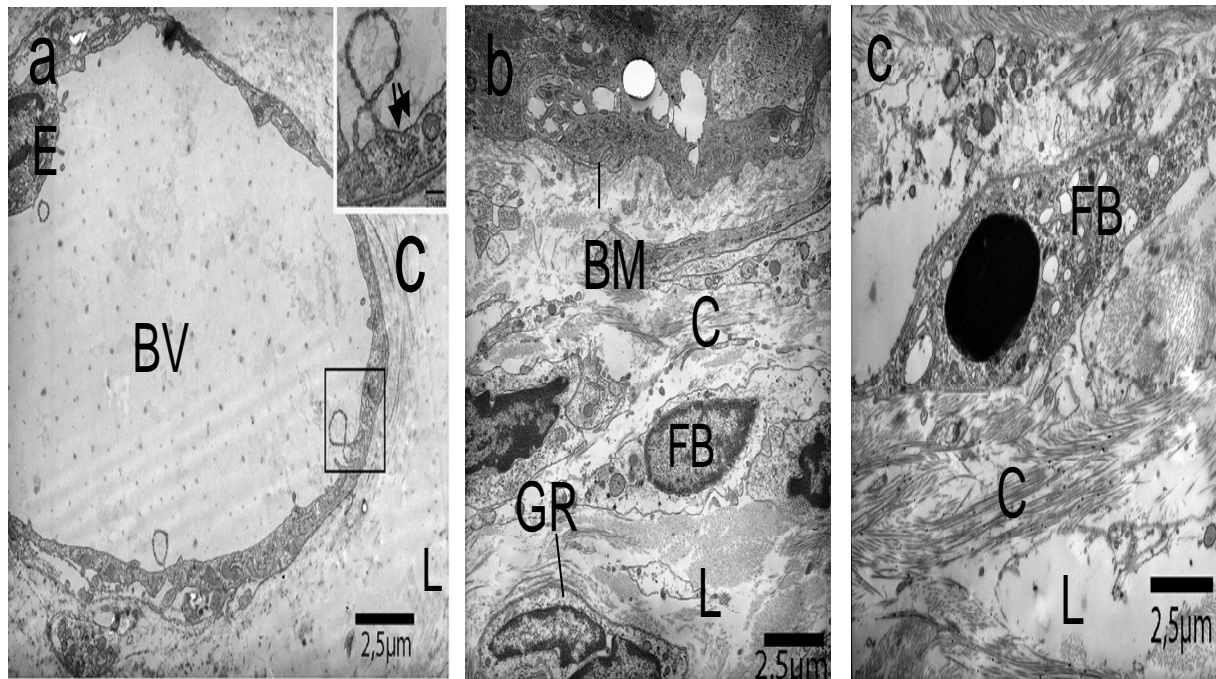


### 3.4 Ultrastructural findings

There were no indications of regional differences in the fine structure of epithelial cells from papillae or interpapillary regions. However, there were some morphological differences detected among the different sheep groups.

#### 3.4.1 Lamina propria

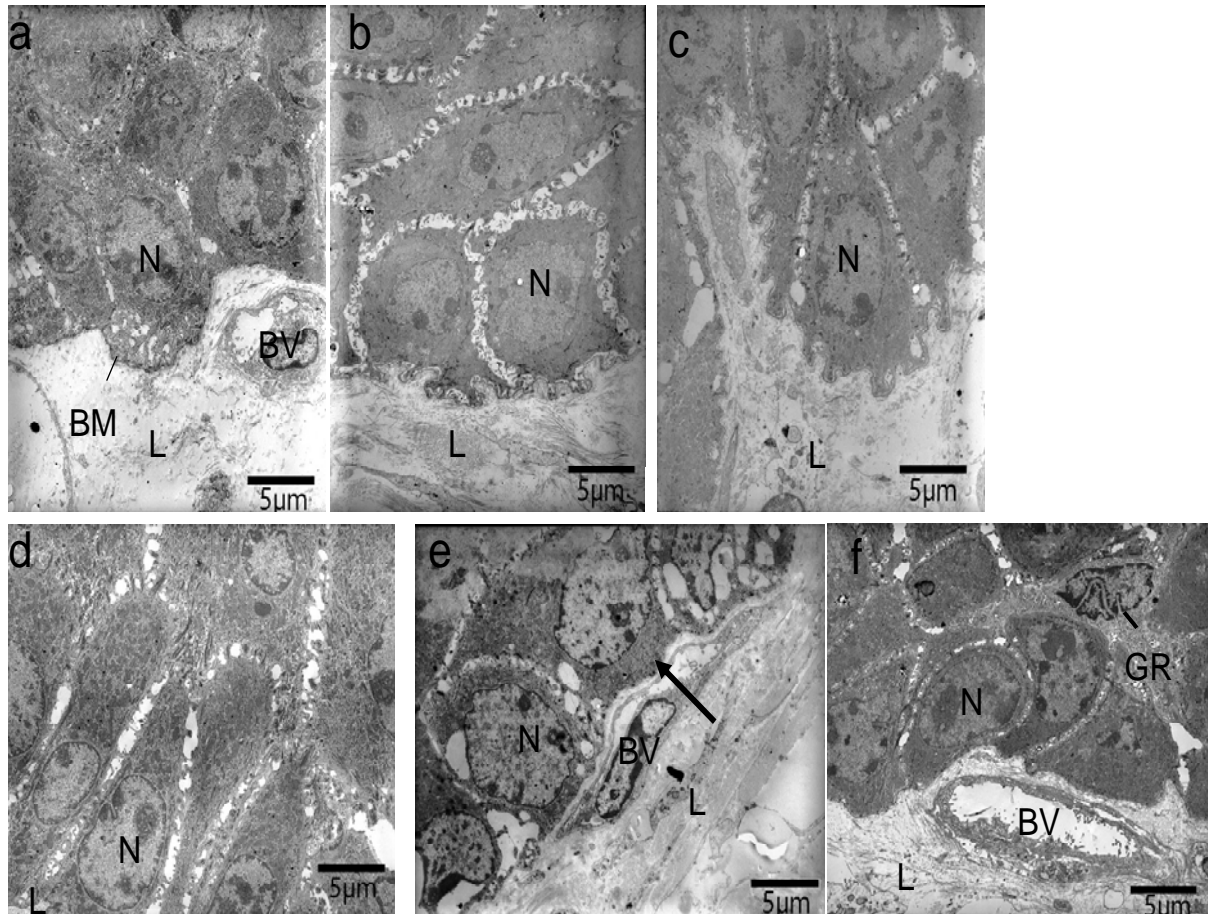
**Hay-fed group:** Lamina propria appeared electron lucent and was composed of a loose network of collagen fibres (fig. 34a).



**Fig. 34: Rumen-lamina propria (L)** a. hay fed-group: The capillary endothelium appeared to have various thicknesses endothelial cell (E), numerous micropinocytotic-like vesicles (→). The lamina propria contained fine collagenous fibres (C) b. 6 weeks concentrate-fed group: Lamina propria composed of a network of collagen fibres (C), fibroblast (FB) and granulocyte (GR). Basal lamina of the epithelium (BM) c. 12 weeks concentrate-fed group: Dense collagenous fibres (C) and large fibroblast (FB) were present. (Scale 2.5 $\mu$ m)

The vascular system was located below the basal lamina (fig. 35a) and in most instances the distance from a basal epithelial cell to a capillary was in the range of 0.72-2.0 $\mu$ m. The

capillary endothelium was varying in thicknesses. Numerous micropinocytotic vesicles were observed in the endothelial cytoplasm (fig. 34a). Interdigitation of lamina propria with the stratum basale (papillary body) was present (fig. 36a).



**Fig. 35: Stratum basale and Lamina propria (L) of different sheep groups** a. short columnar basal cells (hay-fed group) b. wide columnar basal cells (2 weeks concentrate-fed group) c. large pyramidal or columnar basal cells (4 weeks concentrate-fed group) d. thin, large pyramidal or columnar basal cells (6 weeks concentrate-fed group) e. swollen basal cells with large round electron lucent nuclei (N) and f. many basal cells were present with long axis parallel to the basal lamina (→). Notice: the blood vessels (BV) were located in close vicinity of the basal epithelial cells (12 weeks concentrate-fed group). Nucleous (N). (Scale 5 $\mu$ m)

**Concentrate-fed groups:** The lamina propria was composed of a dense network of collagen fibres which was more obvious in 6 and 12 weeks concentrate-fed groups (fig. 34 b and c). In 2, 4 and 6 weeks concentrate-fed groups, the distance from a basal epithelial cell to a capillary was in range of 0.80-0.84  $\mu$ m, 0.60-0.80  $\mu$ m and 0.70-0.75  $\mu$ m, respectively. In 12 weeks

concentrate-fed group, the vascular system was located short distance from basal epithelial cells (0.04-0.20  $\mu\text{m}$ ) (fig. 35e). Many capillaries contained granulocytes (fig. 34 b).

