

3 Results

3.1 Macroscopic and scanning electron microscopic characteristics of ruminal papillae

Sheep fed hay ad libitum (control): Ruminal papillae were present on the whole mucosal surface of the rumen, even on the ruminal pillars, where the papillae were reduced and closely packed, giving a distinctly ridged appearance to the pillars. These papillae were dark brown in color with uniform length and density.

Observations of papillae by scanning electron microscopy (SEM) revealed short, flattened tongue-like appearance with the greatest thickness about two-thirds of the length of the papillae, which were firmly attached at the proximal end (Fig. 2a).

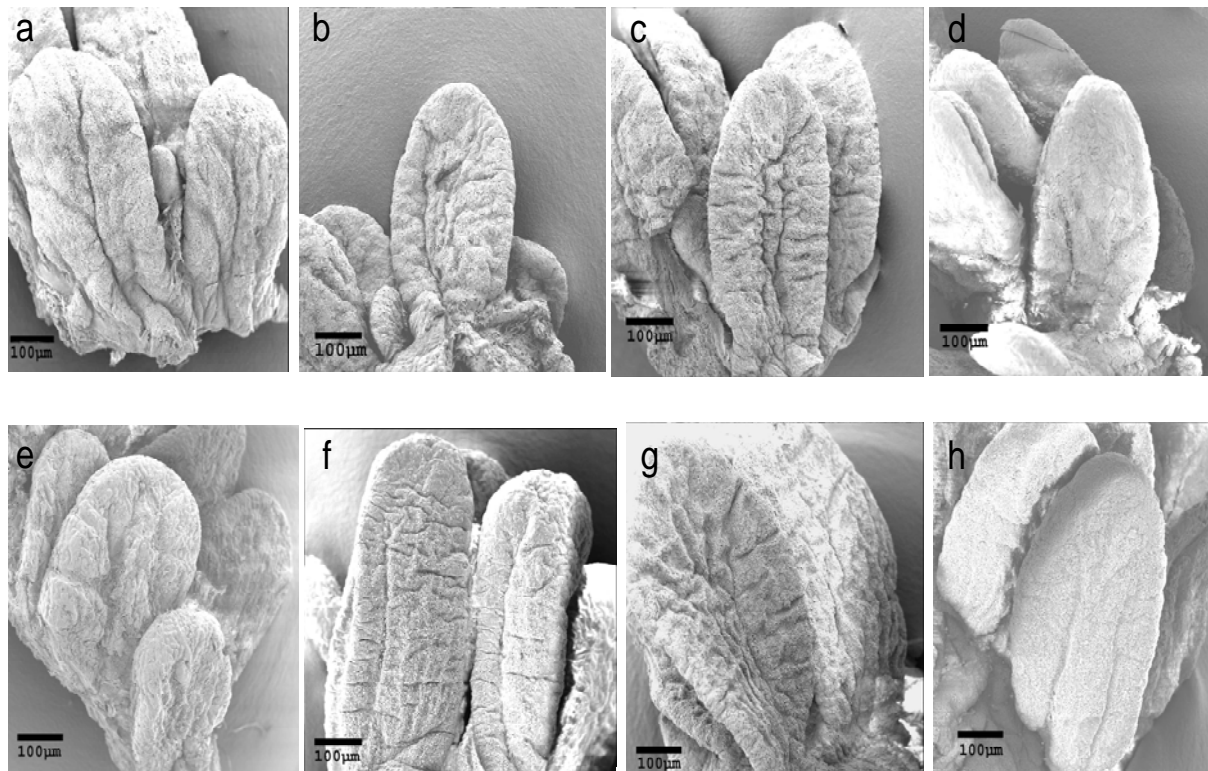


Fig. 2: S.E.M micrographs shows the ruminal papillae isolated from hay and concentrate fed- sheep a. Hay-fed group b. 2 days concentrate-fed group c. 4 days concentrate-fed group d.1 week concentrate-fed group e. 2 weeks concentrate-fed group f. 4 weeks concentrate-fed group g. 6 weeks concentrate-fed group h. 12 weeks concentrate-fed group. (Scale 100µm)

The papillae were generally characterized by the presence of ridges and grooves on their entire surface (Fig. 2a). Scanning electron microscopy revealed details of the surface structure such as deep longitudinal groove and a few shallow grooves arose from the base of the papillae (Fig. 2a). Sometimes, grooves were greater on one side of the papillae than on the other. High magnification of these ridges and grooves revealed highly keratinized squamous cells on the surface of the epithelium (Fig. 3a). The boundaries of these horn cells are clearly visible in fig. 3. At higher magnification, these cells have a distinctly granular appearance due to the presence of nipple-like projections or cytoplasmic protrusions (microvilli-processes) (Fig. 3d and 4a). Moreover, desquamation of the superficial horn cells which is a common feature of rumen epithelium also can be seen. Heterogeneous microflora was present in the trough between the papillary ridges, in association with the outermost layer (Fig.3).

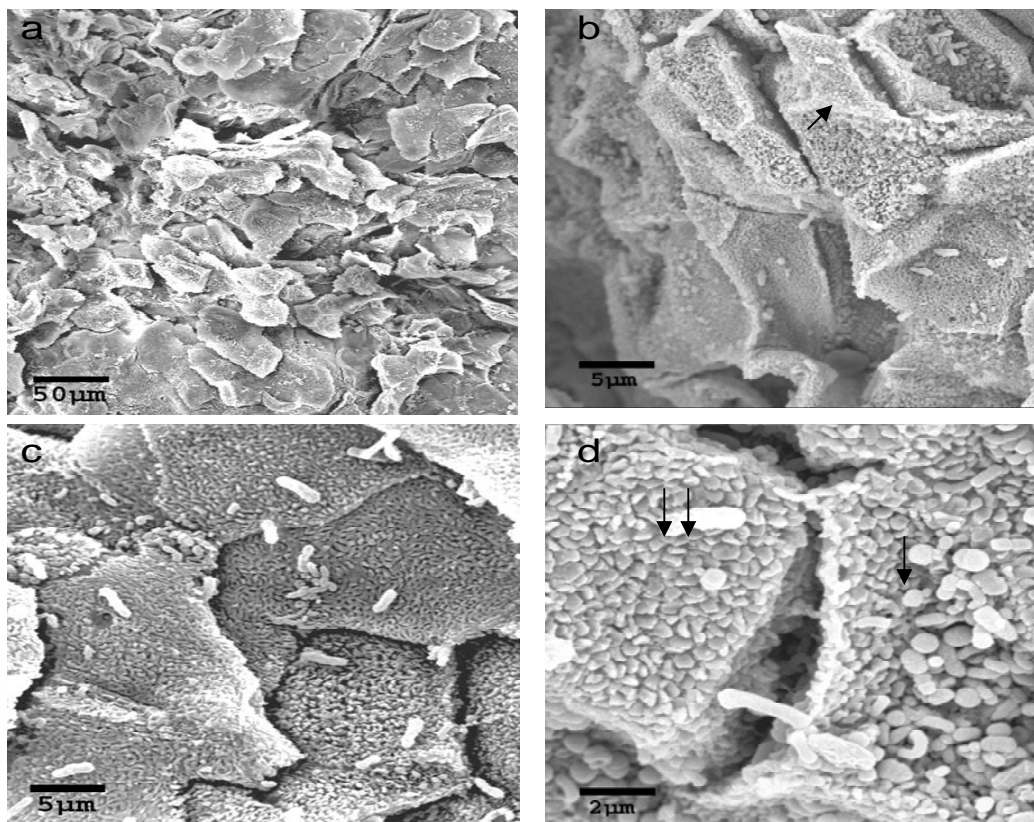


Fig. 3: Surface of the corneal layer of the rumen epithelium and attached microflora.

Note: a. a highly keratinized epithelium (Scale 50 μ m) b. some horn cells showed upturning of their edges (\rightarrow); others were almost completely sloughed off (Scale 5 μ m) c. The boundaries of these horn cells are clearly visible (Scale 5 μ m) d. granular appearance of the cells due to the presence of cytoplasmic protrusions covering cell surfaces. Note the two morphological types of bacteria, short rod (arrows) and cocci (\rightarrow) (Scale 2 μ m)

