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INDIGESTION IN YOUNG CALVES

IV. LESIONS OF RUMINAL PAPILLAE IN YOUNG CALVES FED BARLEY AND BARLEY PLUS HAY

By

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LANDSVERK, THOR: *Indigestion in young calves. IV. Lesions of ruminal papillae in young calves fed barley and barley plus hay.* Acta vet. scand. 1978, 19, 377—391. — Fiftysix calves, in 2 similar experiments, were given 1 of 4 diets including a high lactose milk replacer: ground barley (GB), ground barley plus coarse hay (GB+CH), coarse hay (CH) and the liquid diet only (NS); the 2 last diets were held as controls. The calves were on the experimental diet from 1—2 weeks of age until slaughtered at 7—8 weeks of age. As observed by stereomicroscopy, the ruminal papillae in calves fed on GB were thickened, mostly finger- or club-shaped and sometimes nodular; nodular papillae were always arranged on prominent mucosal folds. Scattered nodular papillae had white coats. For calves fed on GB+CH the papillae were mostly finger- or tongue-shaped. Histologically papillae in calves fed on GB had hyperplasia of secondary papillae and epithelial layers. Microabscesses associated with penetrating hairs were frequent, and some calves had dyskeratosis with infiltration of polymorphonuclear leucocytes at the apex of papillae, identified as the white coats. For calves given GB+CH, abscesses and apical dyskeratosis were absent and the hyperplasia of epithelial layers milder than in the case of the GB diet. An ameliorative effect of hay on papillary changes caused by barley is indicated.

barley; barley plus hay; rumen; papillary lesions;
calves.

In ruminants, various low fiber concentrate diets including barley, are known to cause thickened ruminal papillae with hyperkeratosis (*Jensen et al.* 1954, 1958, *Gilliland et al.* 1962, *Fell et al.* 1968, *McGavin & Morrill* 1976). Calves in their first weeks of life seem to develop more severe papillary deformation than do older calves when fed a low fiber concentrate diet (*McGavin & Morrill*). In a previous paper, *Laksesvela et al.* (1977) reported ruminal hyperkeratosis and ulceration in 7—8 weeks old calves on barley diets, and less hyperkeratosis on bar-

ley plus hay diets. This paper describes the gross, stereomicroscopic and histologic appearance of the ruminal papillae in 2 of the experiments in order to elucidate changes induced by barley in calves given the barley diet in the first weeks of life, and possible modifications by barley plus hay diets.

MATERIALS AND METHODS

Parts of the experimental procedure have been reported in previous papers (*Slagsvold et al.* 1977, *Laksesvela et al.* 1977). In this study, 56 calves comprising the 2 similar Exps. 1 and 2 (*Laksesvela et al.*), were submitted to further examination. All diets contained a high lactose milk replacer; the one denominated I in a previous publication (*Slagsvold et al.*), consisted of 41.4 % skim milk powder, 14 % sweet whey powder, 30 % lactose, 13.3 % butter plus minerals and vitamins. The total intakes of barley and hay on each diet are given in Tables 1 and 2. Ground barley (GB) and ground barley plus hay diets (GB+CH1 and GB+CH2) were considered experimental, diets with coarse hay (CH1 and CH2) and liquid diet only (NS) were held as controls.

Specimens were taken from the ventral part of atrium ruminis, fixed in 10 % buffered neutral formalin and stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS), methylene blue and von Kossa. For stereomicroscopic examinations, paraffin-embedded specimens from Exp. 1 were deparaffinized in xylol and air-dried after being equilibrated in acetone. In Exp. 2, an additional 20 cm² specimen from the ventral part of atrium ruminis was taken, attached to dental wax and fixed as above. These specimens were inspected and photographed submerged in distilled water and formalin. As a control of the procedure, paraffin-embedded specimens from Exp. 2 were treated in the same way as in Exp. 1. Some contraction of the tissue was seen, possibly due to dehydration. However, the general conformation was retained allowing classification according to the outlines used. In addition, the drying procedure diminished light penetration of the tissue and greatly improved contrast.

RESULTS

The results from the gross, stereomicroscopic and histologic examinations are recorded in Tables 1 and 2.

