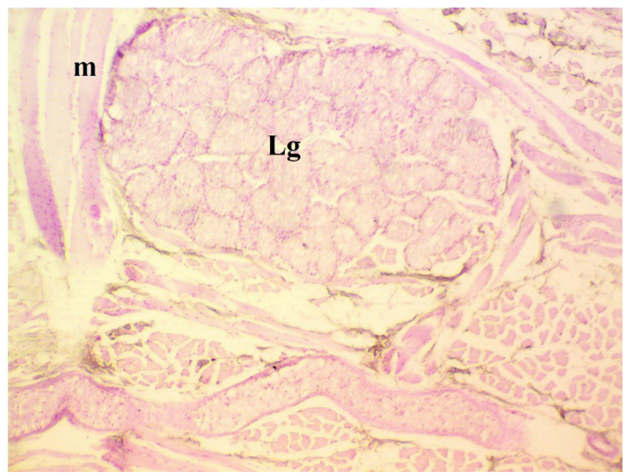

Fig. 4: Tongue of senile crossbred sheep at the level of root. Lingual glands (Lg) and intrinsic muscles (m) showing moderate to strong reaction for neutral mucopolysaccharides. x100 PAS stain

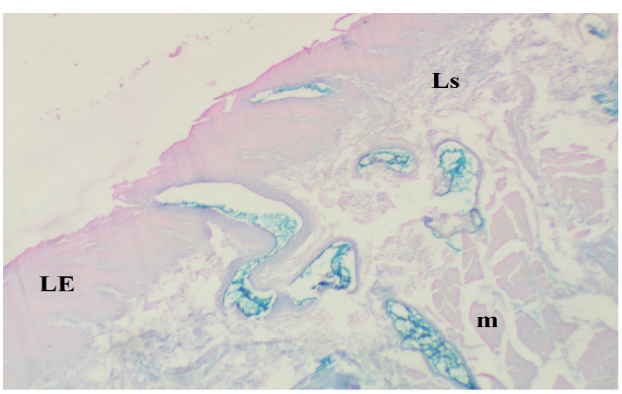

Fig. 5: Tongue of adult non-descript goat at the level of torus linguae. Lamina epithelialis (LE) showing weak reaction for acidic mucopolysaccharides. Lamina propria-submucosa (Ls) and muscle bundles (m) showing negative reaction for Alcian Blue. x100 PAS-AB stain

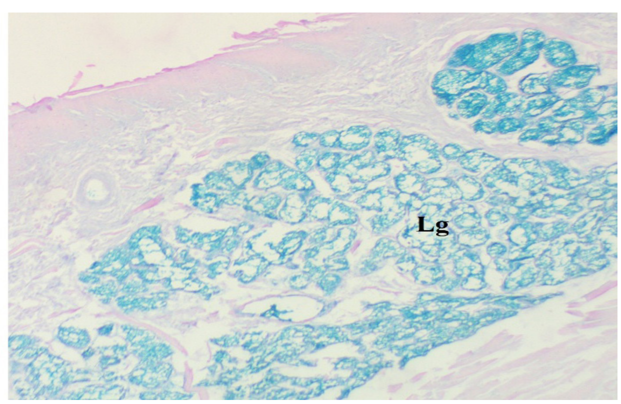

Fig. 6: Tongue of adult crossbred sheep at the level of torus linguae. Lingual glands (Lg) showing strong to intense reaction for acidic mucopolysaccharides. x100 PAS-AB stain

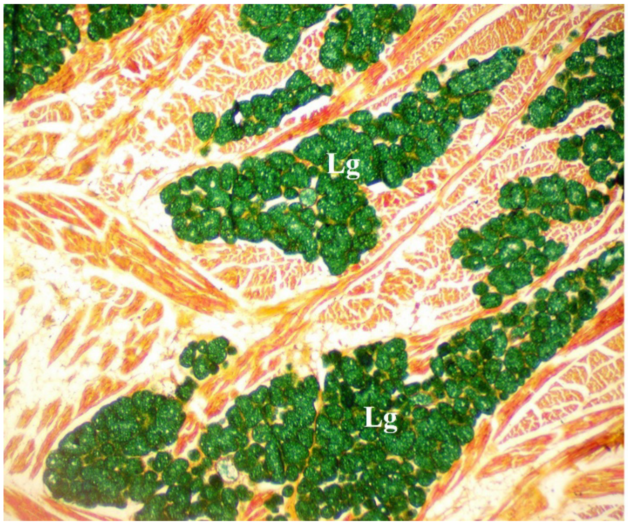

Fig. 7: Tongue of young crossbred sheep at the level of root. Lingual glands (Lg) showing strong to intense reaction for acidic mucopolysaccharides. x40 Dane's method