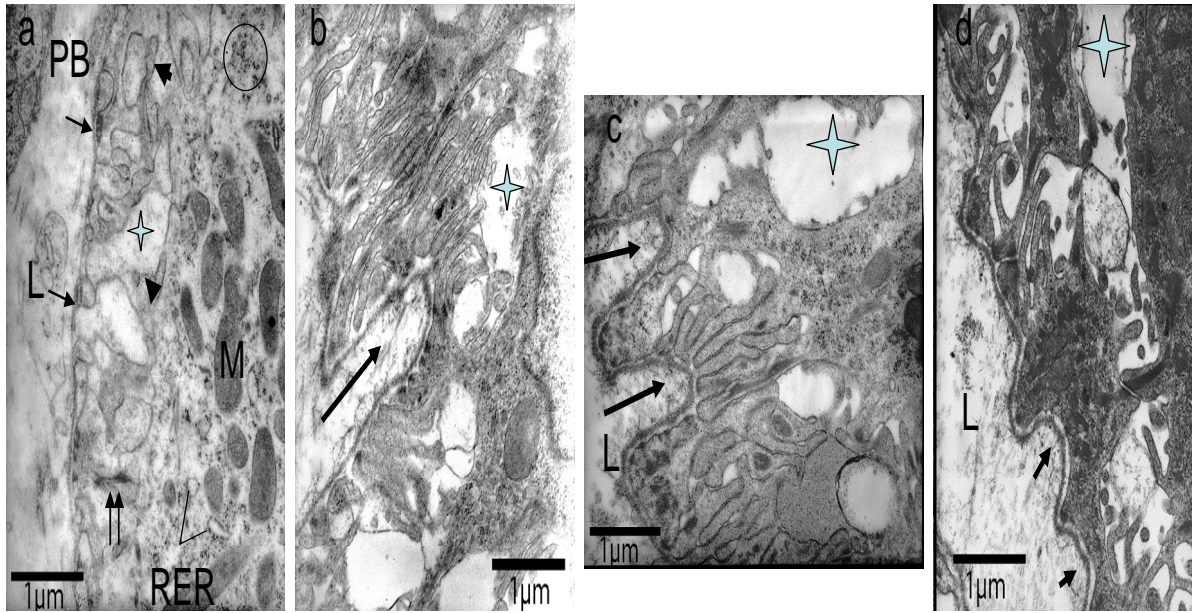
## 3.4.2 Epithelium

### 3.4.2.1 Stratum basale

**Hay-fed group:** The basal lamina was a moderately electron dense structure and did not always follow the basal cell membrane. Instead, the lamina followed a line demarcated by the tips of the processes. The membrane of basal cells adjacent to the basal lamina was convoluted. Hemidesmosomes were observed along the basal cell membrane facing the basal lamina and in irregular distances (fig. 36a). Basal cells were either cuboidal or short columnar with long axes perpendicular to the basal lamina (fig. 35a and 37a). Basal cells were characterized by a large ovoid nucleus, numerous mitochondria with dark staining matrix concentrated at the apical part of the cells, clusters of ribosome, rough-surface endoplasmic reticulum, Golgi apparatus, large vesicles, and small amounts of fine filaments (tonofilaments), which were randomly oriented (fig. 36a and 37a ).

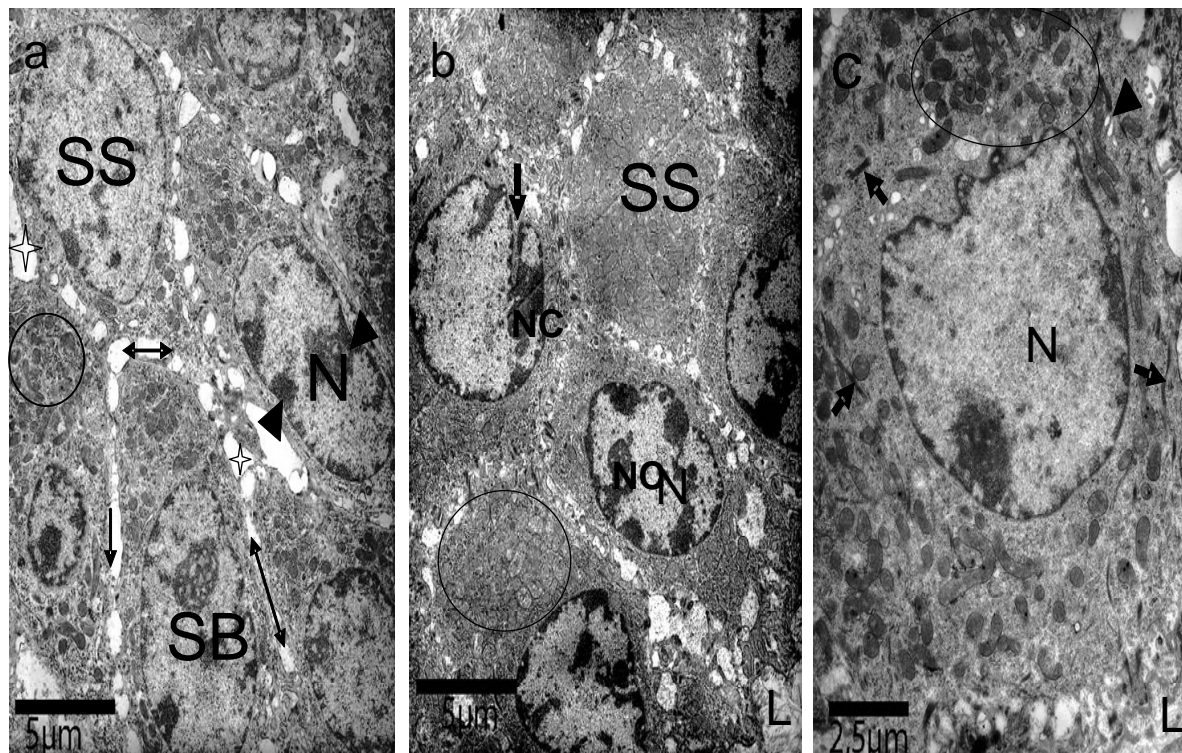
**Concentrate-fed groups:** 2 or 4 days after the addition of concentrate to the diet, the ultrastructural examination of the stratum basale was similar to that of hay-fed group. In 1 or 2 weeks concentrate-fed group, the epithelium showed short columnar basal cells with numerous, light staining mitochondria which were more concentrated at the apical part of the basal cells (fig. 37b).

The epithelial basal cells showed large round electron dense nuclei with prominent nucleoli. These nuclei showed more irregular nuclear membrane and peripheral dark electron dense area than that of hay-fed group (fig. 37b). In 4 weeks concentrate-fed group, similar changes to the previous two concentrate-fed groups were occurred. However, large pyramidal or columnar basal cells with large round, light electron dense nuclei (few dark electron dense area) and nucleoli were observed.



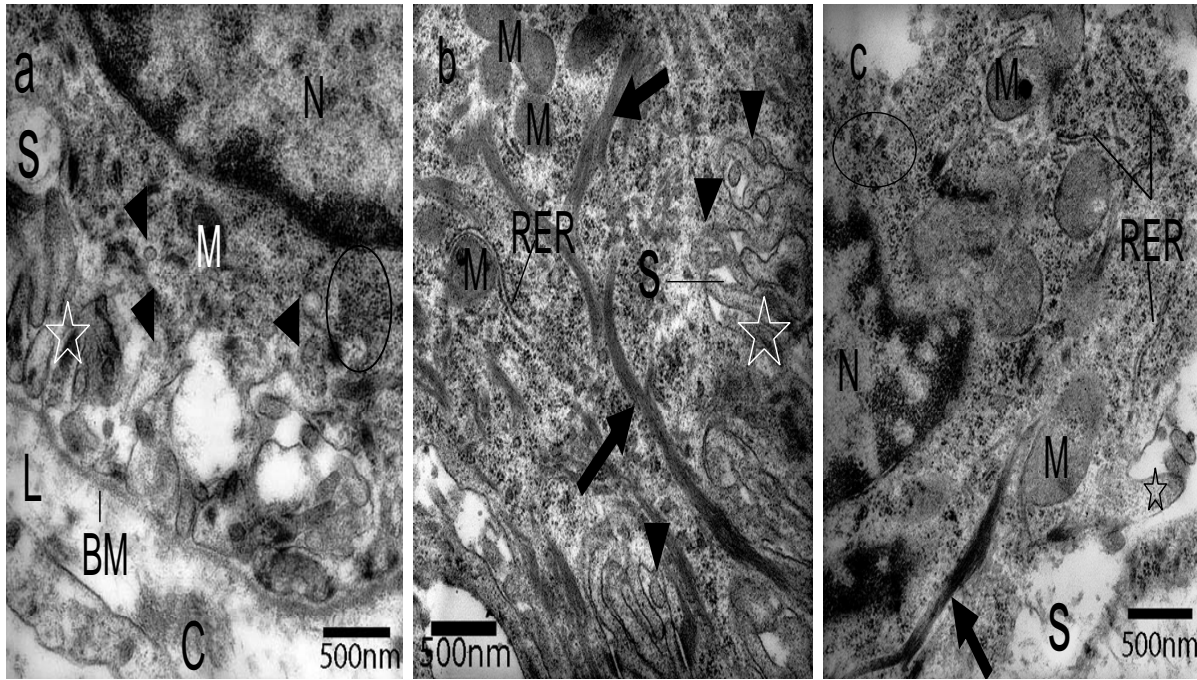
**Fig. 36: Proximal surface of the stratum basale and lamina propria (L).** Notice: a. the proximal membrane of the basal cell was convoluted, interdigitation of lamina propria with the stratum basale (PB), hemidesmosomes ( $\rightarrow$ ), Dilated basal spaces filled with amorphous substance (\*), numerous mitochondria (M), clusters of ribosome (O), rough-surface endoplasmic reticulum, membrane coating granules (arrow head), and small amounts of tonofilaments (arrows) (hay-fed group) b. The numerous, thin basal labyrinth of cell processes and the deeply, folded basal lamina ( $\rightarrow$ ) (4 weeks concentrate-fed group) c. The numerous, wide basal labyrinth of cell processes and the deeply, folded basal lamina ( $\rightarrow$ ) (6 weeks concentrate-fed group) d. less developed microvillus-like projections of the basal cell membrane with wide basal spaces and less folded basal lamina ( $\rightarrow$ ) (12 weeks concentrate-fed group). (Scale  $1\mu\text{m}$ )

The epithelial basal cells showed high density of mitochondria of the both apical and basal part of cells (fig. 37c). The membrane of the basal cell facing the basal lamina, present well developed, numerous, thin basal labyrinth of cell processes and deeply, folded basal lamina (fig. 36 b). In 6 weeks concentrate-fed group, the basal cells were similar to those of 4 weeks concentrate-fed group, being large pyramidal or columnar basal cells. However, they were longer with a larger nucleus (fig. 35 d).