Table 1. Exp. 1. Stereomicroscopic and histologic examination of papillae in atrium ruminis, and intake of barley and hay.

Diet	Ground barley (GB)										Ground barley and coarse hay (GB + CH1)					Coarse hay (CH1)	Liquid diet only (NS)
	8	10	17	19	23	29	1	6	7	18	26	50	All	All			
Calf No.	8	10	17	19	23	29	1	6	7	18	26	50	All	All			
Total intake, kg	18.2	14.4	13.1	16.0	18.5	3.9	16.5	17.1	15.0	13.6	13.6	19.7	—	—			
Ground barley	—	—	—	—	—	—	4.2	4.2	2.0	4.2	3.5	6.6	$\bar{x} = 11.2$	—			
Coarse hay	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
Stereomicroscopic examination of papillae ¹																	
Nodular	—	—	—	+	+	+	—	—	—	—	—	—	—	—			
Thickened, club-, finger- or tongue-shaped	+	+	+	—	—	—	+	+	+	+	+	+	—	—			
Slender, leaf-shaped	—	—	—	—	—	—	—	—	—	—	—	—	+	—			
Small, conoid	—	—	—	—	—	—	—	—	—	—	—	—	—	+			
Histologic examination ²																	
Thickened stratum corneum	(+)	(+)	(+)	+	+	+	(+)	+	+	(+)	(+)	+	—	—			
Hyperplasia of stratum basale and spinosum	+	+	+	+	+	+	(+)	+	+	+	+	(+)	—	—			

¹ No grading was performed.

² — inapparent, (+) slight, + moderate, ++ pronounced.

Table 2. Exp. 2. Gross, stereomicroscopic and histologic examination of ruminal mucosa and intake of barley and hay.

Diet	Ground barley (GB)										Ground barley and coarse hay (GB+CH2)					Coarse Liquid hay diet only (CH2) (NS)			
	18	35	37	38	39	41	46	55	8	16	26	40	42	43	47	54	All	All	
Calf No.	18	35	37	38	39	41	46	55	8	16	26	40	42	43	47	54	All	All	
Total intake, kg	12.2	10.8	9.7	8.3	11.5	8.3	9.6	6.2	6.4	6.0	3.2	5.5	3.8	5.9	5.0	5.0	—	—	
Coarse hay	—	—	—	—	—	—	—	—	5.1	5.4	4.4	5.8	5.4	6.5	5.4	5.0	—	9.36	
Gross examination¹																			
Hard and blunt papillae in atrium ruminis	+	+	+	+	+	+	+	—	(+)	—	—	—	(+)	(+)	—	+	—	—	
Hard and blunt papillae in other ventral and caudal rumen compartments	+	+	+	+	+	+	+	+	+	(+)	—	(+)	+	+	(+)	+	—	—	
Prominent ridges in atrium ruminis	(+)	+	+	—	+	+	(+)	—	—	—	—	—	—	+	—	+	—	(+)	
Prominent ridges in other ventral and caudal rumen compartments	+	+	+	+	+	+	+	+	+	(+)	—	(+)	+	+	+	+	—	(+)	
Stereomicroscopic examination of papillae²																			
Nodular	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Thickened, club-, finger- or tongue-shaped	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	
Slender, leaf-shaped	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	
Small, conoid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	
Histologic examination¹																			
Thickened stratum corneum	(+)	+	+	+	+	+	—	(+)	(+)	—	—	—	(+)	—	(+)	(+)	—	—	
Hyperplasia of stratum basale and spinosum	+	+	+	+	+	+	(+)	(+)	(+)	—	—	—	(+)	—	(+)	(+)	—	—	

¹ — inapparent, (+) slight, + moderate, ++ pronounced.

² No grading was performed.

