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1 Introduction and Literature Review

1.1 Introduction

The squamous, cornified and multilayered epithelium of the ruminant forestomach is involved in absorption, transport, and metabolism of fermentation products (Barnett and Reid, 1961; McGilliard et al., 1965) and in protection of the tissue from the abrasive digested mass (Lavker et al., 1969). Short chain fatty acids (SCFA) are absorbed across the lamina epithelialis mucosae into the blood vessels of the lamina propria-mucosa. Interestingly, 85% of the SCFA produced in the rumen are absorbed in the reticulorumen (von Engelhardt and Hauffe, 1975), and more than 70% of ruminants daily energy requirements are provided by these fermentation products (Siciliano-Jones and Murphy, 1989; Bergman, 1990).

The rumen is covered by a stratified squamous epithelium that consists of leaf like papillae, which greatly increase the absorptive surface area (Steven and Marshall, 1970). High protein and carbohydrate intake appear to increase the papillary size and density via butyrate and propionate regulation of IGF-1 production (Shen et al., 2004).

Four distinct cell layers in the rumen epithelium can be distinguished. These are, from the luminal side, the stratum corneum, the stratum granulosum, the stratum spinosum and the stratum basale (Steven and Marshall, 1970). Thickness of the epithelium, particularly the stratum corneum, and potential effects on transport of nutrients have been linked to diet (Fell et al., 1968; Weigand et al., 1975). For example, increased proliferatition of ruminal epithelial cells caused by availability of excess soluble substrate with a subsequent accumulation of stratum corneum cells has been suggested to determine SCFA transport (Bull et al., 1965; McGavin and Morrill, 1976; Nocek et al., 1980).

The rumen epithelium shows food-dependent morphological, physiological (transport) and biochemical changes (Abdoun et al., 2003; Mentschel et al., 2001; Uppal et al., 2003; Shen et al., 2004). The influence of food on the morphology of the rumen epithelium affects primarily the number and size of the papillae (Liebich et al., 1987). However, little has been done quantitatively to estimate the effect of both type and duration of feeding on the morphology of the rumen mucosa of sheep. Furthermore, the time at which the transport-physiological changes occur is not yet known. Therefore, the objectives of this study are to characterize the effect of concentrate feeding on the morphology, histology, ultrastructure and some immunohistochemistry of the rumen epithelium of sheep which are relevant to the transport

processes and to determine the exact time at which these changes occurs after change of the diet (hay ad libitum → hay + concentrate diet). Furthermore, their localization in the epithelium will be also considered.

1.2 Literature review

1.2.1 Embryonic development of the rumen

The stomach of the ruminants is composed of four compartments: rumen, reticulum, omasum and abomasum. The first three are known, collectively, as the forestomachs (proventriculus). These comparments are derived, however, from the gastric spindle of the embryo without the assistance of a contribution from the oesophagus (Dyce et al., 2002). Ruminal growth involves two major aspects during fetal development; one corresponds to papillary growth and increase of epithelial surface, and the other refers to the growth of the organ as a whole (Panchamukhi et al., 1977). The overall area of the inner surface of the rumen increases with the fetal age (Amasaki and Daigo, 1987). The mucosal appendages (papillae), characteristic of ruminal and reticular mucosa, develop similarly as reported in calves (Warner, 1958), in lamb (Wardrop, 1961a), and in buffalo calf (Tiwari and Jamdar, 1970a-c). It is generally accepted that ruminal papillary formation is initially shaped by mechanical forces which act on the mucosa and which arise from epithelial expansion and from muscular contraction (Wardrop, 1961a). Moreover, the morphogenesis of the ruminal papillae corresponds to epithelial-mesenchymal interaction, which starts around the 5th month of fetal age in bovines (Arias et al., 1978; Kano et al., 1981; Amasaki and Daigo, 1984, 1987). On the other hand, Stinson and Calhoun (1976) observed the primordia of the papillae in bovine's foetuses with crown-rump-length (CRL) 460 mm. However, Michel and Flechsig (1969) found them in foetus with 145 mm CRL and become definite in shape at 480 mm CRL. In buffalo, the first incidence of the ruminal papillary formation is observed in fetuses of 170 mm CRL (Osman and Berg, 1981) or at the middle stage of gestation (Sengar and Singh, 1969). The ruminal papillary formation is observed first in the atrium ruminis and in the caudoventral blind sac simultaneously (Osman and Berg, 1981). In sheep, the papillary primordia are present at 61 days and are fully differentiated compared with birth at 120 to 150 days (37-40 cm CRL) of foetal age (Franco et al., 1992). While in goat, they do not develop up to the 39.5 cm CVR length of foetus (Ramkrishna and Tiwari, 1979). The prenatal papillary shaping corresponds, histologically, to the appearance of undulations involving the deeper cells of the epithelium,

the basement membrane and the underlying lamina propria-submucosa (Wardropa, 1961a; Arias et al., 1978; Franco et al., 1992). Moreover, the arrest of the papillary shaping corresponds with the highest undulation of the basement membrane and the greater proportion of chondroitin sulfate (Vasan et al., 1984; Caplan, 1986). Noteworthy, the histogenic steps of the formation of the ruminal papillae are the aggregation of the cells of the basal layer (redistribution of the basal cells of the epithelium) and an increase of the connective tissue of the lamina propria, especially the fibroblasts of the papillary core (Arias et al., 1980b); the undulations with involvement of the basal layer of the lamina epithelialis, basement membrane and lamina propria; formation of hump from the undulations and lastly the formation of the papillae (Osman and Berg, 1981). Until birth, papillae do not become separated entities and only a few keratinized cells are present (Arias et al., 1978). A progressive maturation of the collagen fibres also occurs and is characterized by an increase in cross linking and helical content, and a change in the collagen type (Arias et al., 1983, 1986). On the other hand, the formation of ruminal papillae seems to follow a series of developmental changes in the subepithelial microvasculature. Therefore, the successive formation of new ruminal papillae is preceded by the formation of subepithelial papillae-like capillary projection (at the latter period of the 4th month of gestaion in bovine) (Amasaki and Daigo, 1987). Interestingly, Tamate et al. (1962) suggested that fetal papillary development is influenced by SCFA from maternal circulation. If ruminal circulation is homogenous, similar development of ruminal papillae in dorsal and cranial sacs should be expected.