**Fig. 37: Stratum basale and stratum spinosum** a. numerous mitochondria (O). The wide intercellular space (↔) desmosomes (→) and inter desmosomal space (\*) and the big rounded nuclei with prominent nucleoli (hay-fed group) (Scale 5 $\mu$ m) b. nuclei (N) with heterochromatin and irregular nuclear membrane (→) (2 weeks concentrate fed-group) (Scale 5 $\mu$ m) c. large, light, electron dense nucleus (N) with irregular nuclear membrane, dense populations of mitochondria (O), Golgi apparatus (arrow head) (→) and increasing amount of fibril material (→). Nucleoli (Nc). (4 weeks concentrate fed-group) (Scale 2.5 $\mu$ m)

Compared to the hay-fed group, the basal cells in concentrate-fed groups were characterized by a high density of mitochondria, cytoplasm rich with ribosome and rough endoplasmic reticulum, membrane coating granules and the appearance of very thick tonofibrils bundles (fig. 38b). Moreover, the proximal membrane of the basal cell present well developed numerous, thin basal labyrinth of cell processes, which were narrow in between spaces (fig. 36b and 38b). Deep folded basal lamina was more evidence than that present in the 4 weeks concentrate-fed group (fig.36c).

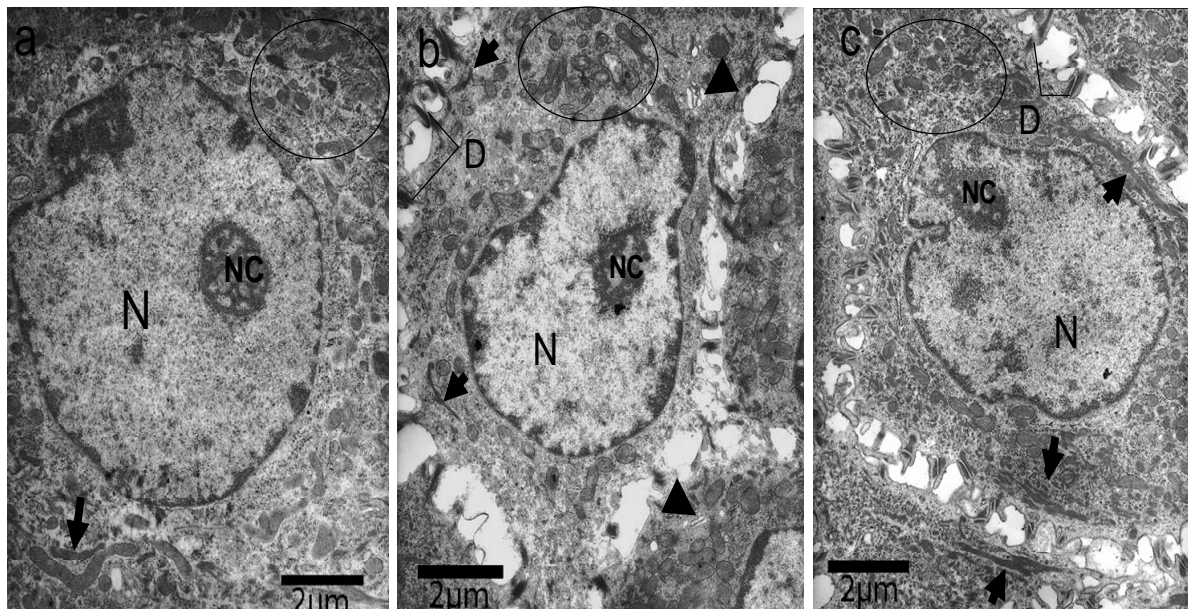


**Fig. 38: High magnification of the proximal part of the basal cells, showed: part of nucleus (N), membrane coated vesicles (arrow heads), and clusters of ribosome (O), basement membrane (BM) and numerous mitochondria (M). Notice: a. the wide spaces between microvilli-like projections (S) (4 weeks concentrate fed-group) b. the extensive wide, proximal projections (\*) with narrow in between spaces (S), high density of rough endoplasmic reticulum (RER) and thick tonofibrils bundles (→) (6 weeks concentrate fed-group) c. short, wide proximal projections (\*) with wide in between spaces (S), thick tonofibrils bundles and high density of rough-surface endoplasmic reticulum (RER) (12 weeks concentrate fed-group). (Scale 500 nm)**

12 weeks after the addition of concentrate to the diet, the basal cells were swollen and oriented with long axis parallel to the basal lamina (fig. 35e). The proximal portion of the basal cell membrane of these cells exhibited short, wide basal processes and wide in between spaces (fig. 36d and 38c). Examination with the electron microscope revealed that the swollen cells have a dense cytoplasm, rich in a lot of free ribosomes and rough endoplasmic reticulum. They contained thick tonofibrillar bundles which were more obvious at the proximal part of the cells (fig. 38c).

### 3.4.2.2 Stratum spinosum

Hay-fed group: Composed of spiny cells, their long axes lie parallel to the basement membrane. The spiny cells present in two layers: parabasal or deep cells and superficial cells. The parabasal cells bordering the basal layer were nearly polygonal or spherical cells with large rounded nuclei and prominent nucleolus and many finger-like processes projected from their surfaces. However, the superficial spiny cells, bordering the granular layer, were more flattened. Cells in the stratum spinosum contained ribosomes, rough endoplasmic reticulum, Golgi apparatus and more filamentous material than cells in the stratum basale. Small bundles of thin tonofibrils (0.08-0.10  $\mu\text{m}$  thick) were seen running randomly throughout the cytoplasm; with no defined association with desmosomes (fig.39a and 40a).



**Fig. 39: Spiny cells characterized by electron lucent nucleus (N) with irregular nucleoplasm and prominent nucleoli (NC), electron dense mitochondria distributed all over the cell (O). Notice: a. large spiny cell with large nucleus (hay-fed group) b. well developed Golgi apparatus (arrow head) (4 weeks concentrate-fed group) c. thick tonofilaments bundles (→) located peripherally (6 weeks concentrate-fed group). Note the distribution and structure of spot desmosomes (D) (Scale 2 $\mu\text{m}$ )**

The membrane-coated vesicles were regularly observed in the peripheral cytoplasm spiny cells especially at the upper part of the most superficial cells boarding to the stratum granulosum (fig. 42c).

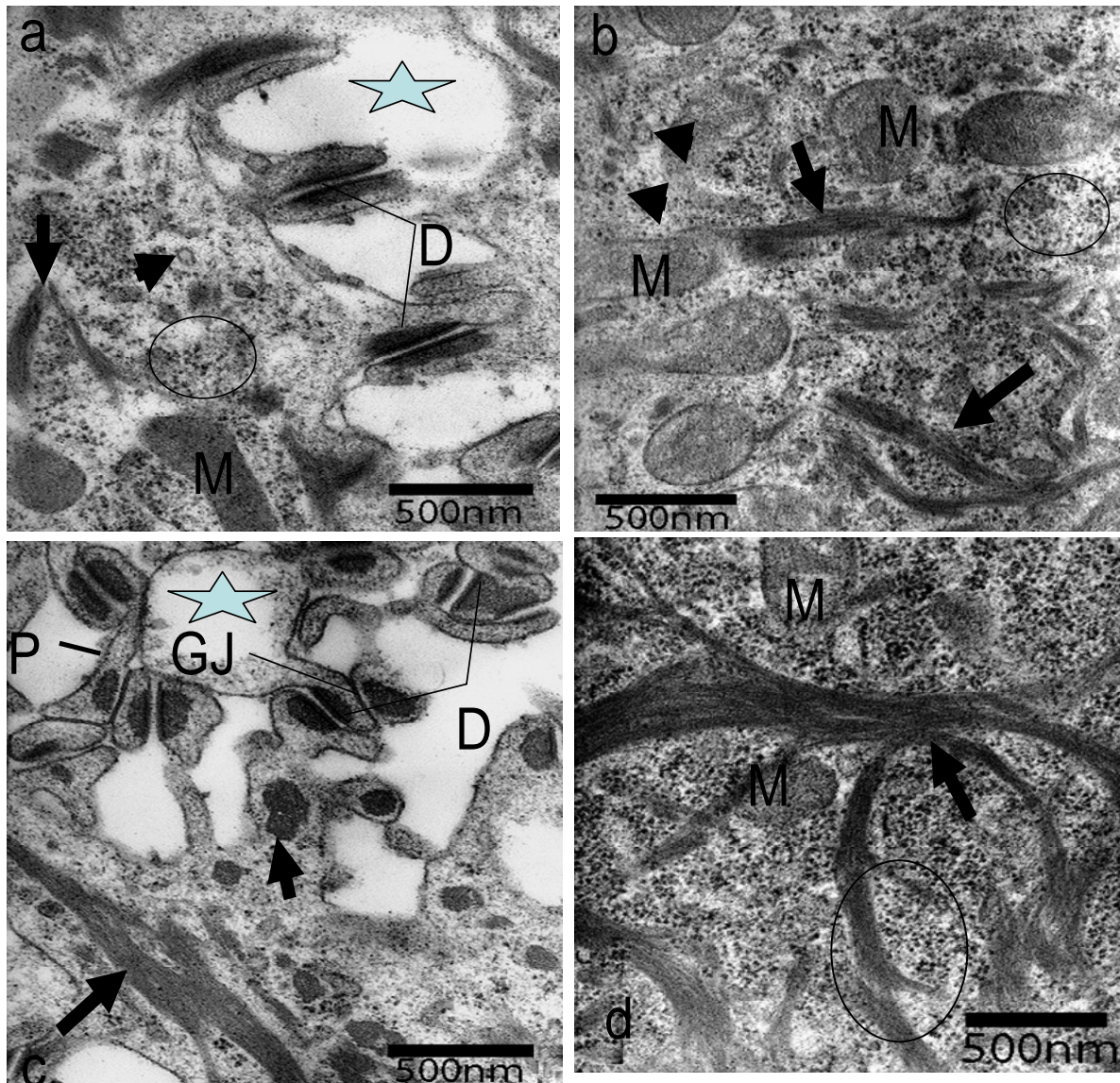
Moreover, high concentrations of mitochondria, with different size, which were not oriented in any particular plane, were found.