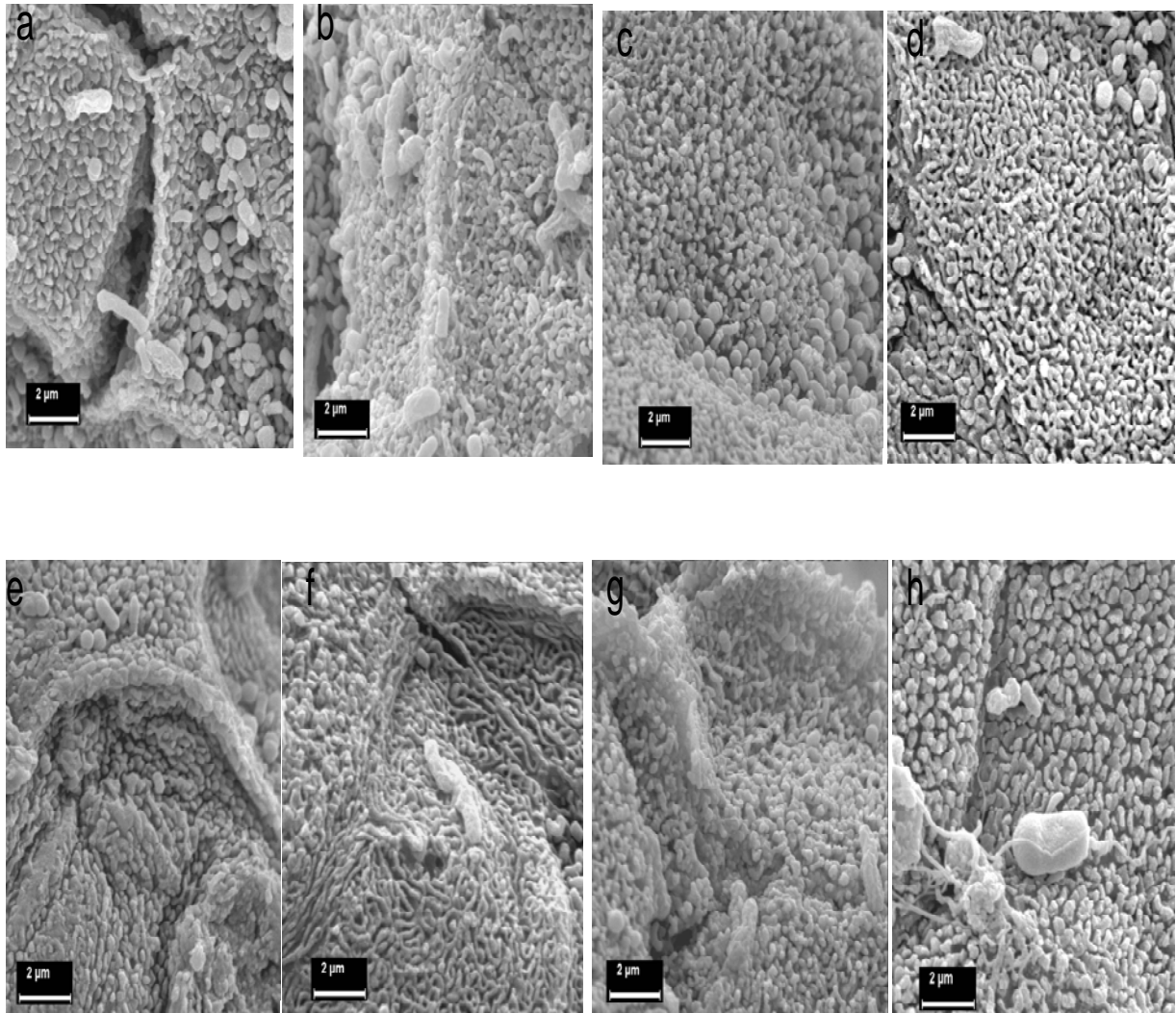


Fig. 4: S.E.M micrographs of sheep-ruminal papillae illustrating the changes in shape and size of cytoplasmic protrusions of horn cells in relation to the type and the duration of feeding (Scale 2 μ m) a. Hay-fed group b. 2 days concentrate-fed group c. 4 days concentrate-fed group d.1 week concentrate-fed group e. 2 weeks concentrate-fed group f. 4 weeks concentrate-fed group g. 6 weeks concentrate-fed group h.12 weeks concentrate-fed group.

Sheep fed hay ad libitum and concentrate-diet for 2 or 4 days: There were minor variations present between these two groups of sheep. On the left lateral wall of the recessus ruminis of the both groups, the papillae were light brown in colour, transitional between the flattened tongue and rounded finger-shaped papillae (Fig.2b and c). SEM examination of these papillae presented two narrow longitudinal grooves arising with one stem from the base of the papilla. Papillae of sheep fed concentrate-diet for 4 days were larger and showed more deep grooves on its surface than those of sheep fed on concentrate-diet for 2 days. The cytoplasmic protrusions were more developed than those of the hay-fed group (Fig. 4b, c).

Sheep fed hay ad libitum and concentrate-diet for 1 week: Three sheep belong to this group, had a considerable variation in the dimension and shape of the papillae. In two sheep, the papillae resembled those of 2 and 4 days concentrate-fed groups, being rounded finger-like papillae. Nevertheless, they were more thickened and brown in colour. Papillae covering the recessus ruminis of the third sheep in this group were similar to those of control group, being tongue-like, shorter and plumper (Fig. 2d). Moreover, the surface of these papillae was smooth except of few, small, shallow grooves which arise from the base of the papilla. The cytoplasmic protrusions were more developed than those of the previous groups and they took more foliate form (Fig. 4d).

Sheep fed hay ad libitum and concentrate-diet for 2 weeks: Two animals in this group had brown, short, very thickened irregular papillae with almost equal dimensions (length and width) (Fig. 2e). The papillary surface present two very deep and wide longitudinal grooves, from which several deep transverse grooves were extended. However, the third animal in this group had long, finger or tongue-like papillae. The surface of the papilla presents shallow longitudinal grooves. The cytoplasmic protrusions were similar to those of one week concentrate fed- group, but they were more developed (Fig. 4e).

Sheep fed hay ad libitum and concentrate-diet for 4 weeks: The papillae covering the lateral wall of the recessus ruminis of the animals in this group were either light or dark brown in colour, very wide, long and finger-like papillae with a uniform in width from proximal to distal end (Fig. 2f). SEM analysis of these papillae revealed one shallow longitudinal groove and a lot of short, deep transverse grooves. Beside the foliate form of the cell processes of the horn cells layer of the rumen epithelium, there were more elongated cell processes forming an arrangement of complex cytoplasmic flaps or folds (contorted arrangement) (Fig. 4f).

Sheep fed hay ad libitum and concentrate-diet for 6 weeks: Papillae belonging to this group were less-well developed compared to those of 4 weeks concentrate-fed group. SEM examination revealed almost black or dark brown leaf-like papillae with a conical apex (Fig. 2g). However, some papillae had a finger like shape. SEM of both types of papillae revealed deep grooves on their surface. However, these grooves were either present in a reticular (leaf-like papillae) or in a transverse form (finger like papillae). The foliate-like cytoplasmic protrusions were well developed.

Sheep fed hay ad libitum and concentrate-diet for 12 weeks: Papillae of the sheep in this group were similar to those of 6 weeks concentrate-fed group, being black or dark brown leaf-like papillae. Nevertheless, they were less uniform in length (Fig.2h). SEM examination of these papillae revealed one deep longitudinal groove, several shallow grooves were branched. Some papillae present reticular grooves on its surface. The cell processes which carried by the horn cell layer, were larger and more foliate in shape than those of hay and all concentrate-fed groups.

3.1.1 Quantitative morphological analysis

The overall differences in the dimensions of the papillae, the number of papillae per cm² mucosa, the surface area of papillae per cm² mucosa of the rumen and the significance are illustrated in Table 5 and 6. A clear effect of the type of diet and the duration of the concentrate feeding on the development of the ruminal papillae was observed (Fig. 5).



Fig. 5: Photograph illustrating changes in shape and size of sheep-ruminal papillae in relation to the type and the duration of feeding. a. Hay-fed group b. 2 days concentrate-fed group c. 4 days concentrate-fed group d. 1 week concentrate-fed group e. 2 weeks concentrate-fed group f. 4 weeks concentrate-fed group g. 6 weeks concentrate-fed group h. 12 weeks concentrate-fed group.

The morphometrical evaluation confirmed that the development of papillae differs significantly between hay and concentrate-fed groups and even among concentrate-fed groups in response to the duration of feeding (Table 5 and 6). Generally, the length, width, number and the total surface of papillae increased in response to the intake of concentrate diet and to increasing the duration of feeding.

3.1.1.1 Length of papillae

Hay-fed group had the shortest papillae (2.21 mm) compared to those of concentrate-fed groups (Fig. 5). They were significantly shorter compared to those of all concentrate-fed groups except 1 week concentrate-fed group. Among concentrate-fed groups, the mean length of the papillae was increased significantly after 2-4 days of feeding concentrate (3.1, 3.23 mm respectively). However, after 1-2 weeks of feeding concentrate, the mean length of the papillae (2.5 mm, 2.6 mm, respectively) was decreased significantly compared to those of 2-4 days concentrate-fed groups. In 4 weeks concentrate-fed group, the mean length of the papillae increased significantly (4.67 mm). It was more than 2 times the length of that

observed in the hay-fed group. Moreover, it had the longest papillae among all concentrate-fed groups, where, the differences were significant. In 6-12 weeks concentrate-fed groups, the length of papillae was decreased significantly (3.89 mm and 3.63 mm, respectively) from that of 4 weeks concentrate-fed group. However, it increased significantly from that of animal fed concentrate up to 2 weeks.

3.1.1.2 Width of papillae

There was a significant difference in the width of papillae between hay (control) and all concentrate-fed groups except that of 2 days and 1 week concentrate-fed groups (Table. 6). Hay-fed group (control) had the thinnest papillae (1.77 mm) compared to those of all concentrate-fed groups. The average width of the papillae of 2 days concentrate-fed group (1.83 mm) did not differ significantly from that of hay-fed group. However, in 4 days concentrate-fed group, the average width of the papillae (2.13 mm) increased significantly compared to that of hay-fed group (control). In 1 week concentrate-fed group the average width of the papillae (1.96 mm) decrease from the previous group, however, the differences were not significant. In 2 weeks concentrate-fed group, the average width of the papillae (2.37 mm) increased significantly from that of animal fed concentrate up to 1 week. In 4, 6 and 12 weeks concentrate-fed groups, the mean width of the papillae (2.75 mm, 2.6 mm, 2.55 mm, respectively) was significantly higher compared to hay-fed group, this was more obvious in 4 weeks concentrate-fed group (it was about 1.5 times the mean width of the hay-fed group) (Table 5).

3.1.1.3 Density of papillae

Density of papillae, which was described by the number of papillae per cm^2 mucosa, increased significantly with the intake of concentrate diet in comparison to that of hay-fed group (38.67 papillae / cm^2 mucosa). However, the increase of density of the papillae was not constant according to the increase of the duration of concentrate feeding (Table 5). It reached the maximum value after 2 days and 1 week of feeding concentrate (64.33, 63.33 papillae / cm^2 mucosa, respectively). It increased significantly ($p < 0.05$) compared to those of hay-fed group (38.67 papillae / cm^2 mucosa) and other concentrate-fed groups.