The main developmental morphologic changes of the epithelium, including number and types of cells, thickness of the epithelium and papillary appearance and growth have been reported in bovine foetuses (Warner, 1958; Arias et al., 1978; Arias et al., 1979; Arias, 1991), buffalo (Tiwari and Jamdar, 1970a-c; Osman and Berg, 1981), sheep (Wardrop, 1961a; Henrikson, 1970a; Franco et al., 1992) and goat (Ramkrishna and Tiwari, 1979). The rumen of the foetus is lined with a non-keratinized stratified epithelium (Wardrop, 1961a; Tamate et al., 1962; Henrikson, 1970a; Ramkrishna and Tiwari, 1979). This epithelium changes, postnatally, from stratified-cuboidal to stratified-squamous cornified type (Arias et al., 1978). The thickness of the foetal rumen epithelium is about ten-cell layers, which are differentiated into thin basal zone (3-4) cell layers and a thicker superficial zone (6-7 or 9) cell layers (Arias et al., 1978; Osman and Berg, 1981). The basal zone is constituted by the stratum basale or germinativum (Arias et al., 1978) and it is reduced later to only one layer, which made of columnar cells (Ramkrishna and Tiwari, 1979; Osman and Berg, 1981). The superficial zone is formed by voluminous polygonal or vertically flattened cells, with their nuclei located on the luminal

pole of the cells (Arias et al., 1978, 1980b). This thickness of the epithelium increases constantly with increasing size of the foetus (Ramkrishna and Tiwari, 1979) and also depending on the ruminal compartments (Osman and Berg, 1981). Noteworthy, the number of epithelial cell layers is larger in the foetal rumen than in adult (Arias et al., 1979). On the other hand, Arias et al. (1978, 1980b) found that in bovine further differentiation of the epithelium during prenatal period evidenced by both; decrease in the number of cell layers and keratinisation of the most superficial cells. Tiwari and Jamdar (1970a-c) reported a decrease in the thickness of the epithelium with age during postnatal forestomach development in buffalo. In sheep, differentiation of the rumen epithelium takes place at 33 days of foetal life and stratification of the epithelium is accompanied by considerable increase in thickness (Franco et al., 1992). Henrikson (1970a) stated that the thickness of the foetal rumen epithelium is about 400 µm and decreases to a thickness of 50-150 µm a few days after birth (Henrikson, 1970a).

Ultrastructural studies on rumen epithelium of the foetus of sheep show that the basal surface of the epithelium is smooth and the intercellular space is narrow (Henrikson, 1970a). In the early stages of bovine foetus (2-7 month), the basal processes are poorly developed, the intercellular space is narrow and there are desmosomes in both basal and superficial cells (Arias et al., 1980a). In the late-stage of bovine foetus, the luminal cells of the epithelium contain abundant filaments, but they lack the keratohyaline granules and fuzzy intercellular material which are characteristic of the cornified cells of the adult (Arias et al., 1979). Moreover, the epithelial cells show a decrease in the appearance of desmosomes between the most luminal cells. These desmosomes have morphological features that reveal to some extend the occurrence of process of keratinization. They have little contrast and defined structure compared to desmosomes of the deeper layers of the epithelium or those present at the luminal cell layer in early stage of bovine foetus. Their tonofilaments appear to fade away and the middle lamella of the desmosomes of some of these cells is apparently absent (Arias et al., 1979). This could indicate that the basis for cell keratinization is already present in the foetus (Arias et al., 1979).

The wall of the rumen is clearly differentiated into mucosal, muscular and serosal layer at three month bovine foetus (Arias et al., 1978) and at 53 days of development in sheep foetus (Franco et al., 1992). The muscularis mucosa is absent in the foetal rumen, and hence the lamina propria and sub-mucosa are continuous with each other (Arias et al., 1978; Ramkrishna and Tiwari, 1979; Franco et al., 1992). The tunica muscularis consists of spirally arranged muscle fibre bundles (Ramkrishna and Tiwari, 1979; Franco et al., 1992). The inner

muscular layer is differentiated earlier than the outer muscular layer and is thicker (Arey, 1966; Ramkrishna and Tiwari, 1979). The thickness of the tunica muscularis increases with foetus age (Wardrop, 1961a; Ramkrishna and Tiwari, 1979).

Histochemichal studies of the foetal rumen epithelium show that large amount of glycogen are present in the fetal rumen as indicated by the PAS reactivity (Wardrop, 1961a; Tamate et al., 1969; Henrikson, 1970a; Steven and Marshall, 1970; Arias et al., 1978, 1980a). It accumulates in the superficial zone of the epithelium (Henrikson, 1970a; Arias et al., 1978, 1980a). Electron microscopic examination of the bovine foetal rumen reveals that the distribution and structure of glycogen are modified during foetal development. Both basal and superficial cells increase their glycogen content in foetuses up to 6 months of age. In older ones, the epithelial glycogen content diminishes (Arias et al., 1980a). Very small amount of glycogen presents in the rumen shortly after birth (Wardrop, 1961a; Habel, 1963; Henrikson, 1970a). On the other hand, Arias et al. (1991) stated that in bovine foetal rumen, there is a decrease in the amount of hyaluronic acid and increase in chondroitin sulfate during fetal development. These results suggest a correlation between glycosaminoglycan composition and the expression of some cellular events resulting in morphological changes which operate during ruminal morphogenesis. Noteworthy, the epithelium lacks secretory capacity during initial embryo-phase; neutral mucopolysaccharides first appear in epithelial cells cytoplasm at 46 days of sheep foetal life, thereafter, the amount decrease gradually and subsequently stabilize in postnatal life (Dellmann and Brown, 1981). Acid mucopolysaccharides, mucins and mucoid compounds are not detected (Franco et al., 1992). The presence of neutral mucopolysaccharides in the deeper epithelial layers is directly related to the gradual adaptation of the ruminal mucosa for protection against chemicals in postnatal life, where it acts as a buffer for neutralization of the acid compounds produced during ruminal fermentation (Dellmann and Brown, 1981).

1.2.2 Gross anatomy of the rumen

1.2.2.1 Topography and relationships

The stomach of the ruminants is very large and it is composed of four compartments; rumen, reticulum, omasum and abomasums (Habel, 1975; Dyce et al., 2002). The rumen of the sheep occupies most of the left portion of the abdominal cavity and extends over the median plane in the middle and to some extent ventrally. Its long axis reaches from a point opposite the ventral part of the 8th intercostal space or 9th rib almost to the pelvic inlet (May, 1970). The