**Concentrate-fed groups:** 2 and 4 days after the addition of concentrate to the diet, the ultrastructural examination of the epithelium in this stratum revealed a picture similar to that of hay-fed group (control). 1-2 weeks after the addition of concentrate to the diet, the epithelium showed more flattened spiny cells with more filamentous material (0.10-0.13  $\mu\text{m}$  thick). Cells in the stratum spinosum rich with mitochondria were more obvious in 2 weeks concentrate-fed group (fig. 37 b). In sheep fed concentrate for 4 weeks, an increasing thickness of tonofilament bundles (0.09-0.20  $\mu\text{m}$  thick) and the development of Golgi apparatus were detected in most cells (fig.39b and 40b). However, the development of tonofilament bundles attached to desmosomes was rather poor. In sheep fed concentrate for 6 weeks, electron microscopy defined thick tonofilaments (0.10-0.32  $\mu\text{m}$  thick) bundles attached to desmosomes and ribosome's-rich stratum spinosum (fig.40 c). In sheep fed concentrate for 12 weeks, the ultrastructure examination revealed similar character of spiny cells of 6 weeks concentrate-fed group. However, the presence of small keratohyaline granules and the appearance of the bundles was more pronounced, which measured approximately 0.08-0.32  $\mu\text{m}$  (fig. 40d and 41a). Moreover, microabscess was found between spiny cells (fig. 42b).

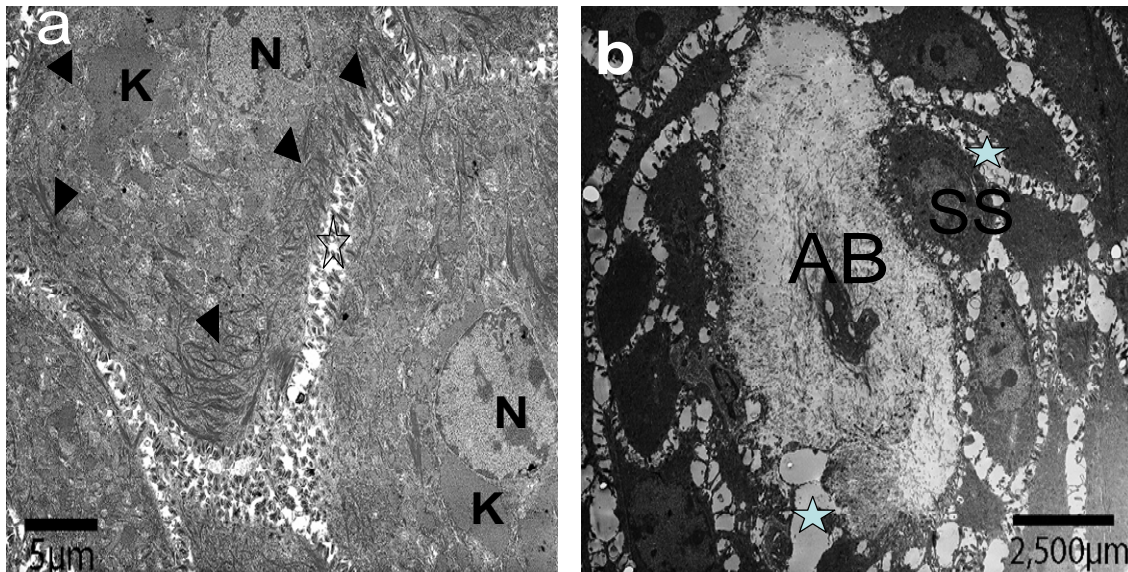
### 3.4.2.3 Stratum granulosum

**Hay-fed group:** The cells of the granular layer contained irregular accumulations of keratohyalin granules, 0.5-0.8  $\mu\text{m}$  in diameter. The deep layer of granular cells (type-A) contained a few keratohyalin granules of varying size, mostly small. However, the superficial granular cells (type-B), boarding to the stratum corneum, were larger in size and contained large keratohyaline granules and more filamentous materials (Fig.42). Cytoplasm of the granular cells appeared moderately electron dense and contained beside the nucleus, tonofibrils, keratohyaline granules, membrane-coated vesicles, and mitochondria (Fig.42). Tonofibrils were in close association with desmosomes and keratohyaline granules (fig. 42b). The membrane-coated vesicles were observed in the peripheral cytoplasm of granular cells especially at the lower part of the deep granular cells boarding to the stratum spinosum (fig. 42c). The cell membranes and intercellular regions showed very marked indentations (fig. 42c).



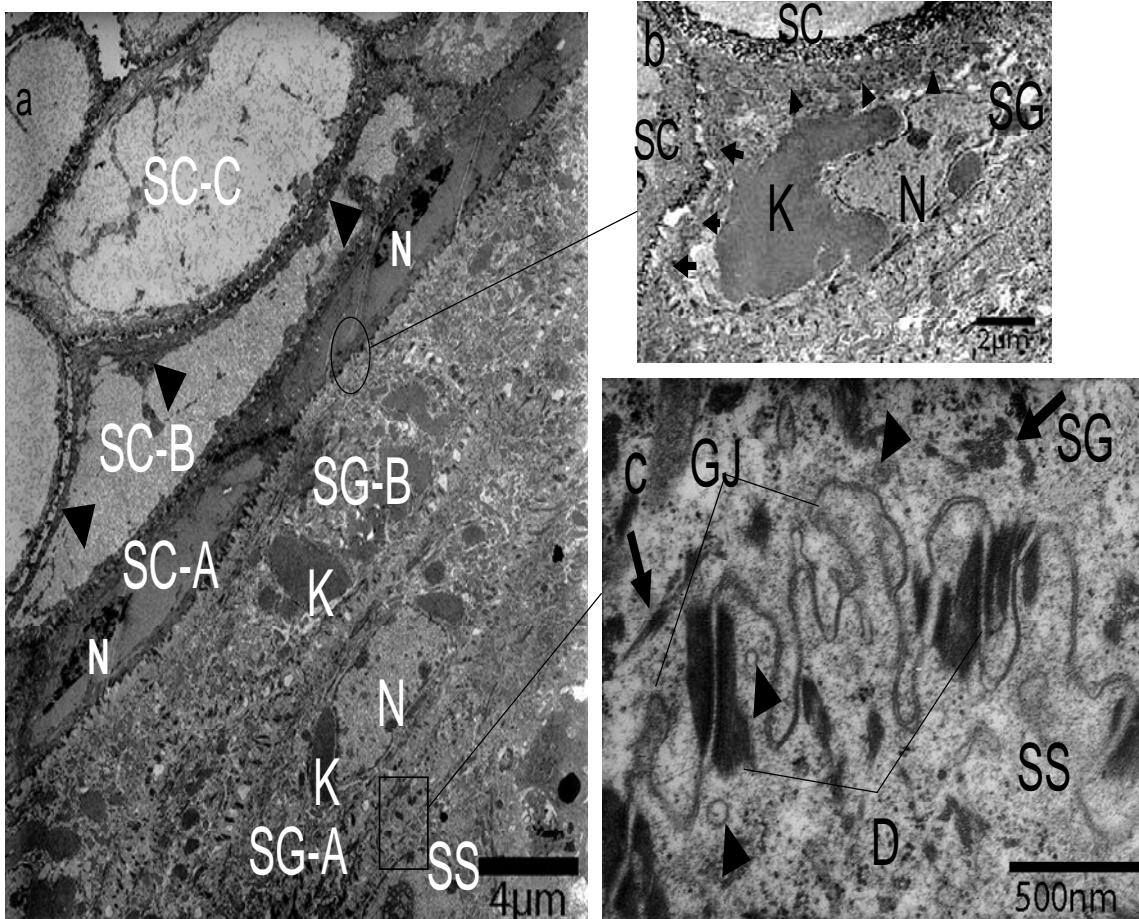


**Fig. 40: Peripheral cytoplasm of spiny cells present mitochondria (M), ribosomes (O), coated vesicles (arrow head).** Notice: a. Small bundles of tonofibrils with no association with desmosomes (hay-fed group) b. Medium sized-tonofilament bundles (4 weeks concentrate-fed group) c. Thick tonofilament bundles and branched finger-like projections (P) carrying desmosomes (D), inter desmosomes-gap junctions (GJ) (6 weeks concentrate-fed group) d. peripheral cytoplasm rich with very dense ribosomes (O) and increased density of thick tonofilament bundles (→) (12 weeks concentrate-fed group). (Scale 500nm)

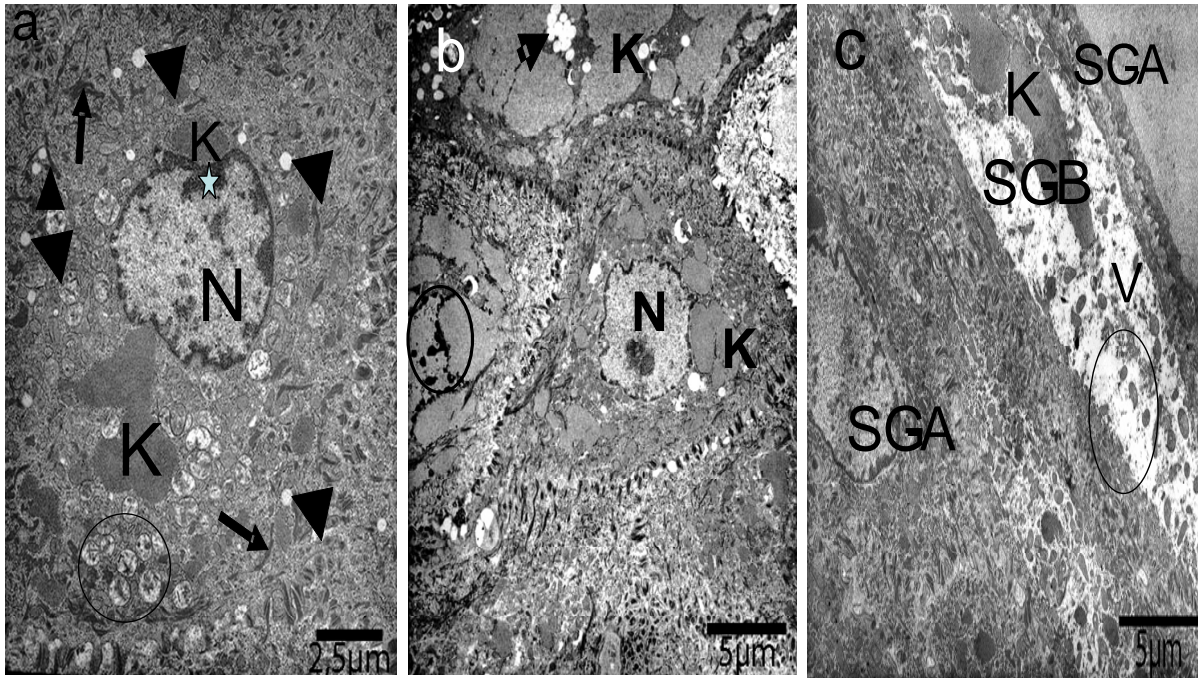


**Fig. 41: Section from stratum spinosum (SS) (12 weeks concentrate-fed group), characterized by a. the presence of small keratohyaline granules (K), very thick tonofilament bundles (arrow head). The wide intercellular space (\*) (Scale 5µm) b. The wide intercellular space contained microabscess (AB). Note: connective tissue fibers surrounding the foreign material. (Scale 2.5µm)**