Table 5: Effects of the duration of concentrate feeding on morphometric parameters of the rumen mucosa (Mean \pm SD) (left wall of the recessus ruminis ventralis sac)

Experimental groups of animals	Papillae		
	Length (mm)	Width (mm)	Number / (cm ²) mucosa
CF0W	2.21 \pm 0.40	1.77 \pm 0.20	38.67 \pm 2.89
CF2D	3.10 \pm 0.50	1.83 \pm 0.18	64.33 \pm 3.06
CF4D	3.23 \pm 0.33	2.13 \pm 0.18	51.00 \pm 2.00
CF1W	2.50 \pm 0.60	1.96 \pm 0.43	63.33 \pm 4.04
CF2W	2.61 \pm 0.42	2.37 \pm 0.32	50.33 \pm 2.52
CF4W	4.67 \pm 0.36	2.75 \pm 0.42	48.33 \pm 3.51
CF6W	3.89 \pm 0.38	2.60 \pm 0.21	49.33 \pm 2.08
CF12W	3.63 \pm 0.29	2.55 \pm 0.37	51.33 \pm 1.53

Table 6: Significant differences of the parameters of the rumen mucosa

Experimental groups of animals	Significant letter	Significant-length of papillae	Significant-width of papillae	Significant-number / (cm ²) mucosa
CF0W	a	-	-	-
CF2D	b	ade	-	acefgh
CF4D	c	ade	a	a
CF1W	d	-	-	acefgh
CF2W	e	a	abd	a
CF4W	f	abcdegh	abcde	-
CF6W	g	abcde	abcd	a
CF12W	h	abcde	abcd	a

^{a-h} Means in the same column and kind of sampling sharing the same superscript letters differ significantly

It decreased and increased irregularly, reaching its lowest value after 4-6 weeks of feeding concentrate (48.33, 49.33 papillae / cm² mucosa) respectively. However, after 12 weeks of feeding hay plus concentrate, the number of papillae per cm² mucosa increased significantly (p<0.05) (51, 33 papillae / cm² mucosa).

3.1.1.4 Surface of papillae

The mean total surface of papillae per cm² mucosa is determined as length × width × 2 multiplied by the number of papillae per cm² mucosa. Concentrate feeding resulted in a significant increase of surface area of the rumen papillae (Diagram 1).

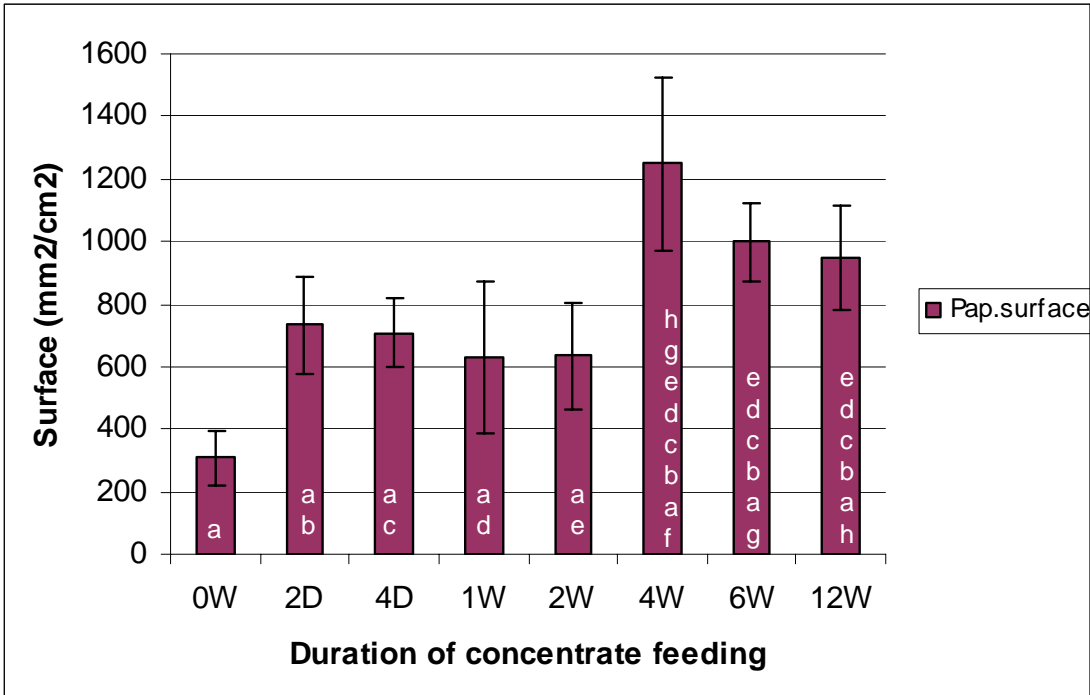


Diagram 1: Total surface of papillae for different animal groups fed hay or hay plus concentrate feeding for different periods. a-h Means in the same column and kind of sampling sharing the same superscript letters differ significantly

With increasing the duration of feeding to 2-4 days, the surface area increased (734.86-706.35 mm² / cm² mucosa) above 2 fold as compare to the hay-fed group (308.21 mm² / cm² mucosa). This value was decreased after 1-2 weeks of concentrate feeding (630.17-633.25 mm² / cm² mucosa) compared to that of 2 and 4 days concentrate-fed groups. After 4 weeks of feeding, this mean value reached the peak about 4 fold (1248.44 mm² / cm² mucosa) in comparison to the hay-fed group. This mean value decreased to 3 fold (997.55-949.66 mm² /

cm² mucosa) after 6-12 weeks of concentrate feeding. Among concentrate-fed groups, the total surface of papillae differs significantly between animal fed concentrate up to 2 week and those for 4-12 weeks concentrate-fed groups (Diagram 1 and fig.5).

3.2 Histological findings

3.2.1 Qualitative histological findings

3.2.1.1 Shape, size and density of the ruminal papillae

Sheep fed hay ad libitum (control): The ruminal mucosa of two animals in this group, had narrow short, tongue-shaped papillae with arched apex and less irregular lateral sides of the papillae where the lower two-third of the papillae were wider than the upper third (Fig. 6a and 7a). However, the ruminal mucosa of the third animal had very narrow and long papillae with wide base (Fig. 7b). The papillae were equal in length and were composed of a core of vascularized connective tissue and covered with epithelium (Fig. 7).

Table 7: Effects of the duration of concentrate feeding on density of ruminal papillae per mucosa field (N = 3 animals for each treatment)

Feeding regime	Density of papillae/mucosa field
CF0W	3-4
CF2D	4-6
CF4D	4-5
CF1W	4-6
CF2W	4
CF4W	4
CF6W	4
CF12W	2-4

Extensions of the epithelium into the lamina propria (epithelial pegs), were more developed at the apex of the papillae compared to the sides and the interpapillary regions (Fig. 8a). The

interpapillary distances were long and had less folded interpapillary mucosa. The density of papillae per field varied from 3 to 4 papillae / field (Table 7).

Sheep fed hay ad libitum and concentrate-diet for 2 or 4 days: No significant morphological differences between these two groups were observed. Therefore, they will be described together.