Gross examination

In Exp. 1, gross findings, apart from those previously published (*Laksesvela et al.* 1977), are not described. In Exp. 2, ruminal mucosa was grey-black in calves fed on GB, brown-grey for GB+CH₂, brown-yellow for CH₂ and yellow-white for NS. Papillae in the GB group were blunt and hard, and the mucosa often had conspicuous ridges (Fig. 1). Papillae were shorter and ridges more prominent in ventral, ventro-caudal and dorso-caudal rumen sacs than in atrium ruminis. Dorsal parts of rumen were unaffected. In the GB+CH₂ group the changes were similar to those observed in calves on GB diet, but less pronounced. In the CH₂ group papillae were delicate and pliable while for the NS group the papillae were small, and there were low pliable folds in the ruminal mucosa.

Stereomicroscopy

Papillae in the specimens from animals on GB diets were nodular, club-, finger- or tongue-shaped, and their surfaces, except in nodular papillae, had small convolutions (Figs. 2, 3, 6, 7). The papillae were sometimes arranged on prominent ridges (Figs. 1, 6, 7). In 2 calves fed GB in Exp. 2, scattered nodular papillae were covered with conspicuous white coats (Fig. 2). Corresponding specimens from Exp. 1 were difficult to evaluate in this respect, because of the different tissue-processing. Hairs were frequently stuck into the papillae (Fig. 6). In animals fed GB+CH, nodular papillae were absent; the appearance of the papillae varied on a sliding scale, from club- to tongue-shaped, the form usually being closer to tongue-shaped than for the GB groups. A few hairs were found in the GB+CH₁ samples while for GB+CH₂ they were absent. Papillae in the CH groups were flat, leaf-shaped and pointed (Figs. 4, 8), whereas papillae in the NS groups were small and conical (Fig. 5).

Histologic examination

The macroscopically observed ridges in calves fed GB diets consisted of folds of submucosa with papillae extending from the lamina of connective tissue. There was an extensive branching of the secondary papillae (Fig. 9). In cases with pronounced epithelial hyperplasia, present especially in nodular papillae, the nuclei of the cells in stratum basale and spinosum were round

and vesicular, and acanthosis was demonstrated. The cells of stratum corneum were mostly flattened and nucleated, and hyperkeratotic areas were seen intermittently, especially at the basis between the papillae (Fig. 16). Penetration of animal hairs was frequent, with micro-abscess formation in the epithelium, lamina propria and submucosa (Fig. 16). Proliferation of fibroblasts and capillaries and a sparse mononuclear cell infiltration in lamina propria and submucosa were sometimes also seen unrelated to abscesses.

The papillae with apical white coats in Exp. 2, and some of the other papillae in the GB groups, showed excessive thickening of polyhedral, sharply outlined cells with a pale, eosinophilic cytoplasm. These cells constituted the stratum corneum analogous layer (Figs. 13—15). The cells had distinct intercellular bridges, and round, well outlined nuclei showing incipient karyolysis. The nuclei were lying in vacuoles which occasionally included the whole cytoplasm. The cells showed the same lack of affinity for methylene blue as stratum corneum in lower parts of the papillae. Abscess formation could be seen centrally in the lesions and penetrating hairs were occasionally observed. In less advanced lesions, pure vacuolation occurred above stratum spinosum (Fig. 15). There was usually a layer of flattened cells above the stratum spinosum where this zone was not obscured by necroses (Fig. 15). The cells of the stratum corneum analogous layer in the lesions differed distinctly from the cells of stratum corneum in the lower parts of the papillae showing secondary swelling. Occasionally, wart-like protrusions of such cells were observed. Generally, the cells showing ordinary secondary swelling were more homogenous, had less marked cell borders and poorly outlined, dotted nuclear debris.

Papillae in the GB+CH groups showed hyperplasia of the secondary papillae (Fig. 12). The hyperplasia of the epithelium was less than for the GB diets. In cases with mild epithelial hyperplasia, stratum corneum was moderately thickened and showed secondary swelling especially between the secondary papillae. Calcification of cells showing secondary swelling was occasionally seen. Papillae for calves on the GB+CH diets showed neither micro-abscesses nor the afore-mentioned apical lesions. Fibroblast proliferation was, however, sometimes seen in lamina propria.