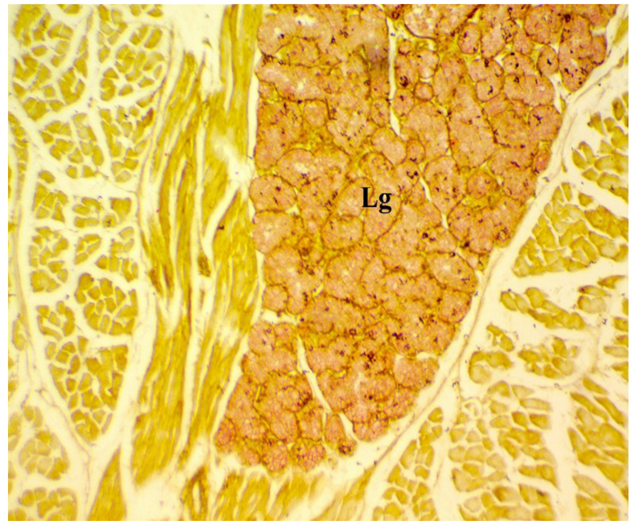

Fig. 8: Tongue of young crossbred sheep at the level of torus linguae. Lingual glands (Lg) showing strong to intense reaction for acidic mucopolysaccharides. x100 Mucicarmine stain

Glycogen

In both crossbred sheep and non-descript goats, the overall pattern of glycogen distribution in the tongue was similar across all age groups. In both species, the keratin layer, lamina epithelialis, basement membrane, lamina propria, and submucosa at the tip, body, torus linguae, and root showed a consistently negative reaction for glycogen using the McManus method (Fig. 9). The negative reaction in the surface epithelium and keratin layer suggests minimal reliance on glucose storage in these terminally differentiating or protective layers (El-Bakary and Abumandour, 2017).

The intrinsic muscle bundles in sheep and goats exhibited weak to moderate glycogen positivity at all tongue levels (Fig. 10), while the lingual glands displayed a strong reaction in every age group (Fig. 11), making them the most glycogen-rich structures in both species. This is predictable, as glands require high energy reserves for continuous secretion, and muscle tissue requires readily available glucose stores for rapid motor function. Similar findings were also reported in Iraqi sheep (Hussein and AL-Asadi, 2010), cattle (Sari *et al.*, 2010), goat (Ammar, 2014; Jabbar, 2014), and Awassi rams and Billy-goats (Kadhim, 2016). Additionally, blood vessels in both animals showed a weak glycogen reaction indicating a negligible glycogen content in endothelial structures. Overall, sheep and goats demonstrated nearly identical histochemical responses showing the same tissue-specific patterns of glycogen localization.

Proteins

In both crossbred sheep and non-descript goats, the keratinized layer of the tongue consistently demonstrated a strong protein reaction across all regions, *i.e.,* tip, body, torus linguae, and root in all age groups (Fig. 12). This uniform intensity reflects the high structural protein content attributed to structural keratins and contractile proteins (actin/myosin), respectively, characteristic of heavily keratinized oral surfaces indicating similar keratinization patterns in both species. The intrinsic muscle bundles also showed strong to intense protein positivity in both sheep and goats, reaffirming the protein-rich composition of lingual musculature regardless of age or species (Fig. 12). Similar observations were also made in Iraqi sheep (Hussein and AL-Asadi, 2010), cattle (Sari *et al.*, 2010), goat (Ammar, 2014), and Iraqi goat (Jabbar, 2014).

In the current study, the lamina epithelialis showed age-dependent variation in its reaction to basic proteins in both species examined. In young animals, the epithelium exhibited only a negative to weak response (Fig. 13), indicating a relatively lower concentration or reduced expression of basic proteins during early developmental stages. As the animals matured, notable species differences became apparent. Adult goats demonstrated a moderate to strong reaction within the lamina epithelialis (Fig. 12), suggesting a more pronounced presence or activity of basic proteins compared to crossbred sheep, which showed only a weak to moderate response (Fig. 14). Moderate to strong reaction to basic proteins was also demonstrated in Muntjac deer (Zheng and Kobayashi, 2006), Iraqi goat (Jabbar, 2014) and Egyptian water buffalo (El-Bakary and Abumandour, 2017). In senile animals of both species, the reaction declined markedly, with the lamina epithelialis appearing largely negative, except for a weak reaction persisting near the basal region. These findings collectively highlight that basic protein expression within the lamina epithelialis is influenced not only by age but also by species-specific physiological characteristics.