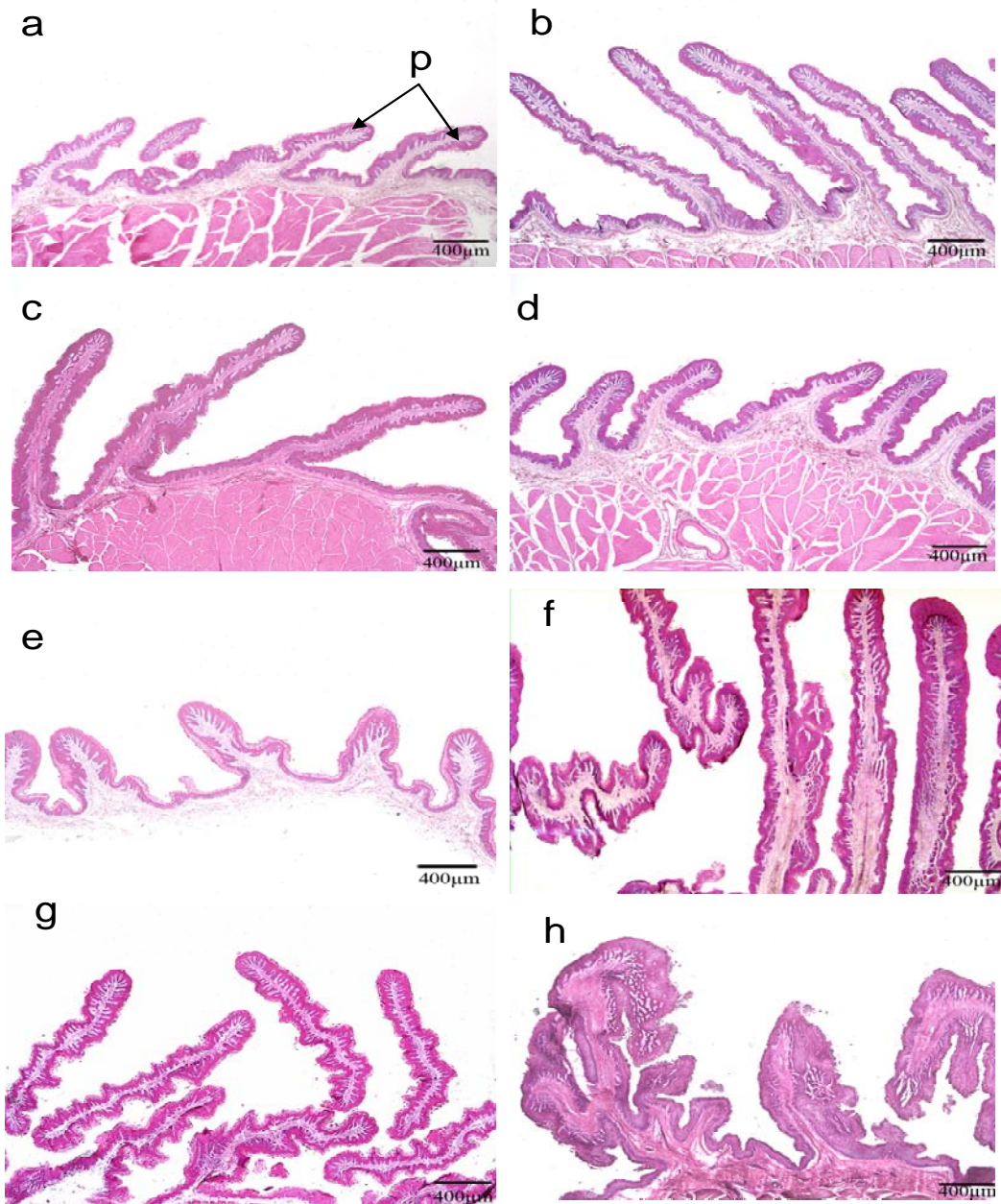


Fig. 6: Sections of sheep rumen (left wall of the recessus ruminis ventralis sac) of eight groups of animals, illustrating different papillary shape and size (Scale 400µm) a. narrow, short tongue-like papillae (control-group) b. narrow, long tongue to finger-like papillae (2 days concentrate-fed group) c. wide, long tongue to finger-like papillae (4 days concentrate-fed group) d. wide, short tongue -like papillae (1 week concentrate-fed group) e. wide, short irregular shaped papillae (2 weeks concentrate-fed group) f. wide, very long finger-like papillae (4 weeks concentrate-fed group) g. narrow, long waved sides papillae(6 weeks concentrate-fed group) h. irregular branched papillae (12 weeks concentrate-fed group). (H and E)

Ruminal papillae lost their smooth tongue form within 2 days of feeding concentrate, where, they became narrow long and showed tongue to finger-like appearance with waved sides, arched apex (equal width along the most length of the papillae) and wide base (Fig. 6 b, 7 b and 8 b). After 4 days of concentrate-feeding, the width of the ruminal papillae were increased, however they had less waved sides (Fig. 6 c). The ruminal papillae of both groups, exhibited mild hypertrophic changes, and showed more developed epithelial pegs into the lamina propria compared to those of hay-fed group (Fig. 8 b, c). The interpapillary distances were short and folded in sheep fed on concentrate for 2 days. However, they were long and straight in sheep fed on concentrate for 4 days (Fig. 6 b, c). The density of papillae per field (5-6 papillae / field) was higher compared to that of hay-fed group (Table 7).

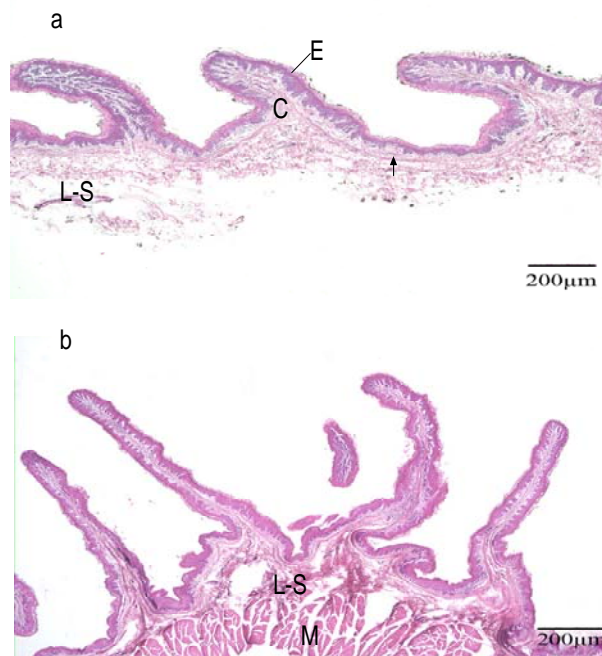


Fig. 7: a. Tongue-like papillae projecting from the luminal surface of the organ (hay-fed group) consisted of keratinized, stratified squamous epithelium (E) and a core of connective tissue (C). The lamina propria-submucosa (L-S) was typical and adjacent to the tunica muscularis (M). (A condensation of connective tissue fibers (arrow) should not be mistaken for lamina muscularis) (H and E) (Scale 200µm)

b. Narrow, long tongue to finger-like papillae (2 days concentrate-fed group) (Scale 200µm)

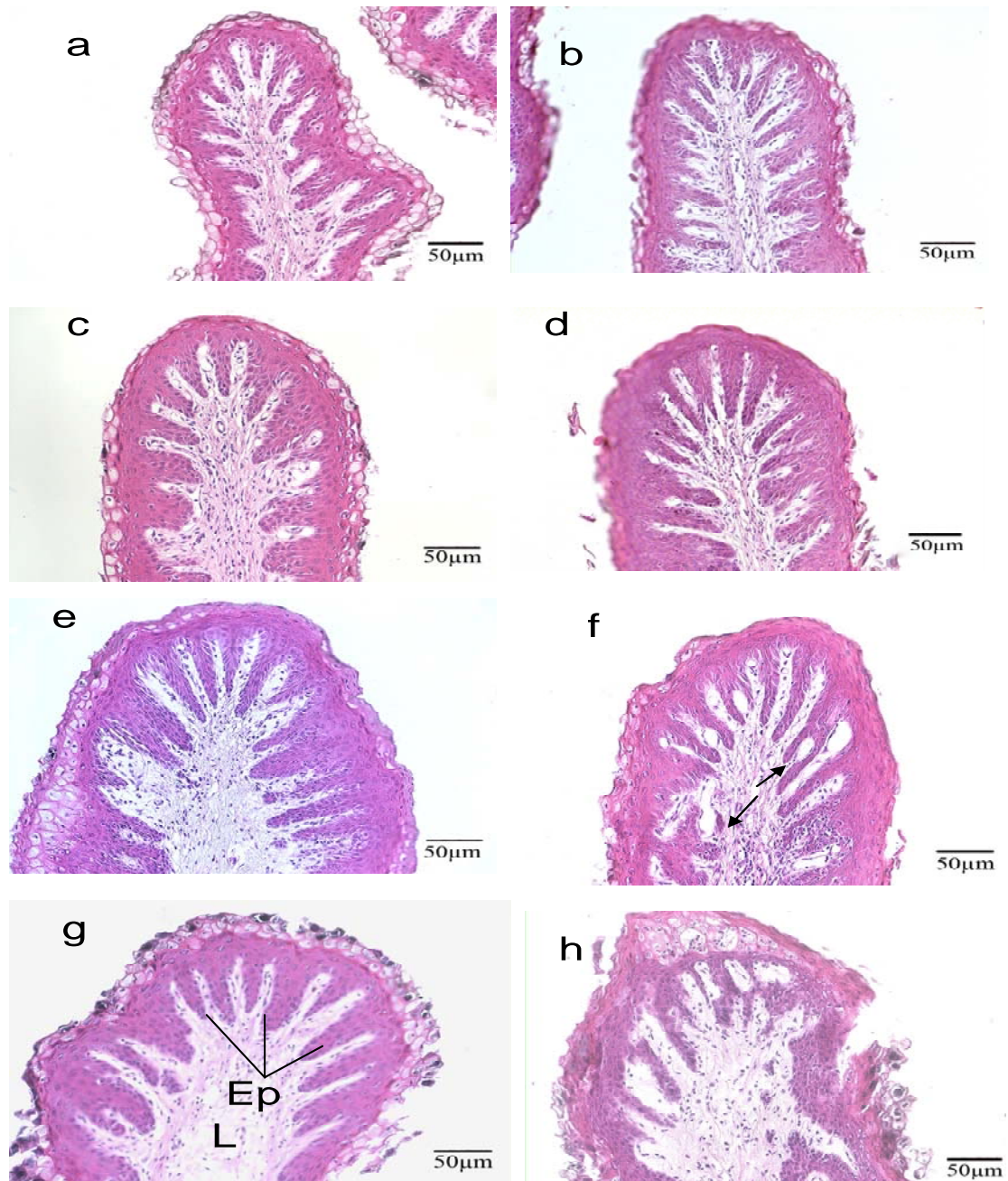


Fig. 8: Sections of rumen papillae from eight sheep groups, illustrating different degree of development of epithelium pegs (EP) into the lamina propria (L) a. Hay-fed group, b. 2 days concentrate-fed group, c. 4 days concentrate-fed group, d. 1 week concentrate-fed group, e. 2 weeks concentrate-fed group, f. 4 weeks concentrate-fed group; notice: The tips of the epithelium pegs branched into 2 or 3 secondary tips (arrow), g. 6 weeks concentrate-fed group, h. 12 weeks concentrate-fed group; notice: The misshaped epithelium pegs. (H and E) (Scale 50µm)

Sheep fed hay ad libitum and concentrate-diet for 1 week- Papillae of two animals in this group were similar to those of hay-fed group, being short tongue-shaped papillae with arched

apex and equal length. However, they were wider (especially at the base of the papillae) with more irregular sides and short interpapillary distances (Fig. 6 d). However, the third sheep had long, leaf-like papillae with very irregular or waved sides and constricted base. The interpapillary distances were short and presented side papillae (Fig. 9). Ruminal papillae from this group, unlike those from hay-fed group and the previous concentrate-fed groups, exhibited moderate hypertrophic changes. There was also a moderate degree of development of the epithelial pegs into the lamina propria (Fig. 8 d). The density of papillae was ranged from 5 to 6 papillae / field (Table 7).