rumen is laterally compressed and extends from the abdominal roof to the floor and from left body wall across the midline, especially caudally and ventrally where it reaches the lower right flank (Dyce et al., 2002). The parietal surface faces the diaphragm, the left wall of the abdomen, and the spleen. The visceral surface is to the right, irregular, and related to the omasum, abomasums, intestine, liver, pancreas, left kidney, aorta, and caudal vena cava. The dorsal curvature is related to the diaphragm and the sublumbar muscles. The ventral curvature lies on the floor of the abdomen mainly to the left of the median plane, especially in the xiphoid region (May, 1970; Habel, 1975). The rumen is partially divided internally into sacs by muscular pillars. The principal pillars encircle the organ dividing it into dorsal and ventral sacs, while lesser coronary pillars mark off the caudal ventral and dorsal blind sacs. The cranial pillar has an oblique direction that partially divides the cranial extremity from the remainder of the dorsal sac, emphasizing the association of the former part (atrium ruminis) with the reticulum (Sisson and Grossman`s, 1975; Dyce et al., 2002). External grooves (left and right longitudinal groove, transverse grooves and coronary grooves) correspond to the positions of all these pillars. The relative proportions of the compartments vary among the domestic ruminants (Dyce et al., 2002). The smaller size of the dorsal sac and extensive caudal projection of the ventral blind sac give the rumen of the sheep and goat an unbalanced appearance when compared with the more symmetrical bovine rumen (Sisson and Grossman`s, 1975; Dyce et al., 2002). In sheep, the dorsal sac extends further forward over the cranial end of the ventral sac, the external demarcation of it's extend being the rumino-reticular groove (well marked ventrally and laterally) (May, 1970). There are also differences in the development of the grooves (visible externally), but these are all together without practical significance (Dyce et al., 2002). The serosa covers the entire surface of the rumen, except dorsally where the ruminal wall is directly adherent to the abdominal roof from the oesophageal hiatus of the diaphragm to the level of the 4th lumbar vertebra (Dyce et al., 2002). The mucous membrane is covered by large papillae (10 to 15 mm in length in the cow); so greatly increase the absorptive surface area (Steven and Marshall, 1970). These papillae vary in prominence according to age, diet and location (Scott and Gardner, 1973; Habel, 1975; Dyce et al., 2002). Normally they are largest and most densely in the blind sacs, fewer and less prominent in the ventral sac and least developed over the centre of the roof and toward the free margins of the pillars (Dyce et al., 2002). In the sheep, the entire surface of the rumen is papillated, including the ruminal pillars, where the papillae are reduced and closely packed, giving a distinctly ridged appearance to the pillars (Scott and Gardner, 1973). Individual papillae vary from low round elevations through conical and tongue like forms to

flatten leaves about 1 cm long (Dyce et al., 2002). SEM studies revealed that ruminal papillae of the sheep are heavily furrowed or grooved over their entire surface. These grooving is greater on one side of the papillae than on the other (Scott and Gardner, 1973). On the other hand, Tamate et al. (1971) described the primary and secondary grooves on the foliate ruminal papillae of cattle, while Scott and Gardner (1973) reported deep grooves on the foliate ruminal papillae of the sheep. Surface of the bovine ruminal papillae present a characteristic ridges and hollows which reveal a highly keratinized squamous epithelial cells. Furthermore, the trough between these papillary ridges contain heterogeneous microflora (Graham and Simmons, 2005). A study of the topography of the surface epithelium of the stratum corneum of the sheep forestomach reveals the presence of cytoplasmic projections of the horn cells which vary in their degree of development in the different chambers (Scott and Gardner, 1973).

1.2.2.2 The blood supply

The stomachs of the ruminants receive blood supply from the branches of the coeliac artery (May, 1970; Sisson and Grossman`s, 1975). In sheep, the right ruminal artery which originates from the splenic artery is running along the right longitudinal groove and ramifies on both surfaces of the rumen. The left ruminal artery which arises usually from the omaso-abomasal artery (in few cases it arises from the right ruminal artery) passes on the anterior part of the right face of the rumen and continuous caudally in the left longitudinal groove and supplies mainly the left surface of the rumen (May, 1970).

The veins of the compound stomach are mainly satellites of the arteries (Dyce and Wensing, 1971). The gastric vein is formed by the union of two trunks: The right ruminal vein and the other trunk are formed by the confluence of the omaso-abomasal; left ruminal and the reticular veins (May, 1970; Sisson and Grossman`s, 1975).

1.2.2.3 The innervation

The compound stomach of the ruminants is innervated by the vagus which has a dorsal and a ventral trunk. The dorsal vagal trunk gives off branches to the rumen, reticulum and the greater curvature of the omasum and abomasums. The ventral vagal trunk innervates the cardia, left surface of the rumen, reticulium, parietal surface of the rumen and right surface and lesser curvature of the reticulum (May, 1970; Dyce and Wensing, 1971; Habel, 1975).

1.2.3 Histology of the rumen

The forestomach is lined by nonglandular stratified squamous epithelium (Dellmann, 1971; Banks, 1986; Mosimann and Kohler, 1990; Liebich, 1999; Dyce et al., 2002). Each has various mucosal projections, namely, the ruminal papillae, the reticular folds and the omasal laminae (Hofmann, 1988). Light microscopy studies in rumen epithelium have characterized this tissue as a keratinized, stratified, squamous epithelium, which resembles epidermal tissue (Dobson et al., 1956; Henriksson and Stacy, 1971). The mucosal surface of the rumen is covered with numerous papillae which vary in size, shape, and distribution through the ruminal wall (Dobson et al., 1956; Liebich, 1999). Their length is greater in the ventral and cranial sacs of the rumen (Tamate et al., 1971), in the blind sacs, and absent or reduced on the pillars and sometimes also in the dorsal wall, where slight folds are present (Dellmann, 1971; Mosimann and Kohler, 1990). In sheep, they are well developed in both atrium ruminis and recessus ruminis (Berg and Edvi, 1976). These papillae are 1.5 cm long (Banks, 1986; Graham and Simmons, 2005) in bovine, and 1-5 mm long in sheep (Henrikson, 1970b). They are formed of a core of highly vascularized connective tissue composed of fine collagenous and elastic fibers or irregular dense connective tissue (Banks, 1986; Dellmann, 1971) and covered by epithelium (Dobson et al., 1956). Ruminal papillae have been described as being luminal extension of the mucosa (Graham and Simmons, 2005), long, wide and flat epithelial-lamina propria protrusions of the mucosa (Dellmann, 1971). They are generally tongue shaped (Banks, 1986; Liebich, 1999), leaf like (Tiwari and Jamdar, 1970a; Graham and Simmons, 2005), spade shaped, hair-like, or triangular (Harrison et al., 1960); finger or paddle shaped (Tamate et al., 1962). Schnorr and Vollmerhaus (1967a) illustrated a variety of ruminal papillae they designated as wart-, keel-, tongue-, rod-, lance-, or leaf-like. Secondary papillae may be present at the bases of primary ones (Bastain and Menon, 1963). The lamina epithelialis mucosa is a very thin layer of stratified squamous keratinized epithelium, of variable thickness (8-10 cells) and consists of four cell layers. These layers are (labeled from the rumen lumen): the intensely stained keratinized outer layer of the stratum corneum, the underlying stratum granulosum, the stratum spinosum, and finally the stratum basale (Lavker et al., 1969; Steven and Marshall, 1970). These layers are not well defined (Dellmann, 1971; Banks, 1986). However, Graham and Simmons (2005) claimed that the limits of both stratum corneum and stratum basale are clearly defined even at low power. Both thickness and depth of the epithelium are decreased with age, but the reverse was true for the stratum corneum (Tiwari and Jamdar, 1970a). In bovine rumen mucosa, 8-10 cell layers comprising the