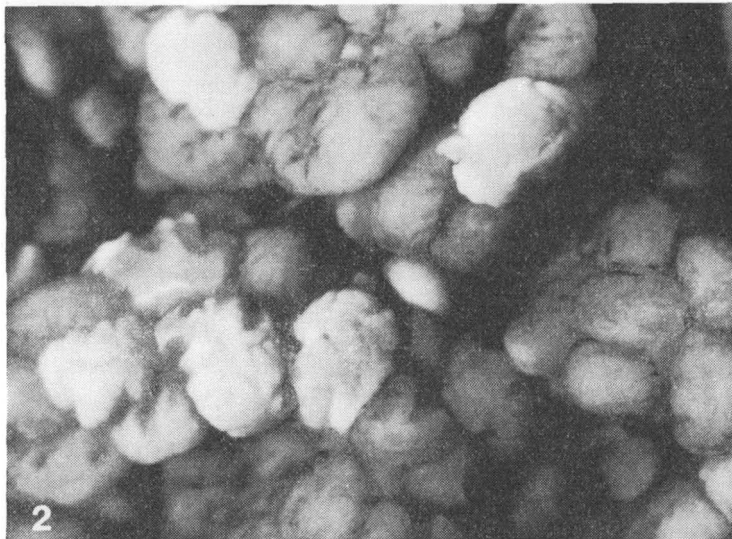
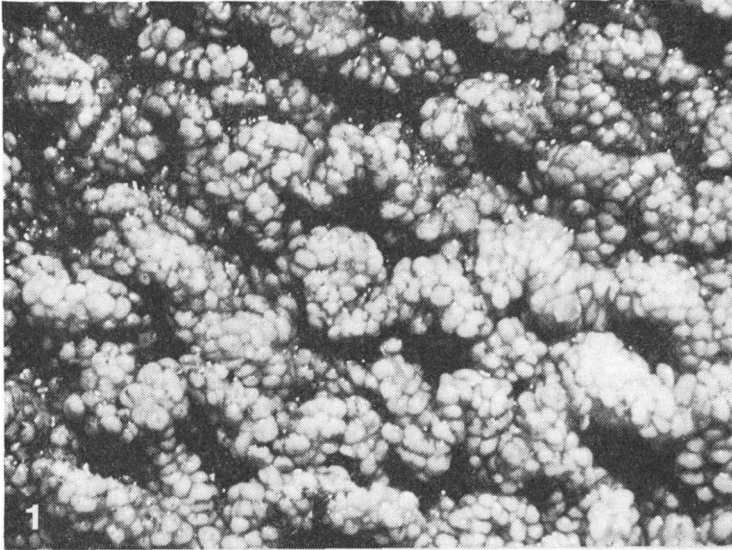


Figure 1. Diet GB, Exp. 2, calf 37, atrium ruminis. Nodular papillae on prominent mucosal ridges. $\times 2.5$.

Figure 2. Diet GB, Exp. 2, calf 37, atrium ruminis. Nodular, hyperkeratotic papillae and some papillae with white dyskeratotic coats. $\times 16$.