Sheep fed hay ad libitum and concentrate-diet for 2 weeks: The ruminal papillae of two animals in this group were thick, short irregular-shaped with very irregular or waved sides and short interpapillary distances (Fig. 6 e and 10 a). A few papillae branched into small secondary papillae at the base of the primary ones. Moreover, interposed papillae were also observed in interpapillary regions (Fig. 10 a). However, the third sheep showed wide, long, leaf-like papillae with very irregular or waved sides (secondary papillae) and less uniform length (Fig. 10 b). The ruminal papillae exhibited a higher degree of development of the epithelial pegs into the lamina propria compared to all previous concentrate-fed groups (Fig. 8 e). The density of papillae per was 4 papillae / field (Table 7).



Fig. 9: Long leaf- like papillae from 1 week concentrate-fed group showed secondary papillae (arrow) and interposed papilla (arrows). (H and E) (Scale 200µm)

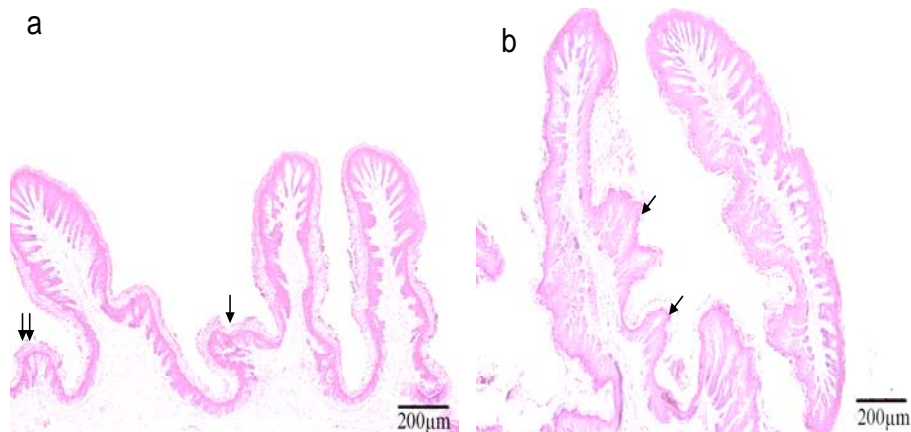


Fig. 10: a. and b. Short and long irregular shaped papillae from 2 weeks concentrate-fed group showed secondary papillae (arrow) and interposed papilla (arrows). (H and E) (Scale 200µm).

Sheep fed hay ad libitum and concentrate-diet for 4 weeks: sheep of this group had the longest and thicker ruminal papillae compared to those of control and other concentrate-fed groups. The papillae varying slightly in length, finger-like appearance with wavy sides, arched or flat apex and wide base (equal width along the most length of the papilla) (Fig. 6 f and 11). Unlike the previous group, the ruminal papillae exhibited moderate hypertrophic changes and moderate degree of development of the epithelial pegs into the lamina propria (Fig. 8 f). The interpapillary distances were very short (Fig. 6 f, 8 f). The density of papillae per field was 4 papillae / field (Table 7).



Fig. 11: Thick, very long finger-like papillae with wide base and waved sides from 4 weeks concentrate-fed group. (H and E) (Scale 200µm)

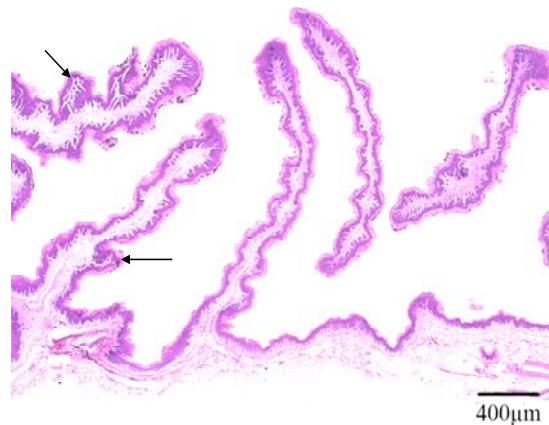


Fig. 12: Narrow, long papillae with waved sides and broad apex (arrow) from 6 weeks concentrate-fed group, showed secondary papillae (arrow). (H and E) (Scale 400µm)

Sheep fed hay ad libitum and concentrate diet for 6 weeks: Sheep of this group showed very narrow and long papillae with deep waved sides (secondary papillae) and broad arched

apex (Fig. 6 g and 12). Many papillae branched into small secondary papillae at the base of the primary ones (Fig. 12). Like 2 weeks concentrate-fed group, ruminal papillae exhibited high hypertrophic changes and high development of the epithelial pegs into the lamina propria (Fig. 8 h). The interpapillary distances were long. The density of papillae per field was 4 papillae / field (Table 7).

Sheep fed hay ad libitum and concentrate-diet for 12 weeks: 12 weeks concentrate-fed group, showed either long irregular shaped - papillae with broad apex or branched irregular shaped papillae (Fig. 6 g and 13 a and b). Most of the ruminal papillae were short and branched into 2-4 secondary papillae with regular length (mushroom like-papilla) (Fig. 13a).

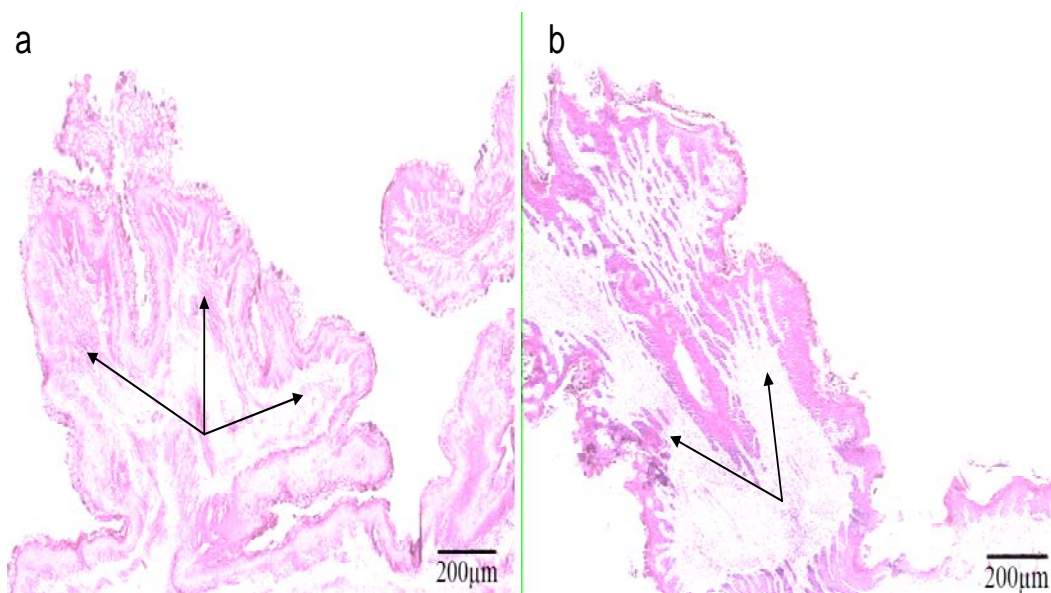


Fig. 13: a. Short and very thick papillae partially branched into three secondary papillae. Note, branching occurred at the apex of the papilla giving a mushroom appearance to the papilla (arrows). (H and E) (Scale 200 µm) b. Long and thick papillae branched into two secondary papillae. Note branching occurred only at the base of the papilla (arrows). (H and E) (Scale 200 µm)

The rest were long branched papillae (Fig. 13b). Like 4 weeks concentrate-fed sheep, the ruminal papillae of this group exhibited moderate hypertrophic changes, and the epithelial pegs showed irregular shape (misshaping) and were less developed compared to that of the previous group (Fig. 6 g, 8 g). The interpapillary distances were long and the interpapillary mucosa was folded. The density of papillae per field (2-4 papillae / field), were similar or less than that of the previous three groups (Table 7)

3.2.1.2 Rumen mucosa

3.2.1.2.1 Epithelium

Sheep fed hay ad libitum (control): Rumen mucosa of sheep in this group was lined by a (stratified, cornified, squamous epithelium) and a lamina propria of connective tissue. The rumen epithelium was composed of five to six layers of nucleated cells and relatively thin stratum corneum formed by two cell-thick layer (Table 8 and 9). From the luminal surface, four cell layers (strata) could be distinguished (stratum corneum, granulosum, spinosum and basale) (Fig. 14 a, b).

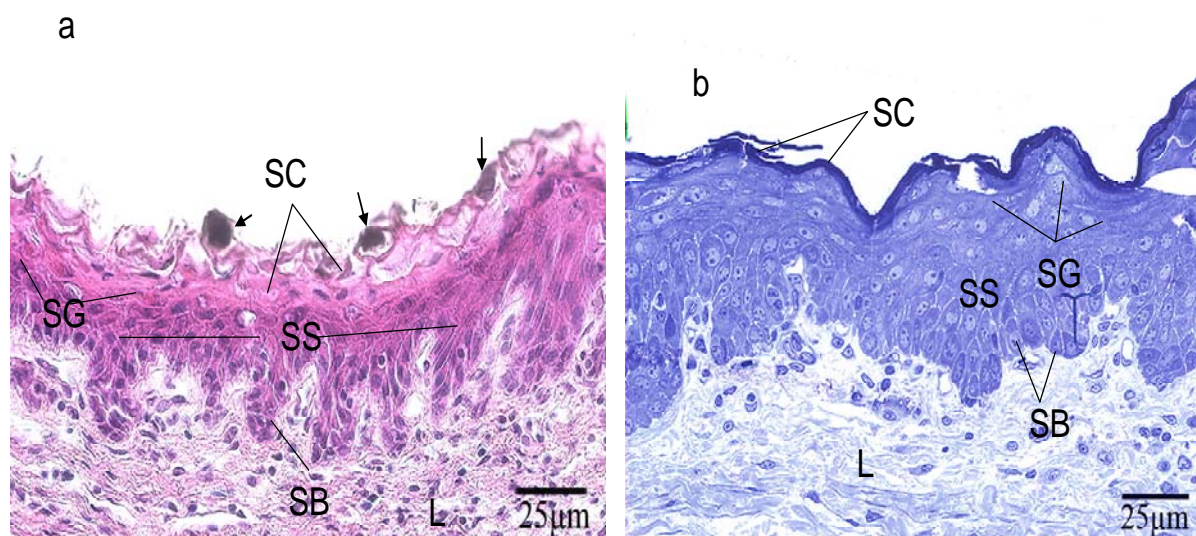


Fig. 14: a. Micrograph of rumen epithelium from hay-fed group, showing stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC). Arrow (→) points to horn cells containing ingesta. (H and E) (Scale 25 µm) b. Semithin-section of rumen epithelium from hay-fed group, showing the above mentioned strata. L: lamina propria (Richardson solution) (Scale 25 µm)

a. Stratum corneum: It was thin, composed of two cell-thick layer of small vacuolated horn cells (balloon cell-like), covering both apex and sides of the papillae or flattened cells at the apex. Flattened cornified cells were occasionally observed in the layer of balloon cells. At the apex of the papillae, mild degree of desquamation of the superficial horn cells was observed. Additionally, ingesta which related to the more superficial horn cells (a few cells), could be observed (Fig. 8 a and 14 a, b).