substrata basale and spinosum (Scott and Gardner, 1973). Cells comprising the stratum corneum are 3 cell layers thick (Scott and Gardner, 1973). They contained nuclei and numerous round or ovoid translucent areas within the cytoplasm (Lavker et al., 1969). Some of these cells are flattened, wherease others are swollen by a large vacuole and often contain lipid droplet (Dellmann, 1971; Henrikson, 1970b; Henriksson and Stacy, 1971). Flattened horn cells are found on the papillary apex, while vacuolated horn cells are regularly arranged along the lateral surface of the papillae and interpapillary epithelium (Henrikson, 1970b; Banks, 1986). Horn cells may accumulate and form a cap over the tips of the papillae (Bastain and Menon, 1963). Henrikson (1970b) found that the thickness of the stratum corneum of the dorsal sac of the rumen is higher than that present in the ventral sac. Cells of the stratum granulosum were flattened and elongated similar to the horn cells (Scott and Gardner, 1973). The lamina epithelialis mucosa of ruminal papillae presents well developed papillary bodies (Tamate et al., 1979; Weyrauch and Schnorr, 1979; Yamamoto et al., 1993; Liebich, 1999). These papillary bodies are formed by folded basal layer of the epithelium (Wardrop, 1961a), and the papillary projections of the subepithelial connective tissues (Liebich, 1999). Typical lamina muscularis mucosa is absent (Dellmann, 1971; Banks, 1986; Mosimann and Kohler, 1990; Liebich, 1999; Dyce et al., 2002). However, a condensation of connective tissue fibers is found in the deep region of the lamina propria-submucosa; which is sometimes mistaken for lamina muscularis mucosa (Banks, 1986). Nevertheless, Dellmann, (1971) stated that single smooth muscle cells are occasionally present. The combined lamina propria-submucosa is generally devoid of lymphatic nodules and is aglandular (Banks, 1986). The tunica submucosa consists of loose connective tissue. The tunica muscularis consists of two smooth muscle layers inner transverse and outer longitudinal (Liebich, 1999). Dellmann (1971) claimed that the tunica muscularis consists of two or three smooth muscle layers, which are especially thick at the pillars. The thin outer muscular layer runs craniocaudally over the rumen (Mosimann and Kohler, 1990; Dyce et al., 2002). The pillars of the rumen are extensive folds of the entire wall that contain a core of muscle from the tunica muscularis (Banks, 1986; Mosimann and Kohler, 1990). The tunica serosa covered the rumen except a part of the dorsal sac adherent to the abdominal roof from the oesophageal hiatus of the diaphragm, where tunica adventitia is present (Mosimann and Kohler, 1990; Liebich, 1999; Dyce et al., 2002). The lamina subserosa is rich in adipose tissue, especially in the ruminal grooves. The lamina epithelialis serosa is simple squamous or low cuboidal epithelium (Dellmann, 1971).

Previous histochemical studies on the rumen mucosa revealed that material stained by the PAS reaction, sharply outlines the horn cells, but is absent from the more proximal layers of the epithelium (Henrikson, 1970b). The stratum corneum contained neutral and both sulfated and none sulfated acidic mucosubstances (Barcroft et al., 1944). Furthermore, the stratum corneum and stratum granulosum have been demonstrated to contain mucopolysaccharides, sulfhydryl and disulfide groups, histidine, acid phosphatase and lipids (Habel, 1959; Henriksson and Habel, 1961; Habel, 1963; Lavker et al., 1969).

1.2.3.1 Vascularisation and nerve supply

Large blood vessels are located in the submucosa. A dense capillary network is observed in the ruminal papillae (Dellmann, 1971), especially, at the papillary bodies (Liebich, 1999). Capillaries penetrated into the papillary bodies from the central core (Dozsa et al., 1964). The lymphatic vessels of the ruminal wall do not originate directly from below the epithelium, but from the deeper layers of the propria mucosa. Furthermore, they are found at the base of the ruminal papillae (Schnorr et al., 1975). The nerve supply of the rumen is parasympathetic (vagus) and sympathetic. The submucosal plexus and myentric plexus are found in tunica submucosa and tunica muscularis (between the outer and middle layer), respectively (Dellmann, 1971).

1.2.4 Ultrastructure of the rumen

No differences are found in the ultrastructure of the epithelium lined the different forestomach-compartments (rumen, reticulum and omasum). They all characterized by having wide intercellular spaces which are lined with numerous desmosomes and cytoplasmic processes (Hyden and Sperber, 1965).

Stratum basale: By electron microscopy, basal cells in the rumen epithelium are surrounded by a prominent and wide intercellular space and the cellular surface opposite to the basement membrane is deeply indented by microvillus processes (Henrikson, 1970b; Henriksson and Stacy, 1971). The basal lamina appeared as a moderately electron dense structure approximately 500 Å thick and does not always follow the contours of the basal cell membrane. Instead, the lamina followed a line demarcated by the tips of the processes. Hemidesmosomes were observed along the basal cell membrane facing the basal lamina (Lavker et al., 1969). Basal cells were either cuboidal or short columnar with long axes perpendicular to the basal lamina. These cells constitute 2-3 cell layer and contained abundant

metabolic organelles and numerous large vesicles (Lavker et al., 1969). Basal cells were characterized by a large ovoid nucleus, numerous mitochondria with dark staining matrix and prominent cristae, clusters of ribosome, rough-surface endoplasmic reticulum, Golgi apparatus, large vesicles, desmosomes and small amounts of fine filaments (Lavker et al., 1969; Henrikson, 1970b; Gemmell, 1973). The cells of the stratum basale contain high density of mitochondria relative to cells of the stratum spinosum or stratum granulosum (Graham and Simmons, 2005). Dense population of mitochondria is found in the cytoplasm of the basal cells, distal to the basal processes (Henrikson, 1970b). The large membrane-contained vesicles which have less electronic dense appearance than the surrounding cytoplasm and contained a mixture of granular and filamentous material (both near the nuclear membrane and isolated in the cytoplasm). Their proximity to the nuclear membrane often resulted in the nucleus appearing crescentic in shape (Lavker et al., 1969). Langerhans cells which are characterized by the dendritic shape and a complete lack of desmosomes or other junctions, are found in stratum basale of the rumen epithelium of sheep (Gemmell, 1973).