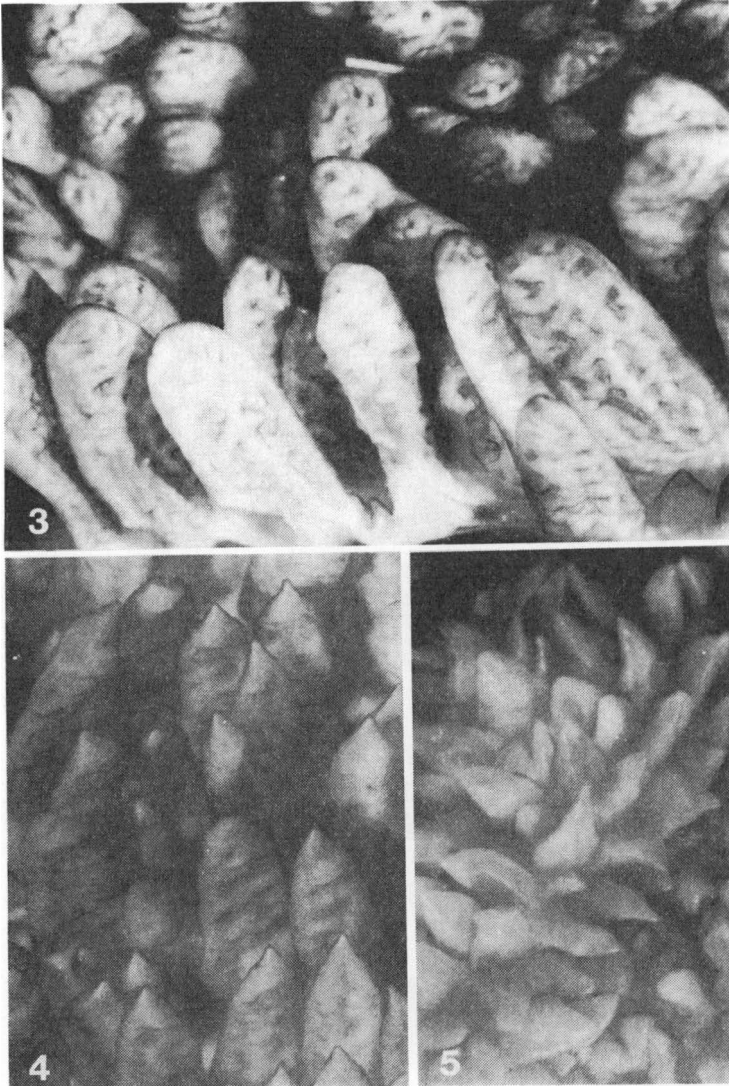


Figure 3. Diet GB+CH2, calf 8, atrium ruminis. Thickened, tongue-shaped papillae. $\times 16$.

Figure 4. Diet CH2, calf 29, atrium ruminis. Normal, leaf-shaped papillae. $\times 16$.

Figure 5. Diet NS, Exp. 2, calf 6, atrium ruminis. Underdeveloped, conoid papillae. $\times 16$.

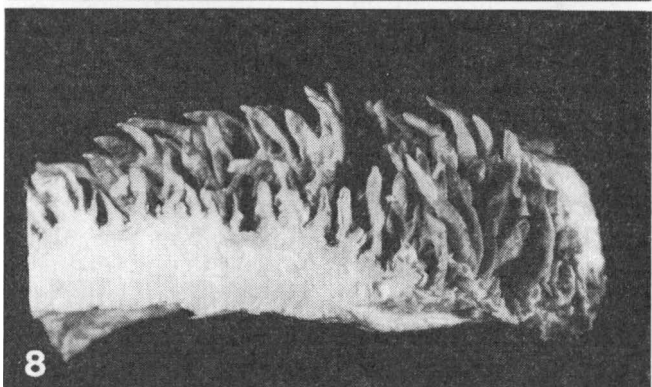
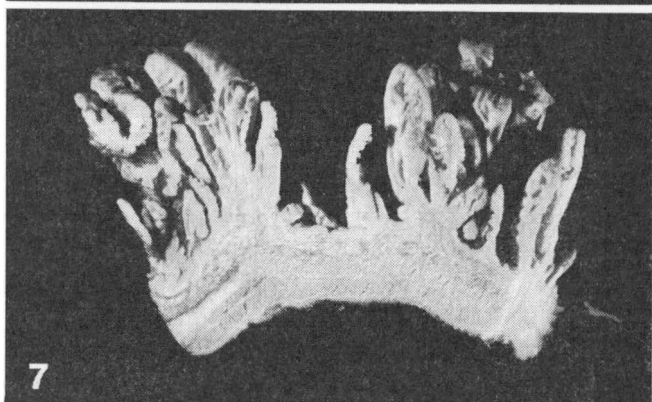
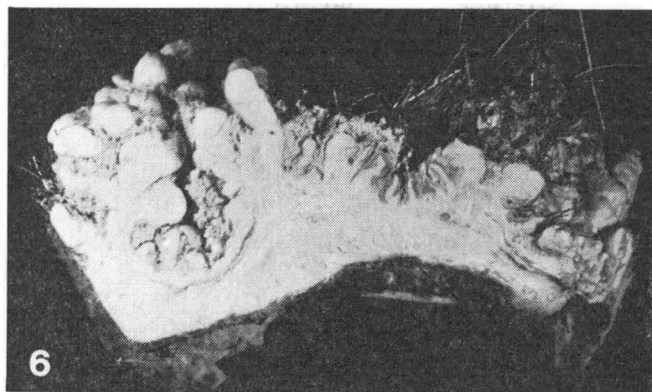


Figure 6. Diet GB, Exp. 1, calf 29, atrium ruminis. Nodular, hyperkeratotic papillae on mucosal folds and penetrating hairs. $\times 6$.