b. Stratum granulosum: 1-2 cell-thick layer and consisted of two types of cells; flattened elongated at apex of the papillae and vacuolated cells at sides and inter-papillary mucosa. The cytoplasm of both types of cells contained fine keratohyaline granules.

c. Stratum spinosum: It consisted of 2-3 cell-thick layer. The cells of this layer had a lighter staining affinity than those of the basal layer and flattened progressively towards the luminal surface. It consisted of superficial and deep (Para basal) spiny cells. The superficial spiny cells were polyhedral cells with large rounded nuclei. Their long axis was oriented parallel to the mucosal surface. They were more flattened near the stratum granulosum. The deep spiny cells neighboring the basal cells (parabasal cells), were almost spherical. The thickness (number of cells) of this stratum at the base of the papillae and the inter-papillary region was more than that at the apex of the papillae (Table 8).

d. Stratum basale: It consisted of a single layer of small columnar or cuboidal cells. Its long axis was perpendicular to the basement membrane. It was in contact with the lamina propria. There was a network of capillaries and loops of capillaries which run through the folds at the interface of the epithelium and the connective tissue (papillary body).

Table 8: Effects of the duration of concentrate feeding on total thickness of rumen epithelium (Thickness was measured as number of cells) (Mean±SD). N = 3 animals for each treatment

Feeding regime	Total thickness of epithelium (cells)		
	Base of papillae	Tip of papillae	Interpapillary area
CF0W	7-8	7	7-8
CF2D	7	7	7
CF4D	7	7	6-7
CF1W	8	7-9	7
CF2W	9	9	8
CF4W	8-9	8-10	7-9
CF6W	7-9	8-9	7-9
CF12W	8-9	8-10	7-8

Table 9: Effects of the duration of concentrate feeding on thickness of epithelial strata

Feeding regime	Thickness of str. spinosum			Thickness of str. granulosum			Thickness of str. corneum		
	Base pap.	Tip pap.	Inter pap.	Base pap.	Tip pap.	Inter pap.	Base of pap.	Tip pap.	Inter pap.
CF0W	2-3	2	2-3	2	2	2	2	2	2
CF2D	2	2	2	2	2	2	2	2	2
CF4D	2	2	2	2	2	2	2	2	1-2
CF1W	2	2	2	3	2-3	2	2	2-3	2
CF2W	2	2	2	3	3	2	3	3	3
CF4W	1-2	1-2	1-2	3	3	3	3	3-4	2-3
CF6W	1-2	1	1-2	3	3	3	2-3	3-4	2-3
CF12W	1	1	1	3	2-3	3	3-4	4-5	2-3

Sheep fed hay ad libitum and concentrate-diet for 2 or 4 days: The thickness of the epithelium after 2 or 4 days of feeding concentrate-diet was not clearly different compared to that of the hay-fed group as shown in table 9. Moreover, the total number of cells in different regions of the mucosa (tip, sides and inter-papillary regions) did not show any remarkable variations.

a. Stratum corneum: consisted of 1-2 cell-thick layer of small horn cells. It showed morphological differences compared to those of control group, where the vacuolated horn cells at the apex of papillae were replaced by small nucleated, flattened cells. At the lateral sides of the papillae and the inter-papillary regions, the horn cells were vacuolated, however,

they were nucleated. Mild degree of desquamation of the superficial horn cells was observed (at sides and inter-papillary regions).

b. Stratum granulosum: It was a distinct layer (1-2 cell-thick layer) of large elongated cells (at the apex of the papillae) and large vacuolated cells (at sides and inter-papillary mucosa). Both types of cells contained accumulations of small to medium-sized granules of keratohyaline.

c. Stratum spinosum: 1-2 cell-thick layer of polyhedral cells with large rounded nuclei.

d. Stratum basale: It was composed of a single layer of small columnar cells.

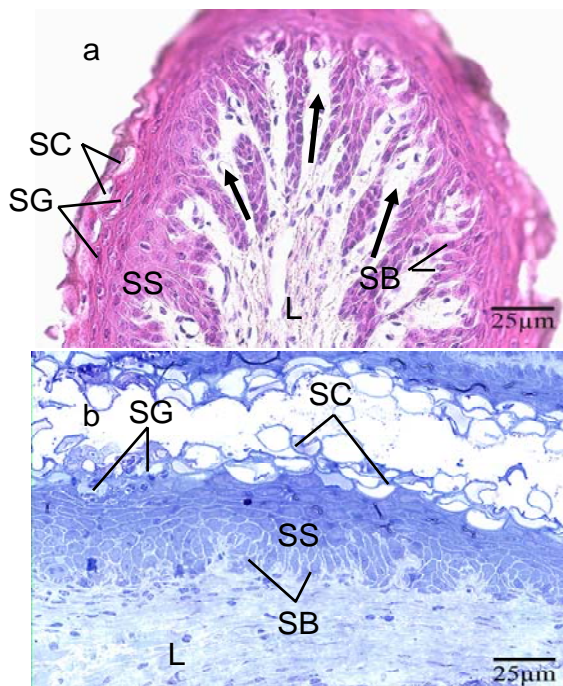


Fig. 15: a. Paraffin-section and b. semithin-section of rumen epithelium from 2 days concentrate-fed group, illustrating the same strata of epithelium as shown in figure14. Arrow (→) points to papillary body. (H and E), (Richardson solution) (Scale 25µm)

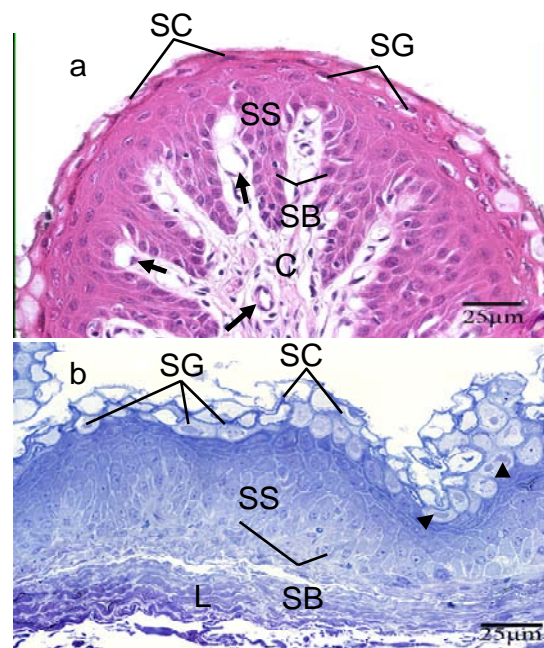


Fig. 16: a. Paraffin-section and b. semithin-section of rumen epithelium from 4 days concentrate-fed group, illustrating the same strata of epithelium as shown in figure14. Arrow (→) points to capillaries located at papillary body and core of the papilla. Arrow's head (▲) pointed to the medium sized keratohyaline granules. (H and E), (Richardson solution) (Scale 25µm)

Sheep fed hay ad libitum and concentrate-diet for 1 week: The thickness of the epithelium ranged from 7 to 9 cells. It was slightly thicker compared to that of the hay-fed group and the previous concentrate-fed groups (table. 8) and (Figs. 8d and 17a, b). The thickness of the epithelium in different regions of the mucosa (apex, sides and inter-papillary regions) showed slight variations. It was less in the inter-papillary regions than that covering the papillae (table 9).

a. Stratum corneum: 2-3 cell-thick layer of medium-sized vacuolated, nucleated horn cells (at the lateral sides of the papillae) and flattened, nucleated cells at the apex of the papillae. There was a moderate desquamation of the superficial horn cells, which was more obvious at the lateral sides of the papillae than at the apex (Fig. 9). Moreover, considerable amount of ingesta were observed related to some superficial horn cells.

b. Stratum granulosum: 2-3 cell-thick layer and composed of large elongated cells at the apex and large vacuolated cells at the sides of papillae with accumulation of fine keratohyaline granules. The thickness of stratum granulosum at interpapillary region was less than that covering the papillae (Table 10).