**Concentrate-fed groups:** 2 and 4 days after the addition of concentrate to the diet, the stratum granulosum showed no large morphological changes from hay-fed group. However, the granular cells containing medium-sized keratohyalin granules were observed. In 1-2 weeks concentrate-fed group, the granular cells were rich in degenerated mitochondria, tonofibrils and, membrane coating vesicles (MCG) and small lipid droplets (fig. 43a and 44a). 4 weeks after the addition of concentrate to the diet, the increase of both size and numbers of irregular, light stained keratohyalin granules (0.40-5.50 µm) in diameter was observed (fig. 43b and 44b). The granular cells included accumulation of small lipid droplets; increasing thickness of tonofilament bundles, numerous membrane coating granules (MCG), degenerating mitochondria and keratin aggregates. 6 weeks after the addition of concentrate to the diet, the appearance of cytoplasmic vacuoles, light stained keratohyalin granules (1.60-5.20 µm), and tonofilament bundles were more pronounced (fig. 43c and 44c). In 12 weeks concentrate-fed group, swelling of the area of the cytoplasm of the stratum granulosum in close proximity to the stratum corneum was noticed (fig. 44d). Thick tonofilament bundles were also present.



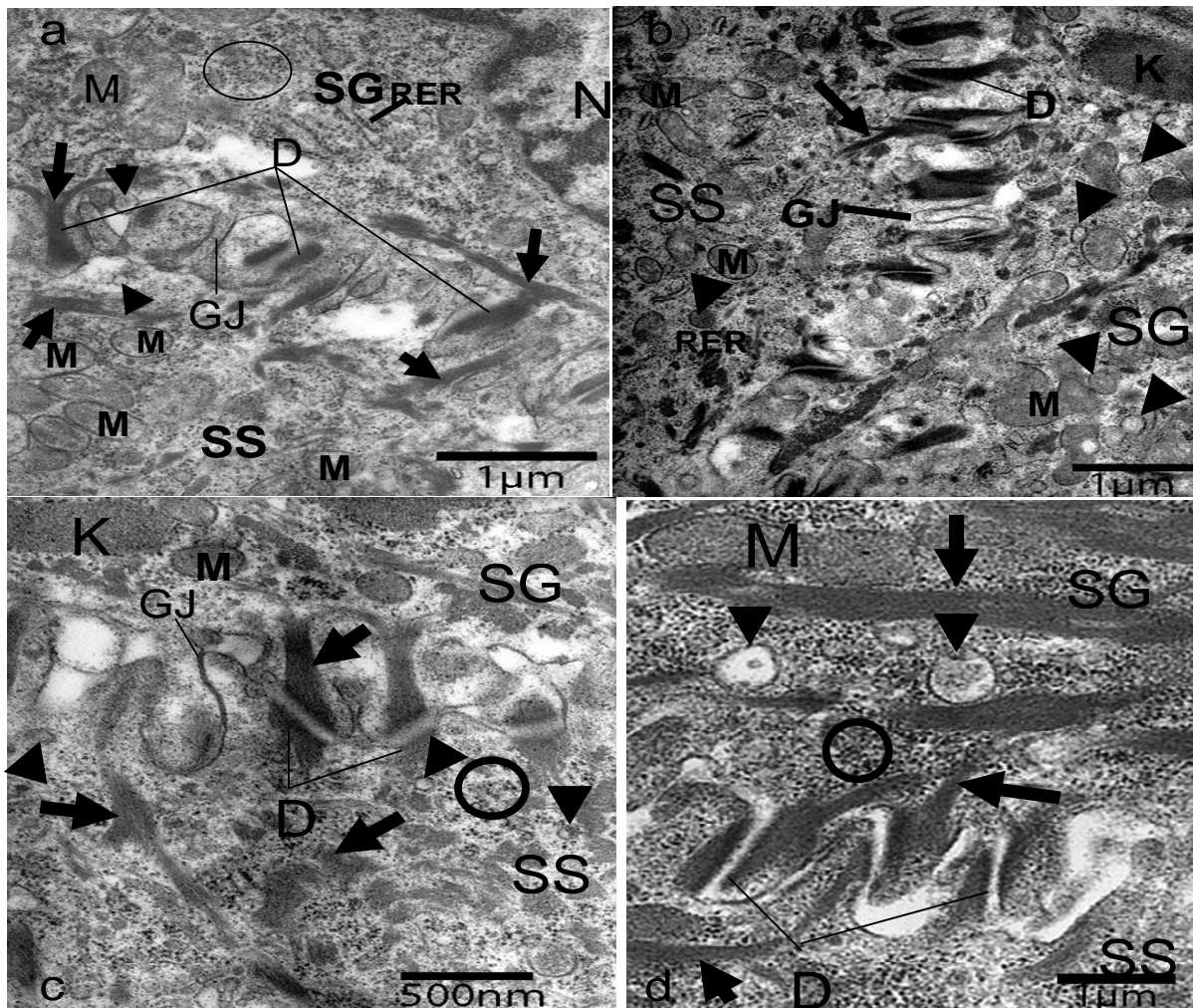
**Fig. 42:** a. Rumen epithelium (hay-fed group). Stratum granulosum type A (SG-A) with few keratohyalin granules (K), while those bordering the horn cells (SG-B), were more flattened and contained large keratohyaline granules. Stratum corneum (SC-A), cells were plate shaped, electron-opaque and present degenerating nucleus (N), while others superficial ones (SC-B) and (SC-C), were distended by a central vacuole. Note: the B-type horn cells were disc-shaped with osmophilic keratin (arrow head) (Scale 4µm) b. stratum granulosum-stratum corneum-junction (O). Condensations of filamentous materials at the upper peripheral cytoplasm adjacent to horn cell (→) (Scale 2 µm) c. stratum spinosum (SS)-granulosum (SG) - Junction ( ). The cell membranes is highly indented and present desmosomes (D), gap junctions (GJ) and membrane-coated vesicles (arrow head) located at both sides of the membranes. (Scale 500 nm)



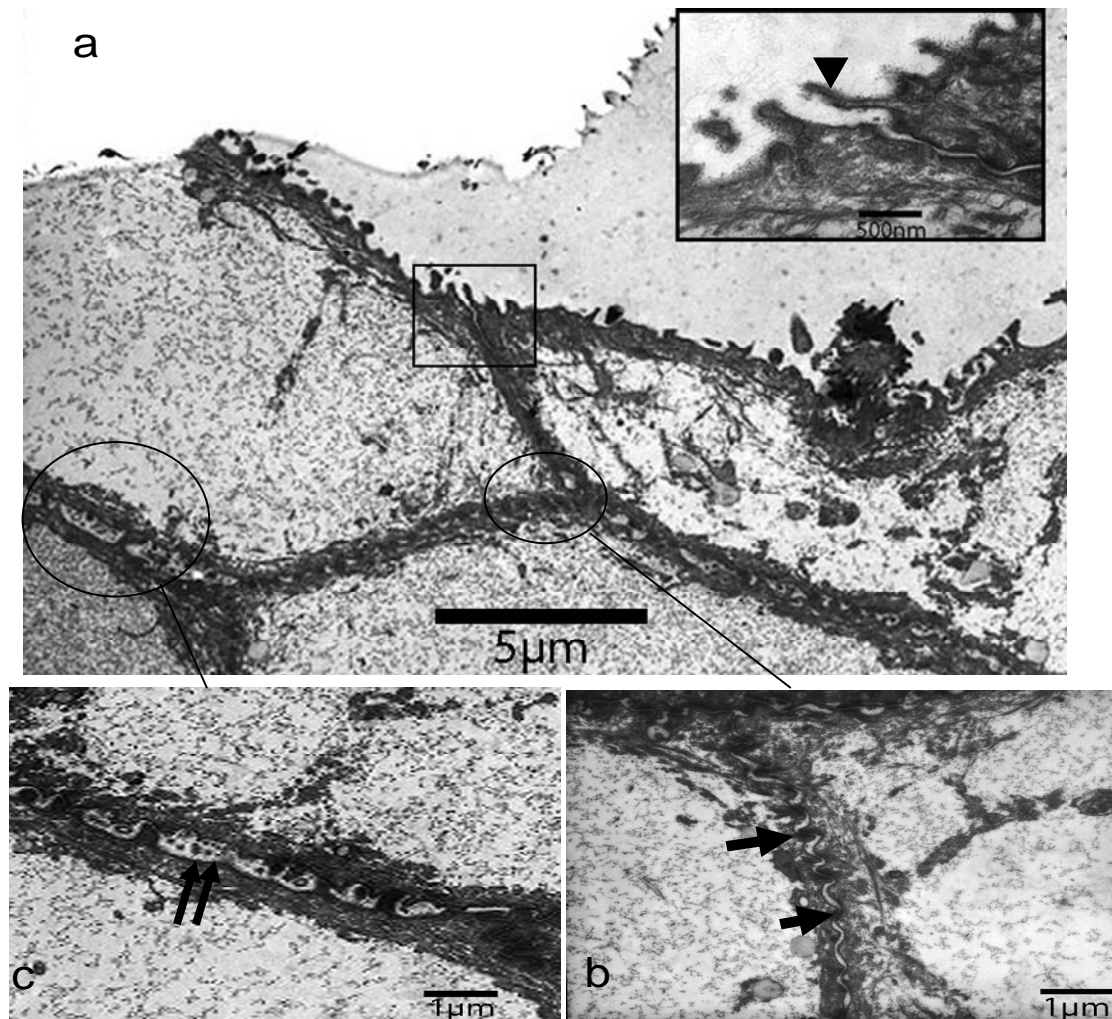
**Fig. 43: Stratum granulosum:** a. large number of degenerated mitochondria (O), numerous, small lipid droplets (head arrow); irregular shaped-keratohyalin granules (K) and thin filament bundles (→). Nucleus (N) showed heterochromatin (\*) (2 weeks concentrate-fed group) (Scale 2.5 $\mu$ m) b. large sized light stained keratohyalin granules (K), accumulation of small lipid droplets (→) and cytoplasmic keratin aggregates (arrow head) (4 weeks concentrate-fed group) (Scale 5 $\mu$ m) c. swollen granular cell-type B (SG-B) located between corneal cell (SC-A) and granular cell type A (SG-A), present large vacuole (V), retained degenerating mitochondria (O), and large keratohyaline granules (K) (6 weeks concentrate-fed group). (Scale 5 $\mu$ m)

