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INTRARUMINAL ADMINISTRATION OF MILK IN THE CALF AS A MODEL FOR RUMINAL DRINKING: MORPHOLOGICAL AND ENZYMATICAL CHANGES IN THE JEJUNAL MUCOSA

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ABSTRACT

Van Weeren-Keverling Buisman, A., Mouwen, J.M.V.M., Wensing, T. and Breukink, H.J., 1990. Intraruminal administration of milk in the calf as a model for ruminal drinking: morphological and enzymatical changes in the jejunal mucosa. *Veterinary Research Communications*, **14 (2)**, 129–140

In order to develop a calf model for studying the syndrome of ruminal drinking (RD) in veal calves, three dual-fistulated calves were used to test the effect of intraruminal administration of milk replacer on the jejunal mucosa. Biopsies of the proximal jejunal mucosa were taken through a jejunal fistula and the mucosal morphology and the activities of two brush border enzymes, lactase and alkaline phosphatase, were determined.

Means of villus length and brush border enzyme activities decreased during the period of intraruminal administration of milk. The hyperplastic villus atrophy in this model was similar to that found in chronic RD patients in previous studies. This could not be associated with isolation of pathogenic micro-organisms from the faeces and is probably the consequence of the intraruminal milk feeding procedure itself.

Clinical recovery from the signs of RD occurred rapidly after intraruminal administration of milk ceased and was followed by restoration of villus length and brush border enzyme activities 3–4 weeks later.

Keywords: calf, enzymes, jejunum, milk, morphology, ruminal drinking

INTRODUCTION

Ruminal drinking (RD) calves are known to have an insufficient reticular groove reflex while drinking milk. This causes deposition of a substantial amount of milk into the rumen (Breukink *et al.*, 1988). During the retention of this milk in the rumen, fermentation takes place and volatile fatty acids and lactate are produced. A distinct reduction in pH is also noticed (van Weeren-Keverling Buisman *et al.*, 1990). At necropsy of chronic RD calves, hyperkeratosis of the rumen is found (van Bruinessen-Kapsenberg *et al.*, 1982; Breukink *et al.*, 1988). Chronic RD calves usually have a reduced growth rate and are small for their age (van Bruinessen-Kapsenberg *et al.*, 1982), apparently the result of disturbed energy and protein balance (Breukink *et al.*, 1988). The latter may be partially due to inappetence but may also be affected by jejunal villus atrophy (de Visser and Breukink, 1984; Breukink *et al.*, 1988; van Weeren-Keverling Buisman *et al.*, 1988a) and brush border enzyme deficiency (de Visser and Breukink, 1984).

The purpose of this study was to determine the influence of intraruminal administration of milk on the morphology of the villi of the proximal jejunum. Information about the functional capacity of the small intestinal epithelium was obtained by determining the activities of the brush border enzymes lactase and alkaline phosphatase (AP).

MATERIALS AND METHODS

Calves

Three clinically normal male Friesian Holstein calves (E, H and S) were used. The housing and feeding regimens were as described by van Weeren-Keverling Buisman *et al.* (1990). They were fed commercial milk replacer twice a day and no additional solid food was given. In week 4 of the fattening period, a cannula was placed in the proximal jejunum under general anaesthesia (van Weeren-Keverling Buisman *et al.*, 1988b). Procain penicillin 10 000 IU/kg and dihydrostreptomycin 10 mg/kg were administered intramuscularly twice daily for four days postoperatively. A rumen cannula was placed under general anaesthesia during week 6 (van Weeren-Keverling Buisman *et al.*, 1990) and this was followed by 20 mg/kg intravenous sodium ampicillin every 8 h for 24 h. This second cannula was used for the intraruminal administration of milk.

Experimental design

The experiment was divided into three periods, the pre-induction period, the induction period and the recovery period. In the pre-induction period (weeks 1–6), during which both operations took place, a commercial milk replacer (Tentofeed I, Tentego BV, Mijdrecht, The Netherlands) containing 23.5% protein (mainly skim milk powder), 16.5% fat, 7.3% ash and 42.9% lactose was administered by an open bucket. In the induction period, from week 7 onwards, the milk replacer was administered intraruminally in increasing proportions until the faeces became clay-like. In week 11, 50% of the ration was infused into the rumen twice daily, the remaining portion being fed from an open bucket. In the recovery period the entire milk replacer diet was again consumed from an open bucket. In calves H and S the induction period was five weeks. In calf E, the induction period was prolonged by two weeks, during which time the percentage of intraruminally administered milk replacer was raised to 63%. Consequently, the recovery period in this calf started two weeks later. During all three periods, milk replacer from the same batch of powder was fed.

Jejunal biopsies

The first sample of the proximal jejunal mucosa was taken in week 4 of the fattening period during the insertion of the cannula. From week 5 onwards, jejunal biopsies were taken twice a week throughout all three experimental periods 40 to 70 cm caudally from the intestinal fistula, using a Crosby capsule (Laméris, Utrecht, The

Netherlands) (van Weeren-Keverling Buisman *et al.*, 1988b). Data obtained in the different experimental periods were compared within each calf.