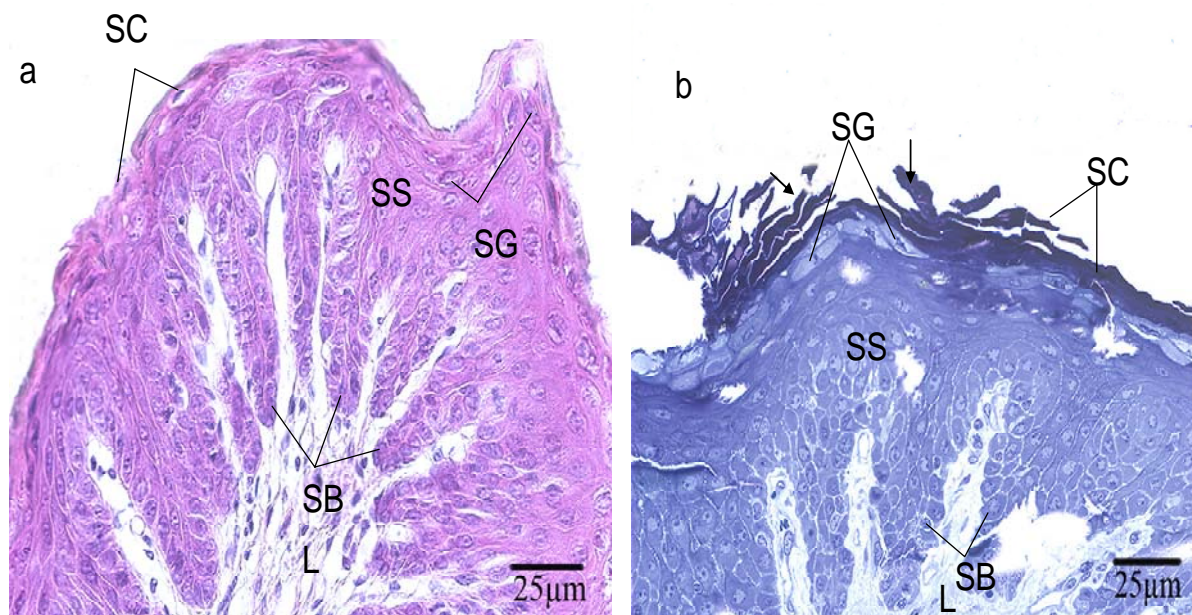


Fig. 17: a. Paraffin-section and b. semi-thin-section of rumen epithelium from 1 week-treated group illustrating the same strata of epithelium as shown in figure14. (Notice, the increasing of the size of the epithelial cells). (H and E), (Richardson solution) (Scale 25µm)

c. Stratum spinosum: 1-2 cell-thick layer composed of polyhedral cells with large rounded nuclei.

d. Stratum basale: It consists of a single layer of columnar cells, which was wider than that of control, 2 days and 4 days treated groups.

Sheep fed hay ad libitum and concentrate-diet for 2 weeks: - After two weeks of feeding concentrate-diet a remarkable increase in the thickness of the epithelium (8-10 cell layers) was observed compared to that of the control and the previous concentrate-fed groups (Table 8). This was due to the increase in the thickness of most of strata of the epithelium (Table 9) and (Fig. 8 e; 18 a, b).

a. Stratum corneum: - 3 cell-thick layer of flattened cell at both the apex and the lateral sides of the papillae. Medium-sized vacuolated cells (at the lateral sides of the papillae) were also observed. Severe desquamation of superficial horn cells was observed. This was more pronounced at the superficial horn cells covering the apex of the papillae. Moreover, a considerable amount of ingesta were observed related to some superficial horn cells.

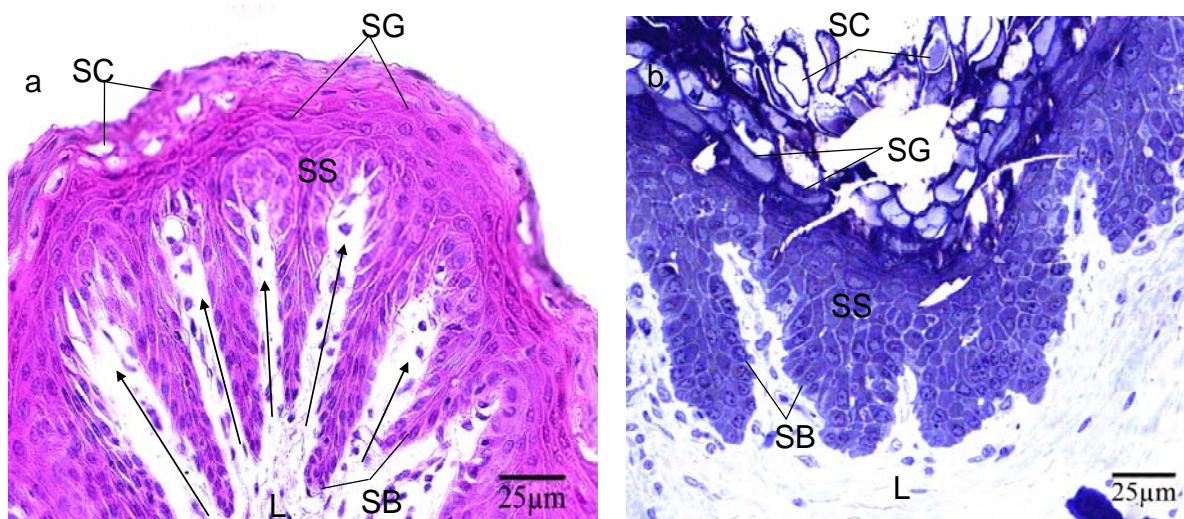


Fig. 18: a. Paraffin-section and b. semithin-section of rumen epithelium from 2 weeks concentrate-fed group illustrating the same strata of epithelium as shown in figure 14. Arrow (→) points to papillary body. (Notice, increasing size of the epithelial cells). (H and E), (Richardson solution) (Scale 25µm)

b. Stratum granulosum: A distinguished 2-3 cell-thick layer of vacuolated cells at the lateral sides and more flattened cells at the apex of the papillae. They contained irregular, medium-sized keratohyaline granules.

c. Stratum spinosum: 2-3 cells layers of large polyhedral cells with large rounded nuclei.

d. Stratum basale: It consists of a single layer of pyramidal cells.

Sheep fed hay ad libitum and concentrate-diet for 4 weeks: The thickness of the epithelium was within the same range of that of the previous concentrate-fed groups (8-10 cell layers) (Table 8). The tip of many epithelial pegs branched into two small secondary tips (Fig. 8 f and 19 a, b).

a. Stratum corneum: 2-4 cell-thick layer, of large vacuolated cells at both the lateral sides and the apex of the papillae (more flattened at the apex). Many papillae showed flattened cells at the upper two layers of the stratum corneum. The lower layer was formed of vacuolated cells. The superficial corneal layer showed mild degree of desquamation and a few ingesta were observed related to some superficial horn cells.

b. Stratum granulosum: A distinct layer (3 cell-thick layer) of large vacuolated cells at the lateral sides and more flattened cells at the apex of the papillae with large keratohyaline granules.

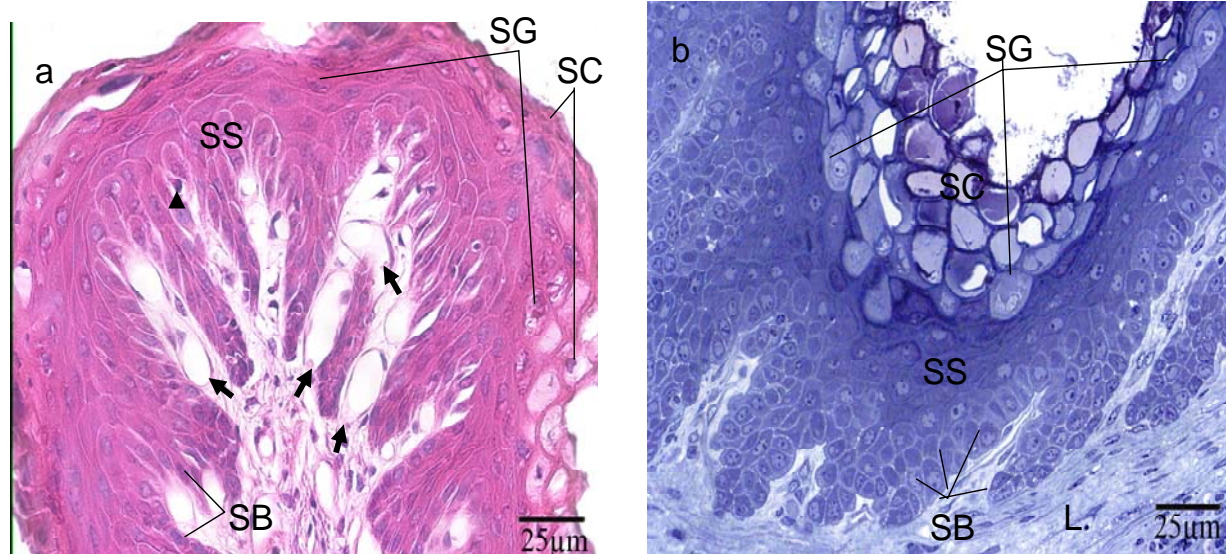


Fig. 19: a. Paraffin-section and b. semithin-section of rumen epithelium from 4 weeks concentrate-fed group illustrating the same strata of epithelium as shown in figure14. Notice, increasing size of the epithelial cells and intercellular spaces. Arrow (→) points to sinusoid-like capillaries. Arrow head (▲) pointed to the lymphocyte-like cell. (H and E), (Richardson solution) (Scale 25µm)

c. Stratum spinosum: It was 1-2 cell-thick layer. Nevertheless, the size of the cells was increased compared to the previous groups.

d. Stratum basale: The size of the basal cells was increased compared to the previous group. It consists of a single layer of large pyramidal cells (Fig. 19 a, b).

Sheep fed hay ad libitum and concentrate-diet for 6 weeks: The thickness of the epithelium was slightly decreased (7-9 cells layers) compared to that of the previous two groups (Table 8) and (Fig. 8 g and 20 a, b).

a. Stratum corneum: The thickness, types and the pattern of cells distribution were similar to that of the previous two groups (1-2 cell-thick layer). However, the cells were larger. More

ingesta were observed related to the most superficial horn cells. Furthermore, a mild desquamation was observed.

b. Stratum granulosum: A distinct layer (3 cell-thick layer) of very large and vacuolated cells at the lateral sides and more flattened at the apex of the papillae. The superficial cells were larger and had larger keratohyaline granules than the deep ones.

c. Stratum spinosum: 1-2 cell-thick layer of large polyhedral cells with wide intercellular space.

d. Stratum basale: It composed of one layer of large pyramidal cells with a wide intercellular space. Lymphocytes were located between the epithelial cells.