#### 3.4.2.4 Stratum corneum

**Hay-fed group:** The stratum corneum was characterized by several layers of electron dense cells. Some of these were plate-shaped and electron-opaque (horn cells of type A and B) and others were distended by a central vacuole (voluminous ballon-shaped type C). The A-type horn cells were present in a simple but not always fully developed layer of plate shaped cells.



**Fig. 44: stratum spinosum (SS) granulosum (SG) - Junction. Highly indented membranes with desmosomes (D) and gap-junctions (GJ). Nucleus (N) keratohyaline granules (K) mitochondria (M), ribosomes (O), rough endoplasmic reticulum (RER), tonofilament bundles (→) and related membrane-coated vesicles (arrow head) which were observed in a. 2 weeks treated-group (Scale 1 $\mu$ m) b. 4 weeks concentrate-fed group (Scale 1 $\mu$ m) c. In 6 weeks concentrate-fed group, Thick tonofilament bundles (→) were strongly attached to desmosomes (Scale 500nm) d. In 12 concentrate-fed group, very thick tonofilament bundles (→) were attached to desmosomes. Large membrane-coated vesicles (arrow head). (Scale 1 $\mu$ m)**



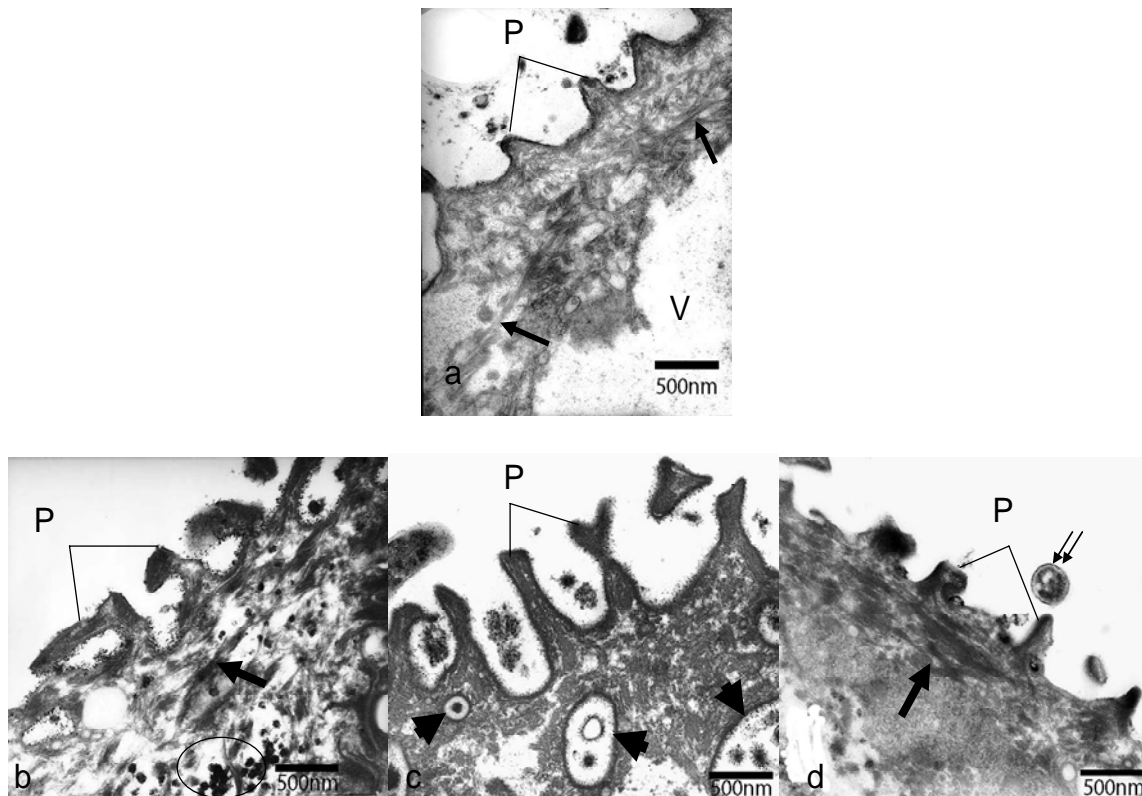
**Fig. 45: Stratum corneum (hay-fed group)** a. The intercellular space between lateral horn cells was narrower than that present between the apical and basal membrane of adjacent horn cells. The thickened plasma membrane (luminal surface of the rumen epithelium) projected as cellular processes which were covered by a fine fibrillar material (Glycocalyx) (arrow heads). (Scale 5µm) b. the lateral surface of these cells was deeply infolded and studded with desmosomes (→) (Scale 1µm) c. the intercellular space between the apical and basal membrane of adjacent horn cells was dilated and contained dense material (arrows). (Scale 1µm)

These cells were filled with osmophilic keratin and generally found closer to the stratum granulosum. The B-type horn cells were disc-shaped with osmophilic keratin and the striped, spongy keratin constituted a wide marginal zone and the internal space appeared to be loosened and of a diffuse light grey colour (fig. 42a). Within the dense cytoplasm, filaments and electron dense bodies devoid of membrane were observed routinely. However, nuclei or their remnants and lysosome-like structures or membrane-coated vesicles were not observed.

The membrane of the horn cell was modified to form finger-like projections which were covered by an amorphous fuzzy coating (glycocalyx) (fig. 45a). Large number of microorganisms was found over the surface of the epithelium.

Concentrate-fed groups: Differences from hay-fed group (Control group) were noticed within 2-12 weeks after the addition of concentrate to the diet. The stratum corneum consisted of several cell layers which showed considerable variations in their cell constituents. Generally, horn cells contained heavy accumulation of thick tonofilament bundles which were more obvious after 6 weeks of the addition of concentrate to the diet (fig. 46c).

Cytoplasmic projections (glycocalyx) increased gradually in size, reaching its maximum size in 4 to 6 weeks concentrate-fed groups (fig. 46b and c). Moreover, in 6 weeks concentrate-fed group, horn cells contained heavy accumulation of thick amorphous cytoplasmic material or dense band at the periphery of the horn cells. The most luminal horn cells exhibited large cavities (lysosome-like structures or membrane-bound granules) and contained numerous types of bacteria. These cavities were lined with a membrane and often covered a complex system of projections as shown in 6 weeks concentrate-fed group (fig. 46 c). After 12 weeks of feeding concentrate, the retention of nuclei and fibrous network in stratum corneum was observed. Many areas of the stratum corneum were detached from the underlying tissue (fig. 47). Sometimes, bacteria were noted in the intercellular space between the most superficial cells. Moreover, the luminal surface of this stratum showed a few, small cytoplasmic projections (fig. 46 d)



**Fig. 46: Luminal surface of the rumen-epithelium and related microflora (double arrows).** a. short cell projections (P), a part of a large vacuole (V) and denser peripheral cytoplasm containing fine fibrillar material (→) (hay-fed group) b. narrow, long finger-like projections (P) and accumulation of some black granule (O) between the fibrillar material (→) (4 weeks concentrate-fed group) c. well developed finger-like projections (P), secondary lysosomes containing deposit material (arrow heads) in the peripheral dense fibrillar material (6 weeks concentrate-fed group) d. short cell projections (P), and dense fibrillar material (→) (12 weeks concentrate-fed group). (Scale 500nm)

### 3.4.3 Intercellular relationships

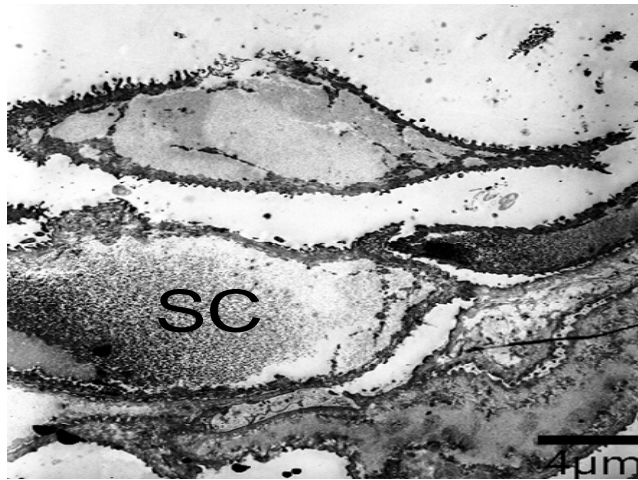
**Hay-fed group:** In stratum basale, the intercellular space surrounding the distal and lateral aspects of basal cells was dilated. The intercellular space between the basal cells had desmosomes and interdesmosomal spaces, which was connected with the vacuoles present between the cytoplasmic processes of the basal membrane and the basal membrane. Desmosomes intermittently arranged along the wavy cell membranes (fig. 37a). In stratum spinosum, the intercellular space was wide at this level. However, in the most superficial layer, the spiny cells became more compactly arranged and the intercellular space was