In this study, the basement membrane along with lamina propria submucosa as well as the lingual glands showed negative protein reactions in both the species

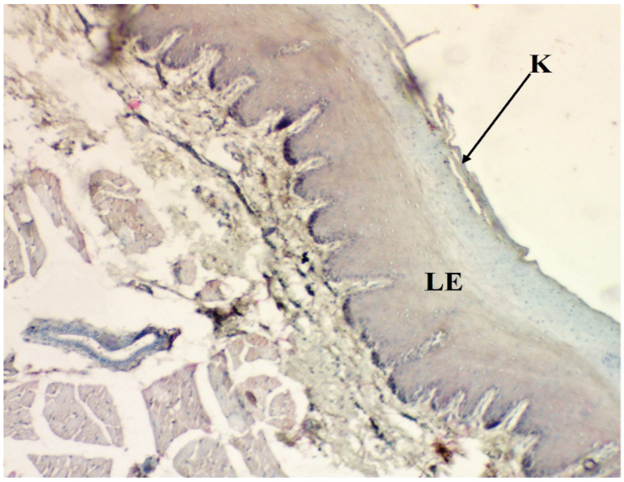

Fig. 9: Tongue of young crossbred sheep at the level of torus linguae. Lamina epithelialis (LE) along with keratin (K) layer showing negative reaction for glycogen. x100 McManus method

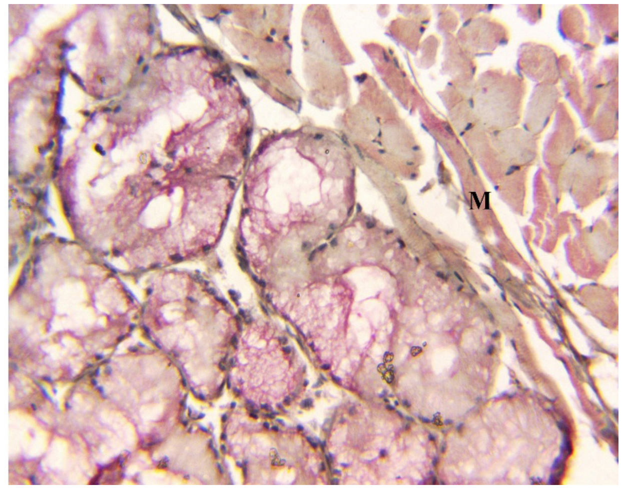

Fig. 10: Tongue of adult non-descript goats at the level of torus linguae. Lingual intrinsic muscles (M) showing moderate reaction for glycogen. x400 McManus method

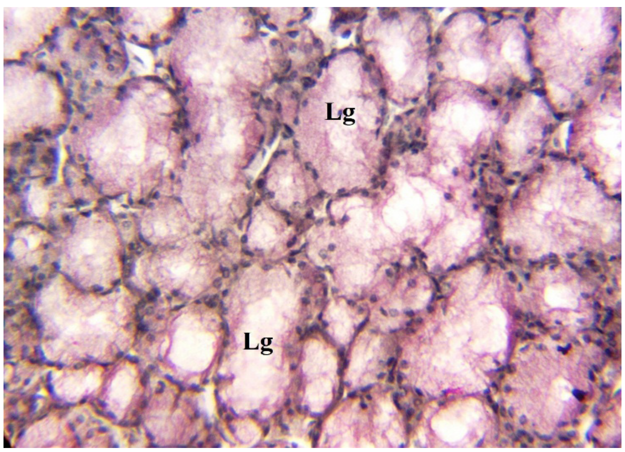

Fig. 11: Tongue of adult non-descript goats at the level of torus linguae. Lingual glands (Lg) showing strong reaction for glycogen. x400 McManus method