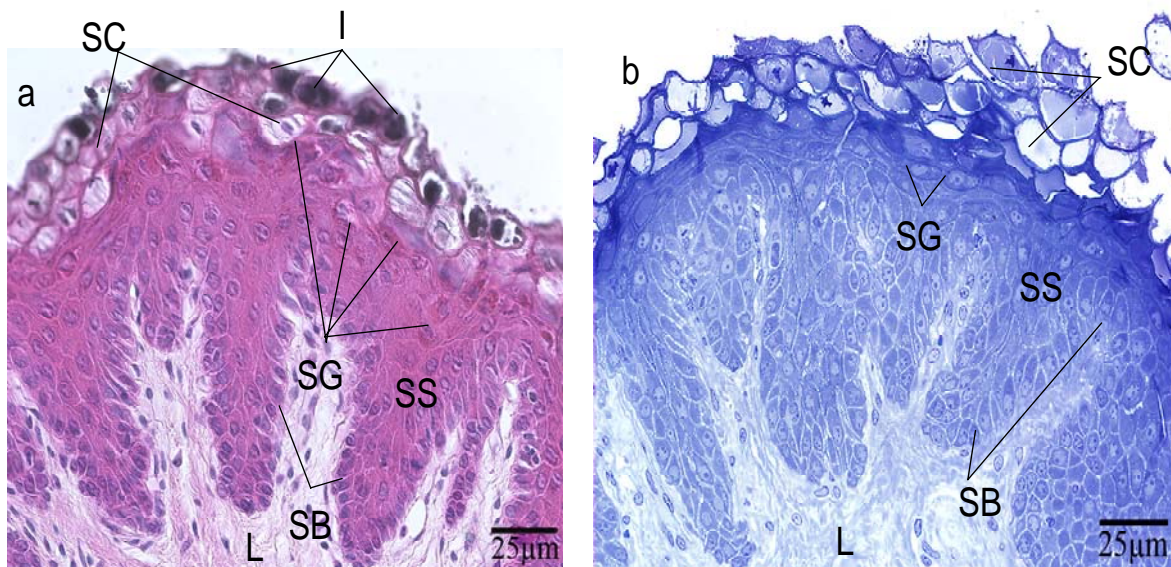


Fig. 20: a. Paraffin-section and b. semithin-section of rumen epithelium from 6 weeks concentrate-fed group illustrating the same strata of epithelium as shown in figure 14. Increasing size of the epithelial cells and intercellular spaces compared with those of previous treated groups. Horn cells contain large-sized digested material. (H and E), (Richardson solution) (Scale 25µm)

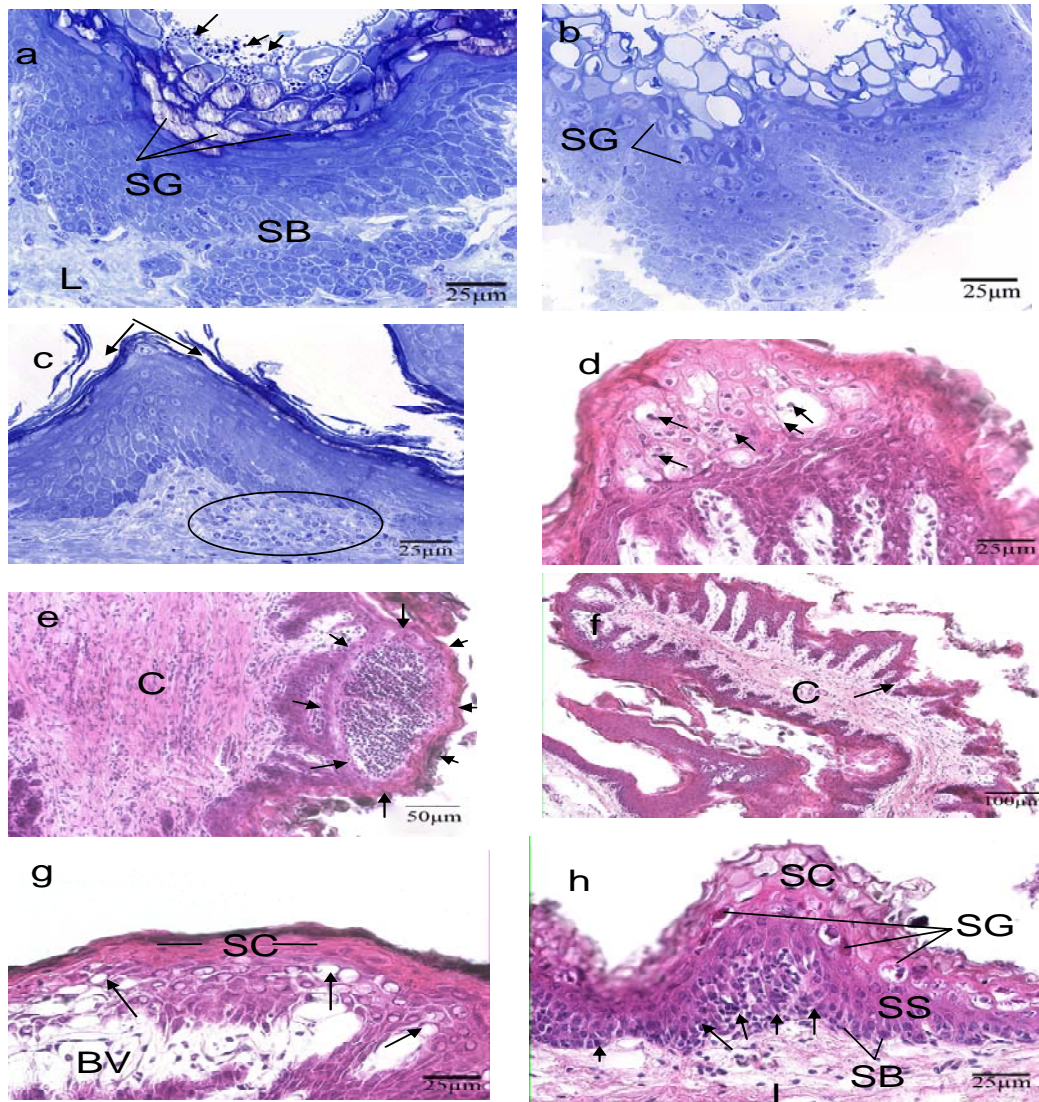


Fig. 21: Semithin-sections and Paraffin-sections of rumen epithelium from 12 weeks-treated group illustrating the same strata of epithelium as shown in figure 14. Notice: a. Increasing size of the epithelial cells compared with those of previous concentrate-fed groups. Arrow (→) points to ingesta related to the superficial horn cells b. stratum granulosum containing large-sized keratohyaline granules (SG) c. desquamated horn cells (→) and accumulation of lymphocyte-like cells in the lamina propria (O) d. horn cells formed a cap over the apex and containing lymphocyte-like cells (→) e. isolated microabscess on the side of the papilla (Scale 50µm) f. high degree of desquamation of horn cells g. thick layer of the flattened horn cells (SC) and undistinguished granular, spinosal and basal layers (adipose-like cells) (arrows). Sinusoid-like capillaries located at papillary body (BV) (Scale 100µm) h. large sized granular cells and accumulation of lymphocyte-like cells adjacent to the basal cells (arrows). (Richardson solution) and (H and E) (Scale 25µm)

Sheep fed hay ad libitum and concentrate-diet for 12 weeks: Although the total thickness of the epithelium was within the same range of sheep fed concentrate-diet for 2, 4 and 6 weeks (7-10 cell-layers) (Table 8 and Fig. 8 h and 21), but the thickness of the epithelial layers showed a remarkable differences (table 9). The thickness of stratum spinosum was decreased to one cell-thick layer, however, the thickness of stratum corneum was markedly increased (2-5 cell-thick) as shown in table 9.

a. Stratum corneum: It was distinct thick layer (2-5 cell-thick layer) and composed of either flattened horn cells (at both the lateral sides and the apex of the papillae), or large superficial vacuolated cells and deep discontinuous flattened corneal layer. Flattened, damaged cells on the outer part of the stratum corneum were frequently seen (Fig. 21 c, f). Histological examinations of stratum corneum of one animal in this group revealed parakeratosis. The horn cells formed a cap over the apex of many papillae and showed accumulation of lymphocyte-like cells (Fig 21 d).

Moreover, isolated microabcess was also observed in the stratum corneum covering one side of the papilla (Fig. 21 e). In some instances severe desquamation of horn cells at the lateral sides of the papillae and at the apex of the papillae was more remarkable. This abnormal degree of desquamation of the surface epithelium led to the exposure of the stratum granulosum or spinosum to the lumen of the rumen (Fig. 21 f). A lot of ingesta or microflora were related to the superficial horn cells (Fig. 21 a)

b. Stratum granulosum: Distinct layer (2-3cell-thick layer) of large vacuolated or flattened granular cells. The superficial cells contained larger keratohyaline granules than the deep one (Fig.21 b, h).

c. Stratum spinosum: A thin one cell-thick layer of large polyhedral cells with wide intercellular space (Fig.21 a, b). An abnormality of this layer was observed in one animal (adipose-like cells), where the spiny cells included large vacuoles (Fig. 21 g).

d. Stratum basale: One cell-thick layer, of very large pyramidal cells with wide intercellular space. The size of the basal cells was larger than that of all other treated groups (Fig. 21a). Accumulation of lymphocytes-like cells at the lamina propria connective tissues adjacent to or penetrating the stratum basale had been also seen (Fig. 21 h).