Figure 7. Diet GB, Exp. 1, calf 8, atrium ruminis. Thickened, slightly hyperkeratotic, club-shaped papillae on mucosal folds. $\times 6$.

Figure 8. Diet CH1, calf 12, atrium ruminis. Normal, leaf-shaped papillae. $\times 6$.

For animals on diets CH and NS, the papillae had short secondary papillae (Figs. 10, 11). The epithelium was normal; the cells of stratum corneum for the CH groups being swollen, whereas those in the NS groups were mostly flattened. Superficial cells of stratum corneum in the CH diets were frequently calcified. Papillae for animals on the NS diets were distinctive from the other diets in showing considerably lower incidence of primary swelling. Lamina propria in the CH and NS groups showed frequent proliferation of fibroblasts and capillaries.

Figure 9. Diet GB, Exp. 1, calf 29, atrium ruminis. Nodular papillae with proliferation of secondary papillae and epithelial hyperplasia. HE \times 68.

Figure 10. Diet CH1, calf 12, atrium ruminis. Normal, leaf-shaped papillae with short secondary papillae. HE \times 68.

Figure 11. Diet NS, Exp. 1, calf 25, atrium ruminis. Conoid, underdeveloped papillae with fibroblast proliferation in lamina propria. HE \times 68.

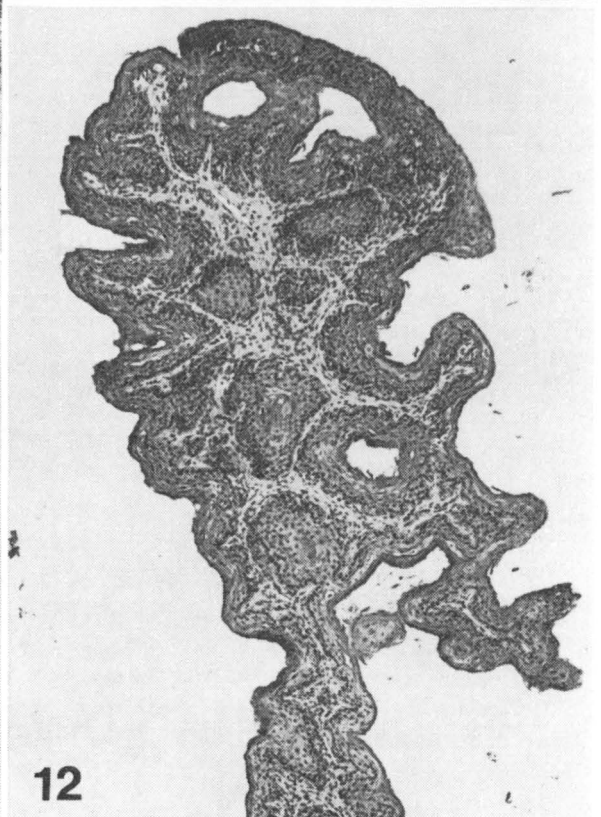
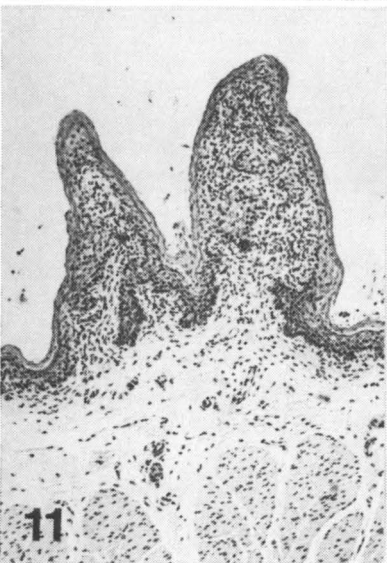
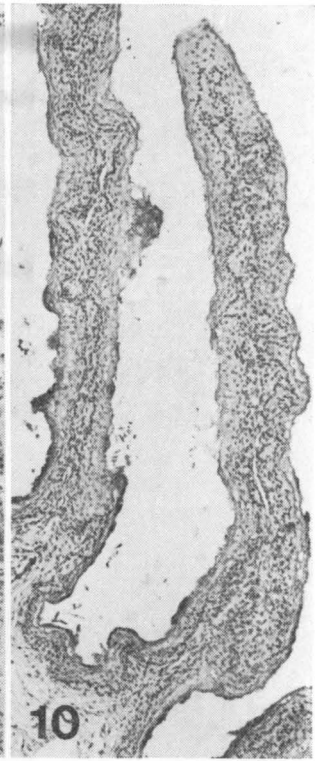
Figure 12. Diet GB+CH2, calf 8, atrium ruminis. Tongue-shaped papillae with lengthening of secondary papillae, epithelial hyperplasia being inapparent. HE \times 68.