Stratum spinosum: The spiny cells are arranged in two layers: parabasal and superficial cells. The parabasal cells (proximal or deep cells), bordering the basal layer, were nearly polygonal or spherical cells with large rounded nuclei, prominent nucleolus and wide intercellular spaces. However, the superficial spiny cells, bordering the granular layer, are small and more flattened cells with very wide intercellular spaces (Schnorr and Vollmerhaus, 1967b). Cells in this stratum contain more filamentous material and membrane-coated vesicles than cells in the stratum basale. The membrane-coated vesicles appeared round to oval and measured approximately 0.2 μm in diameter. The interior of these granules is electron dense, lack any positive lamellar organization and reveal a granular structure. Moreover, high concentrations of mitochondria with different size, which are not oriented in any particular plane, are found in the spiny cells (Lavker et al., 1969). The processes found at this level in the epithelium are shorter and less numerous than those covering the proximal or deep surface of the basal cells and present desmosomes and maculae occludentes (Henrikson, 1970b).

Stratum granulosum: The deep layer of granular cells (type-A) contains a few keratohyalin granules of varying size, mostly small. However, the superficial granular cells, boarding to the stratum corneum, are larger in size and contain large sized keratohyline granules and more filamentous materials (Schnorr and Vollmerhaus, 1967b). The cytoplasm of the granular cells appeared moderately electron dense and contains beside the nucleus, tonofibrils,

keratohyaline granules, membrane-coated vesicles and mitochondria. Tonofibrils are in close association with desmosomes and keratohyaline granules (Lavker et al., 1969; Henrikson, 1970b). The stratum granulosum cells contain developing cytoplasmic keratin aggregates (Graham and Simmons, 2005). These keratohyaline granules (1200-1500 Å) have no defined shape and consist of filaments upon which a dense amorphous material is deposited (Henrikson, 1970b; Lavker et al., 1969). Lavker and Matoltsy (1970) reported the presence of a large number of lysosomes in the granular cells of normal rumen epithelium.

The width of the intercellular space in the stratum granulosum is very much reduced at the more luminal level (Henrikson, 1970b). At the outer limit of the stratum granulosum, the intercellular space is often obliterated by the fusion of the opposing cell membranes and though this form of cell attachment is readily found in some areas of the stratum granulosum (Gemmell and stacy, 1973). Electron microscopy of bovine rumen, defines occluding junction present at the outer-facing side between adjacent stratum granulosum cells (Graham and Simmons, 2005).

Stratum corneum: The stratum corneum is characterized by several layers of electron dense cells, which are classified cytologically into three types: flattened cells (R5A); balloon cells (R5C); and intermediate cells (R5B). The flattened cells form the barrier layer of the epithelium (Schnorr and Vollmerhaus, 1967b; Henriksson and Stacy, 1971; Zitnan et al., 1999). The flattened horn cells are present in a simple but not always fully developed layer of plate shaped cells. These cells are filled with osmophilic keratin and, generally, are found near the keratohyaline-containing cells (granular cells). The intermediate horn cells are disc-shaped with osmophilic keratin (Schnorr and Vollmerhaus, 1967b; Zitnan et al., 1999). Within the dense cytoplasm of these cells, filaments, electron dense bodies devoid of membrane and nuclear remnants are observed routinely, but no membrane-coated vesicles are observed (Lavker et al., 1969). Lysosome-like structures are also not found in horn cells (Henrikson, 1970b), whose membrane is modified to form finger-like projections, covered by amorphous fuzzy coatings (Lavker et al., 1969). Desmosomes and maculae occludentes connect horn cells, but appearently no zonula occludentes, are seen in the epithelium (Henrikson, 1970b). On the other hand, flattened horn cells (R5A) have been found to form the barrier layer of the epithelium (Schnorr and Vollmerhaus, 1967b). In sheep, the zonulae occludentes are seen in the layer of the stratum corneum nearest the stratum granulosum (Gemmell and stacy, 1973). Morphological study of the goat rumen epithelium has revealed zonulae occludentes in the the stratum corneum and on some occasions in the upper stratum granulosum (Schnorr and Wille, 1972).

Numerous clearly-defined points of contact (desmosomes and hemidesmosomes) are present throughout the whole epithelial layer, as well as special features within the intercellular spaces of the lower epithelial layers in ox and goat (Schnorr and Vollmerhaus, 1967b).

1.2.5 Immunohistochemistry

1.2.5.1 Muscularis mucosae

The compartments of the forestomach have peculiar mucosal folds and papillae lined by stratified squamous epithelium. In general, the histology of the compartments is similar except for the mucosal profiles with some distinguishing features such as the muscularis mucosae. The lamina muscularis mucosa is a thin layer consisting of aggregation of smooth muscle cells between the tunica mucosa and the tunica submucosa. In the omasum, it occurs as a continuous muscle layer following contours of the omasal laminae. It is also present in the reticulum but confided to the upper core of the reticular crest (crista reticuli). On the other hand, the rumen is reported to contain a condensed fibrous layer, which mimics the muscularis mucosa (Mosimann and Kohler, 1990; Banks, 1993; Ikemizu et al., 1994; Kitamura et al, 2003). In some studies, this condensed fibrous layer is considered to be the muscularis mucosae of the rumen. In a study on the buffalo rumen, it has been referred to as lamina muscularis mucosa (Taluja and Saigal, 1987). However, other references have described it not as muscularis mucosa, but as a condensed fibrous layer (Banks, 1981; Kitamura et al, 2003), connective tissue band (Bacha et al., 1990) or condensation of connective tissue similar to the stratum compactum (Liebich, 1999; Banks, 1993). On the other hand, Dellmann (1971) mentioned the occurrence of occasional single smooth muscle cells at the limit between propria and submucosa. The absence of lamina muscularis mucosa in the rumen has been reported in prenatal goats (Ramkrishna and Tiwari, 1979), lambs (Wardrop, 1961b) and buffalo (Sengar and Singh, 1969; Tiwari and Jamdar, 1970a). Ikemizu et al. (1994) have indicated that the condensed fibrous layer in the bovine rumen is not as thick and tight as the muscularis mucosa in the other parts of the digestive tract. They reported that the condensed fibrous layer in the bovine rumen is loose and consists not only of mature smooth muscle cells, but also of immature smooth muscle cells and myofibroblasts. Kitamura et al. (2003) have indicated that the muscularis mucosae in both reticulum and omasum of cattle, water buffalo, sheep, goat, Barbary sheep, Japanese serow, sika deer and mouse deer show immunoreactivity for both αSMA and γSMA. Nevertheless, the condensed fibrous layer in the rumen of these mentioned animals is immunoreactive only for αSMA.

Furthermore, the smooth muscle cells of the external muscle layer are immunoreactive for αSMA and γSMA whereas those of blood vessels and pericytes are only for αSMA.