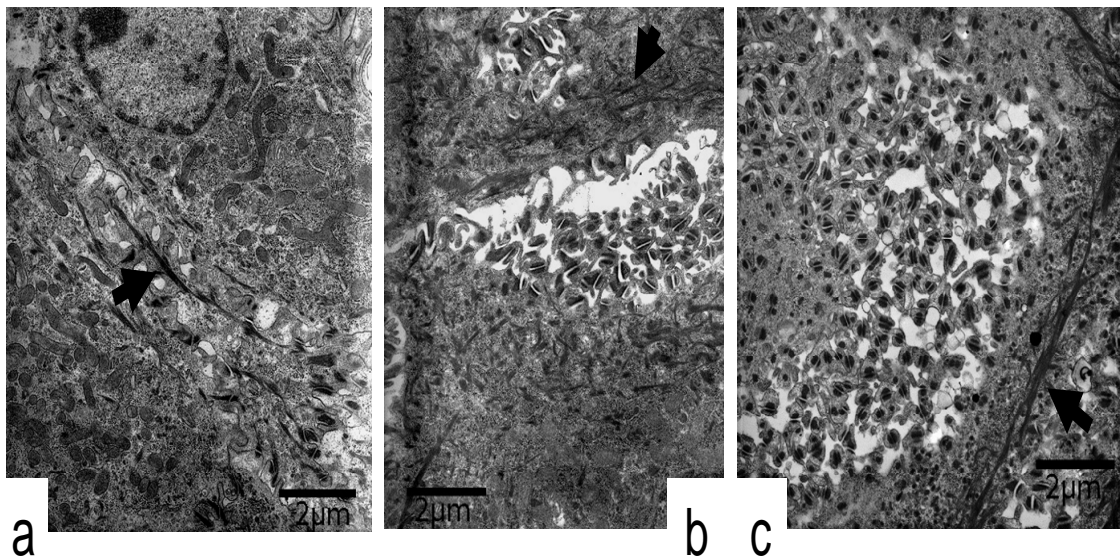
reduced. Desmosomes and gap-junctions were present between processes from adjacent cells (fig. 40a).

In stratum granulosum, the intercellular space was narrow and cell junctions including desmosomes and gap-junctions were present (fig. 42). The intercellular “bridges” disappeared and the cell surface increased many times in size by the deep interdigitations (fig. 42c). Moreover, the intercellular space decreased in width towards the luminal level. In the stratum corneum, the intercellular space between deep horn cells and superficial granular cells was wider than that between granular cells. The intercellular space between lateral horn cells was narrower than that between the apical and basal membrane of adjacent horn cells and the surface of these cells may be deeply infolded, although dilatations of the intercellular space were sometimes seen (fig. 45). The outer lamina of the horn cells was covered by small projections (finger-like cytoplasm processes) (fig. 45a and 46a). Desmosomes (arrows) and macula occludentes connect horn cells (fig. 45b).

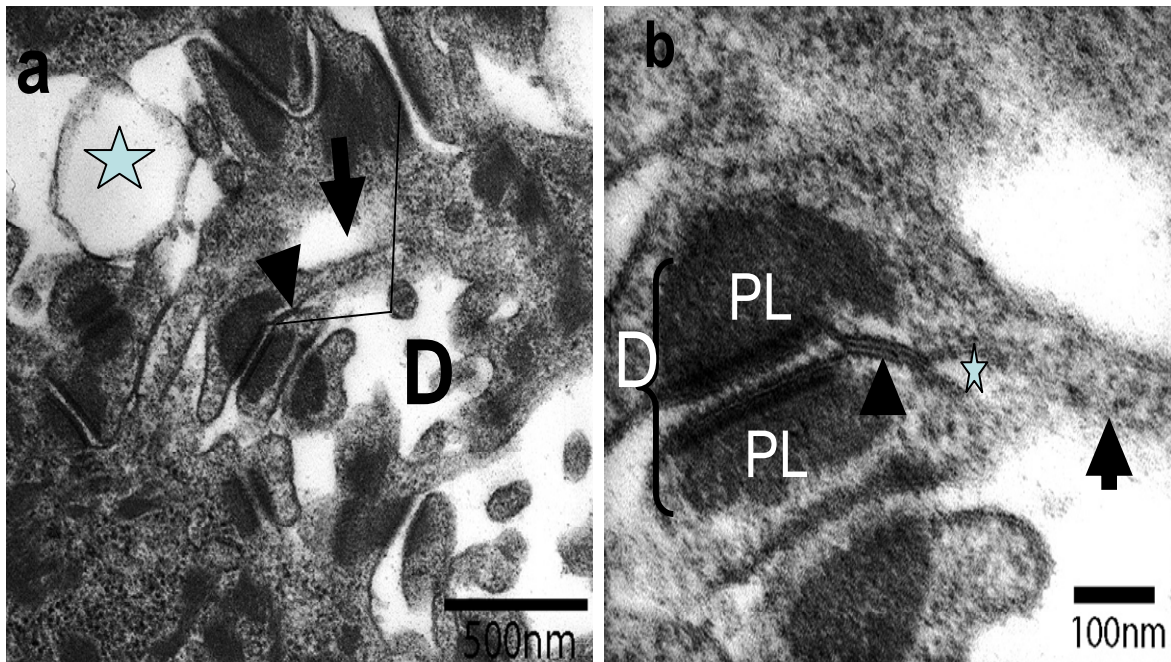
**Concentrate-fed groups:** Addition of concentrate to the diet caused widening of the intercellular spaces in all strata especially in stratum basale and spinosum. 2-6 weeks after the addition of concentrate to the diet, stratum basale exhibited a pronounced widening of the intercellular spaces (fig. 35b, c and d). However, the development of the extensive proximal projections of the basal cells was more noticeable in 6 weeks concentrate-fed group (fig. 36c and 38b). After 12 weeks of feeding concentrate, the finger-like cytoplasm processes extruding from the basal cells to the basal lamina were less developed compared to that of 4-6 weeks concentrate groups (fig. 36d and 38c). The proximal part of some basal cells was straight and in close apposition with the straight basement membrane (fig. 35f). Moreover, leucocytes were seen in the intercellular spaces of this stratum (fig. 35f). In stratum spinosum of the rumen of sheep fed concentrate diet for 4 and 6 weeks, electron microscopy revealed wide intercellular space with gap-junctions and a desmosome-rich stratum spinosum (figs. 48b and 40 c). The cytoplasmic process branched and gave more site for desmosomal attachment (fig. 49).



**Fig. 47:** Stratum corneum (SC) (12 weeks concentrate-fed group): desquamating horn cells. (Scale 4µm)



**Fig. 48:** The intercellular space in the stratum spinosum of the rumen epithelium a. the space was narrow and relatively few desmosomes and related thin tonofilament bundles (→) (hay-fed group) b. the space was relatively wide with desmosome-rich stratum spinosum and related, numerous tonofilament bundles (→) (4 weeks concentrate-fed group) c. the intercellular space was very wide compared to that of all other groups. These intercellular spaces present large numbers of branched desmosomes and tight junctions. Note the very thick tonofilament bundles (→) (12 weeks concentrate-fed group). (Scale 2 µm)

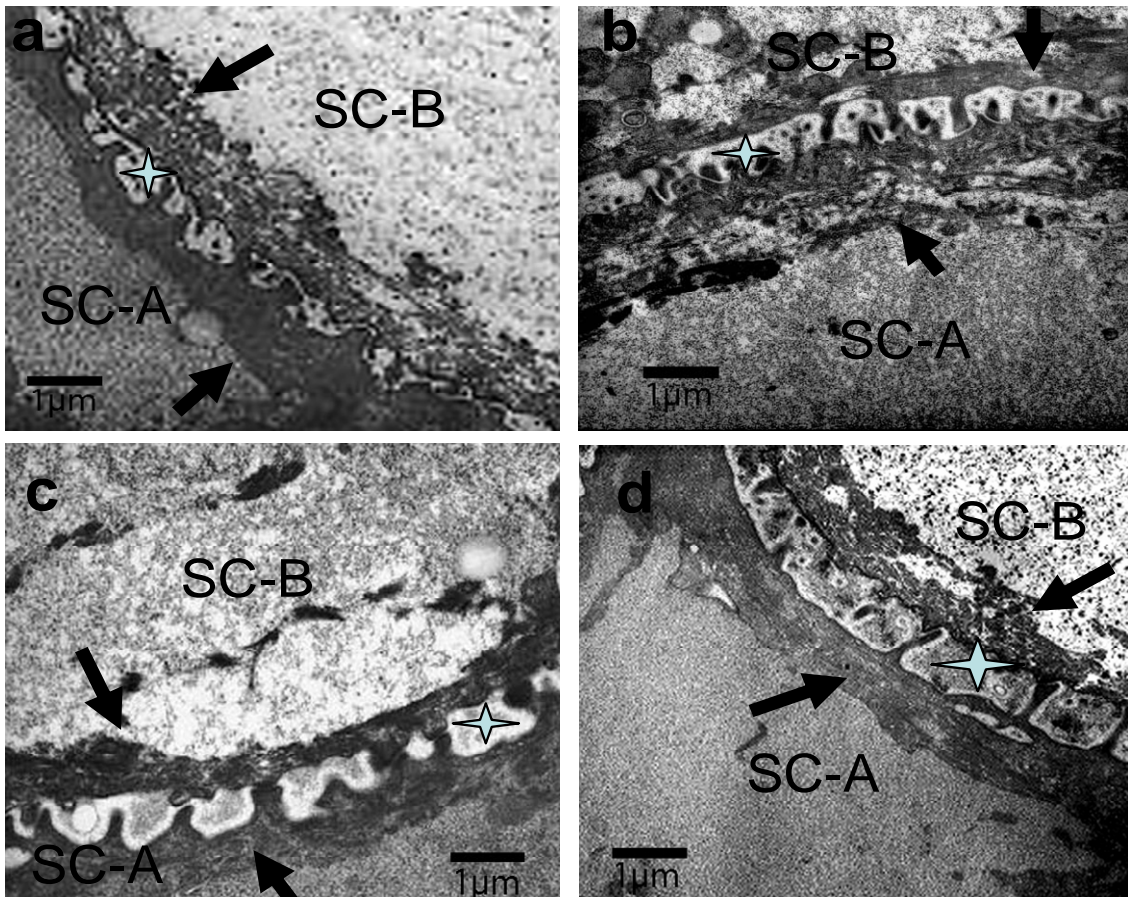


**Fig. 49: Junction between two spiny cells (12 weeks concentrate-fed group)** a. The complex intercellular channel was prominent (\*) with several desmosomes (D) and gap junction (arrow head) (Scale 500nm) b. branched (\*) cytoplasmic processes (→) and desmosomes (D) with gap-junction (arrow head) and attachment plaque (PL). (Scale 100 nm)

In 12 weeks concentrate-fed group, the intercellular space was wider than in all concentrate-fed groups (Fig. 48c).