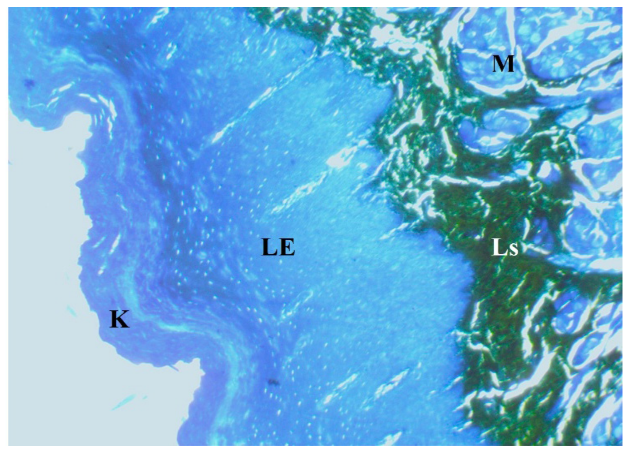

Fig. 12: Tongue of adult non-descript goats at the level of body. Keratin (K) layer showing strong reaction, Lamina epithelialis (LE) showing moderate to strong reaction, lamina propria-submucosa (Ls) showing negative reaction and muscles (M) showing strong to intense reaction for basic proteins. x100 Mercuric Bromophenol Blue method

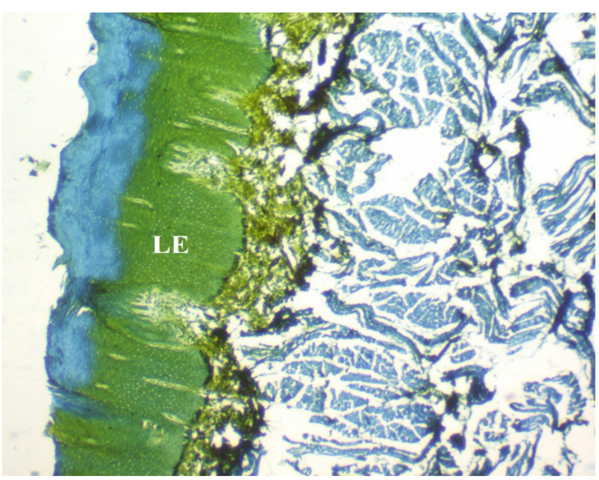

Fig. 13: Tongue of young crossbred sheep at the level of body. Lamina epithelialis (LE) showing negative to weak reaction for basic proteins. x40 Mercuric Bromophenol Blue method

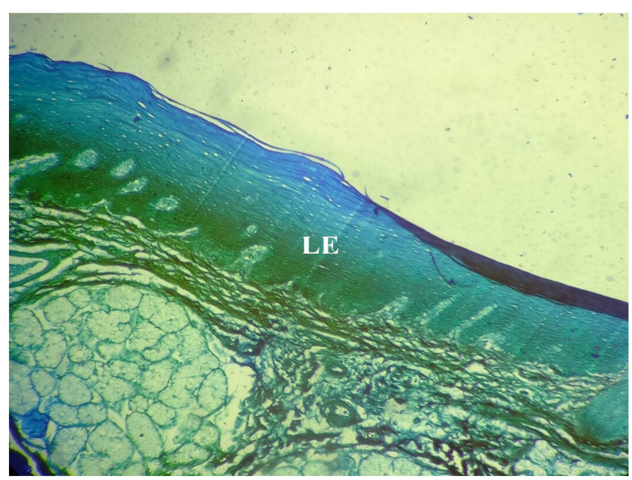

Fig. 14: Tongue of adult crossbred sheep at the level of root. Lamina epithelialis (LE) showing weak to moderate reaction for basic proteins. x100 Mercuric Bromophenol Blue method

under study. Such observations might be due to the fact that the basement membrane is rich in type IV collagen, laminin, proteoglycans, and glycoproteins, not basic proteins. Again, an abundance of ground substance (acid mucopolysaccharides, glycosaminoglycans) and presence of collagen fibers with low basic protein content might be the reason for negative reaction for basic proteins in lamina propria submucosal layer. Moreover, Lingual glands in sheep and goats were predominantly of mucous or mixed type, so their secretion was also rich in mucins (glycoproteins) rather than proteinaceous enzymes, attributing the negative reaction to basic proteins by the lingual glands in both the species. Blood vessels also showed negative reaction for basic proteins in both the species across all the age groups.

Lipids

This study confirmed that in both crossbred sheep and non-descript goats, the overall pattern of lipid distribution within the tongue showed strong similarities across all age groups. In both species, the keratin layer, lamina propria, and submucosa at the tip, body, torus linguae, and root demonstrated a consistently negative reaction for lipids (Fig. 15). However, the lamina epithelialis along with the basement membrane in both the species showed weak to moderate lipid reaction (Fig. 15), indicating minimal lipid presence in this region. The intrinsic muscle bundles displayed moderate to strong lipid positivity in sheep and goats alike, reflecting the metabolic demands of lingual musculature (Fig. 16). Similar findings were reported earlier in Iraqi sheep (Hussein and AL-Asadi, 2010), cattle (Sari *et al*., 2010) and goat (Ammar, 2014). Also, the lingual glands of both species exhibited strong to intense lipid reactions at all levels of the tongue (Fig. 17), highlighting their secretory nature (Hussein and AL-Asadi, 2010). The tunica intima of blood vessels in both the species (sheep and goats) also showed a strong lipid reaction (Fig. 18). Lipids likely serve as structural components (cell membranes) and energy stores (intramyocellular lipids). Overall, the two species exhibited nearly identical lipid distribution patterns in all tongue structures examined, with no major interspecies differences detected.