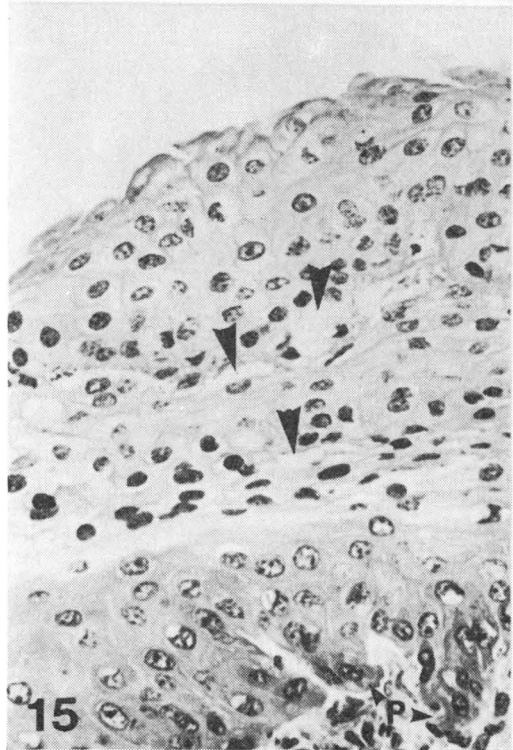
Figure 13. Diet GB, Exp. 2, calf 37, atrium ruminis. Papillae with apical coat or dyskeratosis and abscess formation in the lesion. HE \times 68.

Figure 14. Enlarged superficial area of Fig. 13. Cell borders and nuclei are mostly sharply outlined and nuclei are lying in vacuoles. Lower right: Cell destruction in the periphery of the abscess. HE \times 680.

Figure 15. Diet GB, Exp. 2 calf 39, atrium ruminis. Apical dyskeratosis and vacuolation (large arrows) of the epithelium. Flattened cells above stratum spinosum and small, irregular rete pegs (P). HE \times 425.

Figure 16. Diet GB, Exp. 1, calf 29, atrium ruminis. Nodular papillae with abscess formation in submucosa and hairs (arrows) in hyperkeratotic layers at the basis of the papillae. HE \times 68.





DISCUSSION

The nodular papillae seen in calves on GB diets in this investigation resembled those described by *McGavin & Morrill* (1976) in calves on low fiber concentrates. Nodular papillae have not been described for animals fed a barley diet, and may, in our case, be due to the younger age of the calves than those used by *Fell et al.* (1968). The more frequent club- to tongue-shaped papillae seen in calves on GB and GB+CH diets apparently represent less advanced stages.

Our findings that papillar changes are most pronounced in ventral and caudal rumen sacs are in accordance with the observations of *McGavin & Morrill*. Samples from atrium ruminis in young calves may not, therefore, be entirely typical for other rumen compartments. However, *McGavin & Morrill* state that sequential changes of papillar shape are best observed in atrium ruminis, and in the present Exp. 2 it seemed that individual differences were greatest in this region.