These intercellular spaces present large numbers of branched desmosomes and gap junctions. Two microabscess were found also in the intercellular space between spiny cells (Fig. 41b). In the stratum granulosum of sheep fed concentrate for 1-12 weeks, the intercellular space was wider than that of hay-fed group and cell junctions including desmosomes and gap-junctions were more pronounced (fig. 44).

In the stratum corneum, the intercellular spaces between upper and lower horn cells (apical-basal intercellular spaces) showed gradual dilatation with more dense granular material with increasing the period of concentrate for 2-6 weeks compared with hay-fed group (Fig. 50). After 12 weeks of feeding concentrate, the cell junctions between the stratum granulosum and the stratum corneum and within horn cells became disrupted.



**Fig. 50: The intercellular space between upper (SC-B) and lower (SC-A) horn cells surrounding by the denser peripheral cytoplasm, containing dense fibrillar material (→)**  
 a. the intercellular space between the apical and basal membrane of adjacent horn cells was narrow, folded and contained less dense material (\*) (hay-fed group) b. the intercellular space was relatively wide with dense material (\*) (2 weeks concentrate-fed group) c. wide intercellular space with dense material (4 weeks concentrate-fed group) d. very wide intercellular space with more dense material (\*) (6 weeks concentrate-fed group). (Scale 1 $\mu$ m)

The tables 13-17 summarise the most important findings of the effect of both type and duration of feeding on different morphological criteria in the epithelial rumen of sheep

**Table 13:** Macroscopic and scanning electron microscopic examination

Criteria	Hay-fed group	2 weeks concentrate-fed group	4w weeks concentrate-fed group	6w weeks concentrate-fed group	12w weeks concentrate-fed group
Shape of papillae (Fig. 2)	small flattened tongue-like	small thick and irregular-shaped	large finger-like	large leaf-like	large leaf-like
Surface-grooves (Fig. 2)	a few and shallow	two major, very deep longitudinal grooves	a lot of short, deep-transverse grooves	deep reticular-form	deep reticular-form
Stratum corneum-cytoplasmic projections (glycocalyx) (Fig. 4)	reduced nodular form	less-well developed	large flap-like in form, having a distinctly contorted arrangement	well developed	well developed and more foliate in shape
Mean length of papillae (mm)	2.21	2.61	4.67	3.89	3.63
Mean width of papillae	1.77	2.37	2.75	2.6	2.55
Number of papillae / (cm <sup>2</sup> ) mucosa (mean)	38.67	50.33	48.33	49.33	51.33
Total surface / cm <sup>2</sup> mucosa (mean)	308.21	633.25 (2 fold)	1248.44 (4 folds)	997.55 (3 folds)	949.66 (3 folds)

**Table 14:** Quantitative histological findings

Criteria	Hay-fed group	2 weeks concentrate-fed group	4 weeks concentrate-fed group	6 weeks concentrate-fed group	12 weeks concentrate-fed group
Total thickness of epithelium(cell)	7-8	8-9	8-10	7-9	7-10
Thickness of str. spinosum (cell)	2-3	2	1-2	1-2	1
Thickness of str. spinosum (cell)	2-3	2	1-2	1-2	1
Thickness of str.granulosum (cell)	2	2	3	3	3
Thickness of str.corneum (cell)	2	3	2-4	2-4	2-5
Mean thickness of epithelium (µm)	74.41	113.17	109.98	129.74	106.57
Mean thickness of (germinativum +granulosum) (µm)	47.75	63.85	56.96	69.47	51.75
Mean thickness of str.corneum (µm)	19.30	24.57	32.75	28.86	40.67
Relation (germinativum+granulosum)/ str.corneum (µm)	2.46	2.62	1.76	2.41	1.35
Length of papillary body (µm)	77.24	109.56	106.76	94.33	102.20

**Table 15:** Qualitative histological findings

Criteria	Hay-fed group	2 weeks concentrate-fed group	4 weeks concentrate-fed group	6 weeks concentrate-fed group	12 weeks concentrate-fed group
Shape of papillae (Fig. 6)	small papillae. The lower 2/3 of the papillae was wider than the upper 1/3 ones with arched apex and less irregular sides.	small wide papillae, very irregular sides and flat apex	large papillae equal width along the most length of the pap. with wavy sides and flat apex	narrow and long papillae with deep waved sides (secondary pap.) and broad apex	short or long branched papillae (cauliform like-papilla)
Papillary pegs (Fig. 8)	less developed	well developed	well developed. into two small tips	well developed	misshaped-papillary pegs

Blood vessels in papillary body	few number of small capillaries	few number of small capillaries	large number of wide sinusoid capillaries (Fig. 19)	wide sinusoid-like capillaries	wide sinusoid-like capillaries
Horn cells (Fig. 14-21)	small vacuolated horn cells	small flattened cell at both the apex and sides of the papillae.	large vacuolated cells at both sides and apex of the papillae	large vacuolated cells at both sides and apex of the papillae	flattened horn cells at both sides and apex of the papillae
Granular cells (Fig. 14-21)	flattened and vacuolated cells at sides and apex of the papillae, respectively with fine keratohyaline granules	flattened and vacuolated cells at sides and apex of the papillae, with medium-sized keratohyaline granules.	large vacuolated cells at the lateral sides and more flattened at the apex of the papillae with large keratohyaline granules	very large and vacuolated cells at the sides and flattened at the apex of the papillae with large keratohyaline granules	very large and vacuolated cells at the sides and flattened at the apex of papillae with large keratohyaline granules
Spiny cells (Fig. 14-21)	polyhedral cells	large polyhedral cells	large polyhedral cells	large polyhedral cells with wide intercellular space	large polyhedral cells with wide intercellular space
Basale cells (Fig. 14-21)	small columnar or cuboidal cells	pyramidal cells	large pyramidal cells	large pyramidal cells with wide intercellular space.	large pyramidal cells with wide intercellular space
Desquamation of horn cells	mild	moderate	mild	mild	Sever (Fig. 21e, f)
Inflammation and parakeratosis	-	-	-	-	X (Fig. 21)

**Table 16:** Immunohistochemical finding

Antibody	location	Degree of antibody reaction in				
		Hay-fed group	2 weeks concentrate-fed group	4 weeks concentrate-fed group	6 weeks concentrate-fed group	12 weeks concentrate-fed group
NHE3 (Fig. 31) (Table 11)	present in all strata of the epithelium except stratum corneum with more intense at both stratum granulosum and stratum spinosum (superficial layer), predominantly at the apical surface of the cells	weak	moderate	strong	weak	very weak
Cx43 (Fig. 33) (Table 12)	Plasma membrane connexin 43 immunostaining was most intense at the str. basale and str. spinosum (suprabasal layer) and decreased in intensity through str. spinosum (superficial layers) to str. granulosum. str. corneum was negative. the reaction around the cells gave a syncitial appearance with more apical-immunostaining concentration	weak	strong	very strong	very strong	very strong
$\alpha$ SMA (Fig. 29)	the condensed fibrous layer in the core of ruminal papillae and interpapillar mucosa near the epithelium, at the position equivalent to the muscularies mucosa	weak	strong	very strong	very strong	weak

**Table 17:** Ultrastructural findings

Criteria	Hay-fed group	2 weeks concentrate-fed group	4 weeks concentrate-fed group	6 weeks concentrate-fed group	12 weeks concentrate-fed group
Distance of B.V from basal lamina	0.72-2.0 $\mu$ m	0.80-0.84 $\mu$ m	0.6-0.8 $\mu$ m	0.7-.75 $\mu$ m	0.04-0.20 $\mu$ m



Proximal processes of the basal cell (Fig. 36)	convoluted	short and wide finger like-projections	extensive long and thin finger like-projections	extensive, long and wide finger like-projections	short and wide finger like-projections
The basal lamina (Fig. 36)	less folded	folded	folded	deeply folded	less folded
Cytoplasmic organelles(all strata) (Fig. 39)	rich with mitochondria	high density of mitochondria	high density of mitochondria and well developed golgi apparatus	high density of mitochondria and ribosomes	high density of mitochondria and ribosomes
Tonofibrils bundles in str. spinosum (Fig. 38 and 40)	thin bundles (0.08-0.10 $\mu\text{m}$ thick)	Medium sized-bundles (0.10-0.13 $\mu\text{m}$ thick)	Medium sized-bundles (0.09-0.20 $\mu\text{m}$ thick)	thick bundles (0.10-0.32 $\mu\text{m}$ thick)	a very thick bundles (0.08-0.32 $\mu\text{m}$ thick)
Membrane-coated vesicles (Fig. 44)	few	numerous	numerous	few	few (large-size)
Intercellular spaces (all strata) (Fig. 48 and 50)	narrow	wide with desmosome-rich stratum spinosum	wide with desmosome-rich stratum spinosum	wide with desmosome-rich stratum spinosum	wide with large number of desmosomes
Stratum corneum-cytoplasmic projections (glycocalyx) (Fig. 46)	Small-sized	Well developed	Well developed	Well developed	Small-sized
Abnormalities (Fig. 41)	-	-	-	-	presence of small keratohyaline granules and microabscess in Str. spinosum