1.2.5.2 Connexin 43 (CX43) expression in the rumen epithelium

The members of the connexin family are integral membrane proteins that forms hexamers called connexons. Most cells express two or more connexins. Gap junction channels are formed by docking of two connexons and are found at cell-cell appositions. Gap junction channels are responsible for direct intercellular transfer of ions and small molecules including propagation of inositol trisphosphate-dependent calcium waves (Saez et al., 2003). In transmission electron micrographs of ultrathin tissue sections, gap junctions appear as regions where the plasma membranes of adjacent cells closely approach each other, but appear to be separated by a small gap of 2-3 nm (Benedetti and Emmelot, 1965, Revel and Karnovsky, 1967). Connexin 43 is the most ubiquitously expressed vertebrate gap -junction protein (Saez et al., 2003). Gap junctions, connexins, and functional intercellular coupling have been identified in multiple portions of the mammalian gastrointestinal tract. Along the gastrointestinal tract, connexin 26 and Connexin 32 are found in many of the epithelial cells, whereas connexin 43 is found in some epithelial cells but in most of the smooth muscle cells (Saez et al., 2003). In human skin, connexin 43 is found to be the predominant gap junction protein expressed in the stratum granulosum and stratum spinosum; however, it is minimal in stratum basale or absent in the stratum corneum (Salomon et al., 1994). In human skin, immunoelectron microscopy has localized connexin 43 to close membrane apposition (e.g., pentalaminar junctions adjacent to desmosomes) and has shown that the cellular distribution mapped by immunofluorescence matched that immunoelectron microscopy (Tada and Hashimoto, 1997). All gap junctions contain the same proteins; however, they differ in their electrophoretic mobilities (21-70 KDa) of band detected by SDS-PAGE in gap junction-enriched preparation of different tissues (Henderson et al., 1979, Hertzberg and Gilula, 1979, Kistler et al., 1985). In bovine, western analysis confirmed expression of connexin 43 with a single band of ~ 43 KDa in the rumen epithelium. Plasma membrane connexin 43 immunostaining is most intense at the stratum granulosum and decreases in intensity throughout stratum spinosum and stratum basale. Meanwhile, the stratum corneum is negative. Pattern of staining is consistent with intercellular communication within and between the cells of the stratum granulosum, spinosum and basale, forming a syncytium (Graham and Simmons, 2005). Connexin 26 immunofluorescence was present in cells of the stratum granulosum and stratum spinosum, whereas the stratum corneum and stratum basale

were negative (Graham and Simmons, 2005). However, Shahin and Blankemeyer (1989) studied the morphology and distribution of the intercellular junctions in isolated skin of Rana pipiens using various electron-microscopic techniques. They demonstrated the presence of gap junctions and suggest that the distribution of gap junctions is not homogeneous among the epithelial strata. Gap junctions are less frequent in the stratum corneum and stratum granulosum than in the stratum spinosum and stratum germinativum. A key concept of the arrangement for a multicellular-stratified epithelium capable of active Na^+ transport (such as the frog skin) is that the epithelial barrier layer communicates with lower basal cell layers via low-resistance intercellular gap junctional pathway (Mills et al., 1977).

1.2.5.3 Na^+ / H^+ Exchanger type 3 (NHE3)

Na^+ / H^+ exchangers (NHE) of mammalian cells are plasma membrane intrinsic proteins mediating exchange of Na and H ions in various tissues. The NHE catalyzes the electroneutral transport of extracellular Na^+ for intracellular H^+. They play a major role in regulation of intracellular pH (pHi) in addition to transcellular absorption of Na^+, cell volume regulation, cell proliferation, adhesion, shape determination and migration (Grinstein et al., 1988; Yun et al., 1995a; Lang et al., 1998; Putney et al., 2002). Nine NHE isoforms (NHE1-9) have been cloned so far (Yun et al., 1995b; Noel and Pouyssegur, 1995; Ritter et al., 2001; Masereel et al., 2003; Goyal et al., 2003; de Silva et al., 2003). They are all similar in their primary structure and predicted to have N-terminal sequence, a highly hydrophilic C-terminal segment and 10-12 transmembrane domains (Wakabayashi et al., 1994). The cytoplasmic domain is highly divergent among all isoforms and contains several putative regulatory consensus sequences (Wakabayashi et al., 1994). Amiloride is a common NHE inhibitor and it is considered as a non specific inhibitor for many of them. Nevertheless, other specific inhibitors have been developed for each isoform.

There are many physiological evidences that support the existence of NHE in the rumen. Hypertonic buffer solution induces a decrease of the intracellular pH (pHi) in isolated ruminal cells, which could be due to inhibition of Na^+ / H^+ exchange, probably, NHE3 which is known to be inhibited by high osmotic pressure (Kapus et al., 1994; Nath et al., 1996; Wakabayashi et al., 1997; Schweigel et al., 2005). On the other hand, the intracellular pH (pHi) recovery from acidification by butyrate in the rumen tissues is reduced by more than 60% in the presence of NHE inhibitor (Müller et al., 2000). Recently, RT-PCR demonstrates expression of multiple NHE family members in the rumen tissue, including NHE1, NHE2, NHE3, and NHE8 (Graham et al., 2007). Immunostaining showed that NHE1 was predominantly

localized to the stratum granulosum, with a progressive decrease toward the stratum basale. NHE2 immunostaining was observed mainly at an intracellular location in the stratum basale, stratum spinosum, and stratum granulosum, meanwhile the study did not mention the localization of NHE3 (Graham et al., 2007).

NHE3 is present and active in recycling subapical endosomes (Biemesderfer et al., 1997; Biemesderfer et al., 1998; Janecki et al., 1998). It undergoes constitutive uptake into clathrin-coated vesicles and is recycled back to the plasma membrane in a phosphatidylinositol 3-kinase (PI3K)-dependent manner (Kurashima et al., 1998; Chow et al., 1999). The modulation of vesicular traffic of NHE3 could contribute to the regulation of Na^+ absorption across epithelia (D'Souza et al., 1998). A variety of hormones and physical parameters, such as osmolality, influence NHE3 function, thereby contributing to the fine control of electrolyte and fluid homeostasis (Wakabayashi et al., 1997; Orlowski and Grinstein, 2004). Noteworthy, the transport of the NHE3 protein and its insertion on the apical membrane has been reported to occur in short-term situations (Yang et al., 2000; Peng et al., 2001).