The epithelial hyperplasia described for calves on barley diets is in agreement with the description of *Fell et al.* In pronounced cases, there is a concomitant hyperplasia of inner epithelial layers and stratum corneum. As stratum corneum may be easily lost during tissue processing (*Gilliland et al.* 1962), the associated changes in the inner layer are preferably taken into account when evaluation is given. The proliferation of secondary papillae noted here, and by *McGavin & Morrill*, appears to be an important cause of the changes in gross and stereomicroscopic appearance of the papillae. Several papillae, characterized as blunt or finger-shaped, showed slight or no additional epithelial hyperplasia. The proliferation of secondary papillae, best assessed through the stereomicroscopic papillar appearance, seems to be the most sensitive index of the influence of barley.

There are no clear descriptions in the literature of lesions similar to those observed at the apex of papillae on GB diets. *Fell et al.* mentioned, without any further comments, apical parakeratosis with diffuse leucocyte infiltration of papillae in calves on barley diets. We wish to avoid the term parakeratosis in connection with rumen epithelium, as nuclear remnants, also in the control groups, remained stainable throughout stratum corneum, confirming the description of *Hauser* (1929) and *Henriksson & Habel* (1961). For the lesion described in this study, we prefer the term dyskeratosis, as we interpret the term as covering

any disturbance in the process of keratinization in stratified epithelia. A defective keratinization is probable, both because of the abnormal appearance of the outer epithelial cells in these lesions and the frequent abscess formation, possibly caused by a reduced resistance of the epithelium to invading bacteria.

Proliferation of fibroblasts in lamina propria in calves on barley diets has been reported previously (*Fell et al.*). In this investigation similar fibroblast proliferation was also found in the control groups, possibly due to the immature state of the papillae in the young calves.

The high lactose milk replacer used in these experiments was originally designed to provoke diarrhoea (*Laksesvela et al. 1977*). It is not very likely that the ruminal changes found in calves on GB and GB+CH diets were modified by the milk replacer used, as calves on the NS diets had underdeveloped but otherwise normal rumens.

As previously reported (*Laksesvela et al.*), a rise of rumen pH was noted in calves on GB+CH diets in the last week of the experiments, while the pH remained low on GB diets. In Exp. 1 this occurred in spite of a similar intake of barley in calves on the GB+CH1 diet. The amelioration seen in morphologic changes when feeding GB+CH may accordingly be a direct effect of hay, the rise in rumen pH being a possible effector mechanism. This coincides with the findings of *Kay et al.* (1969) who noted that papillary changes induced by barley were almost abolished by feeding bicarbonate simultaneously. The graded response to various levels of barley intake in calves on GB diet in Exp. 2 indicate a relationship between the quantity of barley ingested and papillary changes.

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SAMMENDRAG

Indigestion hos unge kalver. IV. Lesjoner i vompapillene hos unge kalver føret med bygg og høy.

Femtiseks kalver i 2 like forsøk ble gitt én av 4 dietter inkludert laktoserik mjølkeerstatning: Byggrøpp (GB), byggrøpp og grovt høy (GB+CH), grovt høy (CH) og bare mjølkeerstatning (NS). Kalvene stod i forsøket fra 1—2 til 7—8 ukers alder da de ble slaktet. Stereo-mikroskopisk var vompapillene hos kalver gitt GB for det meste finger- og kølleformede eller nodulære; nodulære papiller var alltid plassert på folder i mucosa. Apex av enkelte nodulære papiller var dekket av hvite kapper. Hos kalver gitt GB+CH var papillene mest finger- eller tungeformede. Histologisk hadde kalver gitt GB hyperplasi av sekundærpapillene og epitelet. Penetrasjon av hår med mikroabscessdannelse var hyppig, og noen kalver hadde dyskeratose med infiltrasjon av polymorfkjernede leukocytter på toppen av papillene; denne lesjon viste seg å være identisk med de hvite kapper. Vompapillene hos kalver på GB+CH diett hadde ikke abscessdannelse eller dyskeratose og hyperplasien av epitelet var mindre enn på GB diett. Høy synes å motvirke den skadelige effekten av bygg på vompapillene.

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