Fig. 15: Tongue of senile crossbred sheep at the level of root. Keratin (K) and lamina propria-submucosa (Ls) showing negative reaction. Lamina epithelialis (LE) along with basement membrane showing weak to moderate reaction for lipids. x40 Sudan Black B

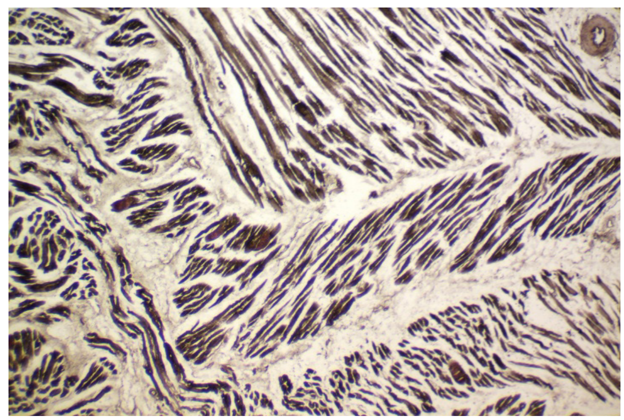

Fig. 16: Tongue of adult non-descript goats at the level of body. Intrinsic muscle bundles displayed moderate to strong reaction for lipids. x40 Sudan Black B

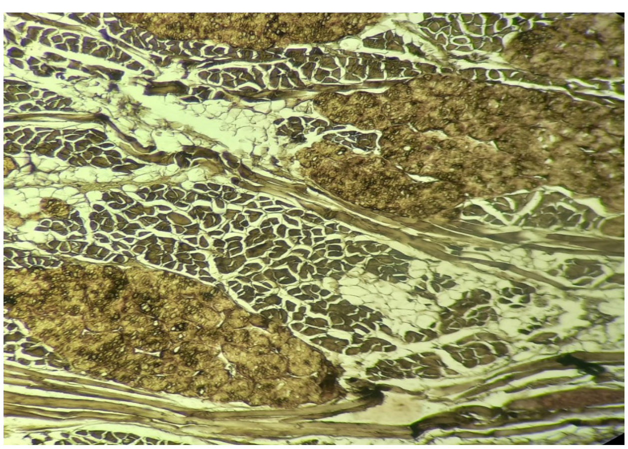

Fig. 17: Tongue of adult non-descript goats at the level of root. Lingual glands showing strong to intense reaction for lipids. x100 Sudan Black B

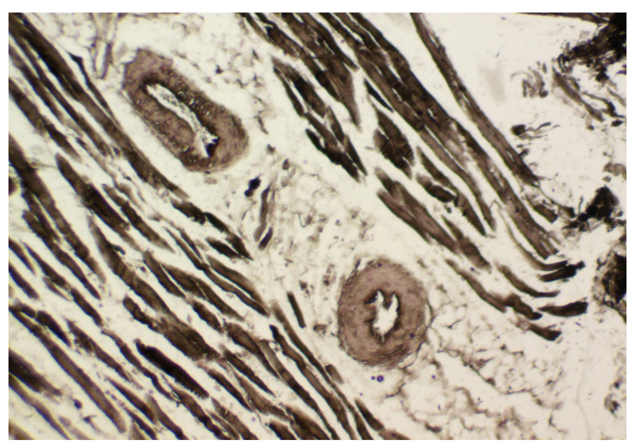

Fig. 18: Tongue of adult crossbred sheep at the level of body. Tunica intima of the blood vessels showing strong reaction for lipids. x100 Sudan Black B

Conclusion

The present study revealed that the tongue of crossbred sheep and non-descript goats in Jammu region exhibits largely similar histochemical distribution patterns of major biochemical constituents suggesting comparable structural organization and functional adaptation of the tongue. These findings provided useful baseline information for comparative anatomical and functional studies in small ruminants.

Acknowledgement

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