1.2.6 Physiology of the rumen epithelium

The rumen serves as a large fermentation vat in which micro-organisms (bacteria and protozoa) break down the ingested feed stuffs resulting in a product which mainly comprises of short chain fatty acids (SCFA; mainly acetic, propionic, and butyric acids) and ammonia. The rumen of sheep and cattle do not act solely to ferment and to prepare feed for subsequent digestion in the abomasum and intestine but also allow selective uptake of large quantities of nutrients. Therefore, the rumen should have efficient mechanisms to transport the product of microbial fermentation, especially SCFA (Stevens and Settler, 1966; Sehested et al., 1996; Gäbel and Sehested, 1997; Moller et al.; 1997; Sehested et al., 1999; Muller et al., 2002). Considerable amounts of intraruminally produced SCFA are metabolised during absorption by the epithelium (Kristensen et al., 1996), namely, the stratum spinosum (Baldwin et al., 1992). Noteworthy, the transport of SCFA is found to be higher in the rumen of concentrate-fed animals compared to hay-fed animals (Dirksen et al., 1984; Gäbel et al., 1987, 1991). The efficiency of nutrient transport across the epithelium depends to a large extend on the integrity and degree of keratinization of stratum corneum (Dirksen and Garry, 1987). Dirksen et al. (1985) described faster absorption of acetate, propionate and butyrate in animal with thick ruminal mucosa. It was assumed that structural changes of the rumen epithelium are associated with the corresponding effects on its function such as absorptive capacity and

SCFA metabolism (McGilliard et al., 1965). So, mucosal proliferation and parakeratosis are indications for physiological adaptations to the increased SCFA production (Dirksen and Garry, 1987). Moreover, in addition to the electrolytes in feed, large quantities of electrolytes are provided into the rumen with saliva. Rumen epithelium may develop spontaneous electrical potential differences and short circuit currents when isolated and clamped in Ussing chambers bathed in identical medium (Martens and Gäbel, 1988); thus absorption of many components is likely to be mediated by active and secondary active transport processes (Sehested et al., 1996). Interpretation of such rumen transport events has relied on the Koefoed-Ussing model (Koefoed and Ussing, 1958). The transport mechanisms of electrolytes such as Na^+, Cl^-, $HCO3^-$ and ammonia across rumen epithelium and the possible interactions with different nutrient components are the main topics in many studies. Consequently, a text book model for the transport mechanisms in the rumen has been well established. Na^+ is transported across the rumen epithelium by two mechanisms; electrogenic, which is amiloride insensitive and electronuetral one by NHE; amiloride sensitive (Martens and Gäbel, 1988). Interestingly, NHE works in parallel with HCO3/Cl exchanger (Martens et al., 1991). SCFA are absorbed as undissociated and as anion in exchange with HCO3, which a mechanism helps in buffering the ruminal milieu (Kramer et al., 1996). Amonia is transported into two forms: lipophilic form as NH3 and as $NH4^+$ via putative K channel (Abdoun et al., 2006). The rumen has several important physiological functions: absorption and transport of nutrients, metabolic activity, and protection.

1.2.7 Dietary effects on the morphology and physiology of the ruminal mucosa

At birth, the ruminant stomach is prepared for the digestion of milk. The relative proportions of stomach chambers are not the same as in adult. The abomasum is the predominant chamber and may already exceed 60% of the total weight of the stomach or comprise as much as 56% of the total organ weight at birth (Banks, 1986; Dyce et al., 2002). In contrast to abomasum, the rumen and reticulum of the newborn calf are very small; they are bypassed by milk (Dyce et al., 2002). Furthermore, the wall of the forestomach is thin and deficient in muscle, but their mucosa possess the characteristic adult features, especially in the rumen, where the papillae project barely 1 mm above the surroundings surface and are fused together at their bases (Banks, 1986). No striking changes in proportions and structure are to be observed before the young calf shows serious interest in solid food (2-3 weeks old; Dyce et al., 2002).

Postnatal development, including the increase of epithelial cell mass, is related to their mitotic rate and life-span (Sakata and Tamate, 1978c). In 1 to 4 week-old lambs, the papillary surface is relatively smooth and epithelial cell is relatively thin and flat. While in 6 to 10 week-old lambs, the tissue topography is typically rough (Zitnan et al., 1993). The length and surface characteristics of papillae change dramatically over the 10 week period post birth.

Generally, the development and growth of ruminal papillae and their metabolism are modulated by diet; due to mechanical stimuli provided by the roughage volume and chemical stimuli caused by short chain fatty acids, age of the animal and the time of weaning (Warner et al., 1955; Flatt et al., 1958; Loe et al., 1959; Harrison et al., 1960; Stobo et al., 1966; Banks, 1986; Anderson et al., 1987; Franco et al., 1992; Zitnan et al., 1999; Swan and Groenewald, 2000). An increase in dietary roughage accelerates the development of the rumen, whereas maintenance on milk or similar constituted diet retards its development (Brownlee, 1956; Banks, 1986). The early intake of hay and concentrate, which is connected with the development of microbial fermentation, might positively influence the functional development of the forestomach (Zitnan et al., 1999). On the other hands, the rate of development of ruminal fermentation is identical irrespective of weaning calves at 3, 5 or 7 weeks of age (Winter, 1985). The transition from the milk diet of the newborn lamb to feed with medium to high fiber concentration requires increased competence of the ruminal wall, giving rise to a two folds mechanism: strengthening of the mucosa (morphologically evident in epithelial expansion); and muscle contraction as a direct result of contraction of inner bundle. These phenomena govern the active growth of papillae that are initially rudimentary elements (Troner et al., 1971; Scott and Gardner, 1973; McGavin and Morril, 1976; Franco et al., 1992). The mean length, width and surface of papillae in early weaned animal increased with both age of the animal and the elevated intake of solid feed than in late weaned ones, whereas the number of papillae decreased (Zitnan et al., 1999; Swan and Groenewald, 2000). Moreover, the time of weaning can affect the development of different types of cell in the ruminal epithelia. In addition to type A and B horn cells (found in milk-fed calves), type C cells are found in the earlier weaned calves (Zitnan et al., 1999). In contrast to Zitnan et al. (1999), Lane et al. (2000) found that the delay of initiation of solid feed consumption results in rumen morphological development but does not stimulate rumen metabolic development.

Dietary effects on the rumen-papillae were also observed by scanning electron microscopy (McGavin and Morrill, 1976; Yamamoto et al., 1994). Ruminal papillae from milk-fed calves showed simpler structure than that of calves fed on roughages (McGavin and Morrill, 1976). On the other hand, Scheurmann and Weyrauch (1983) noted the possible effects of feeding on

the organization of the sub-epithelial connective tissues. They stated that the subepithelial connective tissues of starter-fed and milk-fed calves have a ride-like and a papillary organization, respectively.

The physical structure of the diet has been also reported to affect papillary size and shape, but does not influence the muscle thickness of rumen (Beharka et al., 1998). Roughage in adequate amounts and consistency is required to maintain the growth of the rumen epithelium and papillae in mature cattle, which effect is diminished if the roughage is ground (McGavin and Morrill, 1976). Thus, calves fed ground diet have shorter papillae with lesser total surface area than do calves fed ungrounded diet (Beharka et al., 1998). Feeding greater amounts of non-structural carbohydrates increases the absorptive surface of the rumen epithelium in calves (Zitnan et al., 1998). On the other hand, papillae from shopped-fed calves show a uniform in length and width but differ in shape (Nocek et al., 1984). Köhler et al. (1997) observed that the papillae and rumen musculature are better developed in calves from conventional bucket feeding.

A diet rich in concentrate, generally is associated with high concentration of SCFA and consequently, this will lead to increase the transport of SCFA across the ruminal wall (Dirksen et al., 1984; Gäbel et al., 1987, 1991), stimulating some aspects of rumen morphologic and metabolic development (Lane and Jesse, 1997; Dyce et al., 2002), leading to stronger development of rumen papillae than observed with diet based on forage (Weigand et al., 1975; McGavin and Morrill, 1976; Goodlad, 1981; Lane and Jesse, 1997; Zitnan et al., 1999; Swan and Groenewald, 2000).

SCFA affect enlargement of the papillae firstly, by increasing the mitotic division of stem cells in the stratum basale of the ruminal mucosa (Mentschel et al., 2001). Secondly, SCFA, butyrate in particular, act locally to increase the blood supply to the papillae, indicating the possibility of increasing the transfer of promoting factor, which may participate in rumen development (Thorlacius, 1972). It has been shown that under standard conditions, the mitotic index of the epithelial basal cells of the rumen does not exceed 1.0% and that it increases significantly after intraruminal administration of butyrate (Sakata and Tamate, 1976, 1978a, b), propionate and acetate (Sakata and Tamate, 1979). In addition, the acetate-propionat ratio can also influence the development of the rumen mucosa (Zitnan et al., 1998). However, both propionic and butyric acid stimulate much more papillary growth than did acetic acid (Sander et al., 1959). Nevertheless, butyric acid is far more effective than propionic acid (Sander et al., 1959; Sutton et al., 1963; Kauffold et al., 1977). The difference between these two fatty acids could be mainly explained by different apoptotic rates, which is only one third for

butyric acid compared to propionic acid (Mentschel et al., 2001). Therefore, this explains the differential effect on papillary length caused by the two fatty acids (Mentschel et al., 2001). Butyrate in particular appears to be the most potent SCFA in promoting the development of rumen epithelium (Sander et al., 1959; Tamate et al., 1962; Sutton et al., 1963; Kauffold, 1975; Kauffold et al., 1977; Sakata and Tamate, 1978c). This finding has been also supported by the demonstration of an increased mitotic index and a decreased cell deletion (apoptosis) in sheep fed barley (Tamate and Fell, 1977). Nevertheless, the opposite is found in animals fed hay (Tamate and Fell, 1977). Hofmann and Schnorr (1982) described changes in the barrier layer (flattened horn cells) in relation to diet. The barrier layer of the rumen epithelium becomes thicker in winter or in dry seasons when animals eat food with large amounts of fibre. On the other hand, Nocek et al. (1984) found that the ruminal epithelial lining is thicker and shows more vaculation of stratum granulosum in concentrate-fed animals and has more mucosa to muscle compared to rumen of calves fed ground or chopped hay. However, papillary length, number of papillae per field, and surface area per field are greater in calves fed hay.

Generally, the morphology of the mucosal structures of the ruminant forestomach is related to the feeding habits of the different species, which can be classified into three categories: concentrate selectors, grass-roughage eaters and an intermediate type (Hofmann, 1973, 1985, 1988). In addition, seasonal changes and dietary effects on the mucosal morphology have been reported in red deer (König et al., 1976) and in domestic cattle (Warner et al., 1956; Flatt et al., 1958; Tamate et al., 1962). In winter, when only a high-fibres and low-nutrient diet is available, the ruminal papillae on the dorsal sac, in particular, decrease in number (Hofmann and Schnorr, 1982; Hofmann, 1985, 1988). In wild ruminants striking changes in papillary prominence, size, and thus in the ruminal surface area, accompany seasonal changes in forage quality. In contrast, in domestic species, whose diet is subjected to human influence to a greater degree, changes in papillary development tend to be more restrained (Dyce et al., 2002).

Alterations of the rumen epithelium caused by inadequate roughage intake or intake of concentrate alone have been described by several workers (Jensen et al.,1958; Ward, 1962; Hinders and Owen, 1965; Oltjen and Davis, 1965; Hentges et al., 1966; Nockels et al., 1966; Rowland, 1966; Haskins et al., 1967; Fell et al., 1968; ElSabban et al., 1971; Berg and Edvi, 1976; McGavin and Morrill, 1976; Nocek et al., 1984; DiGiancamillo et al., 2003). These morphological changes have revealed abnormalities, such as: presence of small, nodular papillae, unusual branching of papillae, excessively long, dark and unhealthy appearing

papillae, papillae with irregular length and shape, ruminal parakeratosis, extensive clumped-papillae with embedded hair, feed particles or lesions on their tips, reduction or crusting of papillary tips, excessive pigmentation, an unfavourable ratio of the thickness of the rumen epithelium to the thickness of the papillary body, a decrease in the level of metabolic activity and pronounced lymphocytic infiltration. Among all these observations hyperkeratosis and clumped papillae are the most reported. Moreover, absorptive capacity of calves fed only concentrate diets decreases compared to calves fed diets with 40% hay (Nocek et al., 1984). Noteworthy, Nockels et al. (1966) found that a scabrous textured roughage is needed to prevent accumulation of access keratin, feed residues, and hair on the surface of mucosa and thus to prevent the growth of clumped papillae. Importantly, to avoid the abnormalities in the rumen (e.g. ruminitis) the fibre content of the ration must not be less than 12% of the ration (Berg and Edvi, 1976).

The morphological and functional adaptations of the rumen epithelium due to change in diet, particularly evident in atrium, are rapid (4-6 days or 2 to 3 weeks) and are reversible (Wardrop, 1961b; Palmquist and Ronning, 1963; McGavin and Morrill, 1976, Gäbel et al., 1987). Noteworthy, the atrium ruminis is the last part of the rumen to show papillary changes (from tongue-shaped to nodular-shaped) in response to feeding regime (McGavin and Morrill, 1976).

Conclusion for the present study

The rumen epithelium exhibits diet dependent alterations of histology and transport properties. However, little is known about the time course of the alterations. It was the aim of the present study to follow morphological adaptations to a change of diet. Therefore, the gross morphology of the ruminal papillae including their shape, color and clumping of the papillae was determined. Light microscopy was used to study the histology of the ruminal papillae including their shape, sides, apex; the fodder precipitations on the papillae and to calculate the number of cells constitute the different epithelial strata. S.E.M was used for observations of surface structures, with attention paid to grooves covering papillary surface and the surface topography of the flattened horn cells. Quantative morphometrical analysis was carried out for measurements of number, length and width of papillae with calculation of the total surface of papillae. On the other hand, qualitative morphometrical analysis was carried out for measurements of the thickness of the different epithelium strata; length of papillary body and the relation between dead horny and living epithelial cells. T.E.M was applied to study the differences in the fine structure of different epithelial strata between the different sheep groups. Immunohistochemical staining was done for detection of NHE type 3, gap junction-connexin 43 and lamina propria-α smooth muscle actin.

Well developed differences among groups were evident in sheep fed concentrate for 4 to 6 weeks; changes in 12 weeks were similar, but less developed.