Morphological studies

The jejunal biopsies were fixed in 4% buffered formalin. Stereomicroscopic examination of all biopsies was carried out with a Zeiss stereomicroscope at a magnification of 10–20× in order to determine the shape of the villi (Mouwen, 1971). Thin slices of the biopsies perpendicular to the mucosal surface were then embedded in paraffin and serial sections, 5 μm in thickness, were stained with haematoxylin and eosin. The lengths of 10 full-length villi and crypts were measured with an ocular micrometer (Mouwen, 1972). The same 10 crypts were used to determine the index of mitosis, so as to indicate the proliferative status of the crypt epithelium. The number of mitotic cells in metaphase and anaphase was counted and the total number, multiplied by 100, was divided by the total crypt depth in μm.

Chemical analysis

The other jejunal biopsies (one biopsy from each week) were immediately frozen and stored in liquid nitrogen at −196°C during the experimental period. The biopsy was homogenized (IKA-Thyristor speed controller TR50, Janke and Kunkel, Ika Werk, Stauben, FRG) for 3 min in 2 ml of buffer solution (50 mmol/L mannitol and 2 mmol/L tris at a pH of 7.1), in melting ice. The homogenate was centrifuged at 15,000 rpm for 5 min at 4°C (Eppendorf 5414S, Merck BV, Amsterdam, The Netherlands) and the supernatant was stored at −70°C for up to two days prior to analysis.

Determination of the protein content in the supernatant was carried out by the method of Lowry *et al.* (1951).

The method for determining the lactase activity of the biopsies was first assessed using a commercially available lactase (EC 3.2.1.23). A mixture containing 0.05 ml of the 3-fold diluted supernatant in the same mannitol-buffer and 0.05 ml of lactose solution (300 mg/5 ml) was incubated at 37°C for 40 min. Lactose hydrolysis was then ended by putting the mixture in ice. The amount of glucose was measured by the gluco-quant method (Boehringer Mannheim, FRG). Lactase activity was expressed in mmol glucose/g protein.

Alkaline phosphatase activity (AP, EC 3.1.3.1) in the supernatant was determined colorimetrically by the optimized standard method (Boehringer Mannheim, FRG) on the Multistat III (Instrumentation Laboratory (Benelux) BV) and expressed in U/g protein.

Examination of faeces and blood

Throughout the experimental period a weekly sample of the faeces was taken directly from the rectum of each calf. Bacteriological examination for *Salmonella* and *Pseudomonas* was performed by direct isolation on modified brilliant green agar or after selective enrichment in sodium selenite–brilliant green–mannitol broth (Oxoid,

Hampshire, UK), followed by an antimicrobial sensitivity test (Neo-sensitabs, Rosco, Taastrub, Denmark). The samples were examined for cryptosporidia by the modified Ziehl Neelsen technique. The presence of rotavirus was tested for by latex agglutination (Wellcome Diagnostics, Dartford, UK) (Microbiologic Diagnostic Centre, State University of Utrecht). Virological examination for the presence of coronavirus in the faeces was carried out using a double antibody-sandwich (DAS) ELISA test from week 8 onwards (Central Veterinary Institute, Lelystad, The Netherlands).

Serological examination for antibodies against rotavirus, coronavirus and bovine diarrhoea virus (BDV) was performed by blocking ELISA in weeks 5 and 15. A tube agglutination test with H and O antigen was done to detect antibodies against *Salmonella dublin* and *Salmonella typhimurium* (Central Veterinary Institute, Lelystad, The Netherlands).

RESULTS

Clinical aspects

In the induction period, the faeces developed a clay-like consistency with greyish-white colour. Faeces with these characteristics, typical for chronic RD calves, were produced in week 10 by calves H and S and in week 13 by calf E. During the recovery period, the faeces of calves E and S regained their normal character within a few days. In calf H, recovery from the clinical signs of RD was delayed for about three weeks after intraruminal administration of milk stopped. In this calf, clinical recovery was only achieved after modification of the feeding regimen by increasing the feeding frequency, giving smaller meals and using a floating nipple.

The results of the microbiological examination of the weekly samples of the faeces are shown in Table I. No salmonellae were isolated during the entire experiment. In weeks 5 and 7, other pathogens (*Pseudomonas*, cryptosporidia, rotavirus) were isolated in all three calves. In most cases no diarrhoea was observed. From weeks 8 to 11, all the faecal samples were negative for potential pathogens and no diarrhoea was observed. From weeks 12 to 15, *Pseudomonas*, rotavirus and coronavirus were again isolated.

Diarrhoea was observed sporadically on a few days during the experiment. Only calf S experienced diarrhoea during the larger part of the recovery period. In no case was there any need for treatment or adjustment of the feeding regimen. *Pseudomonas* species were found in large numbers in calf E from weeks 5 to 7 but were never associated with diarrhoea. Three of the six diarrhoeic samples from calf S contained *Pseudomonas*. All *Pseudomonas* isolated were resistant to most of the antibiotics tested.

The antibody titre against both types of *Salmonella* and BDV was negative in all three calves. Although the titre against rotavirus (calves E and H) and coronovirus (calf S) was unchanged or slightly elevated in week 15 compared to the titre in week 5, no seroconversion occurred in any calf.

TABLE I
Micro-organisms isolated from the faeces of the three experimental calves and the incidence of diarrhoea

Period	Week	Calf E	Calf H	Calf S
Pre-induction	4	Neg	Neg	Neg
	5	*Pseud.*	*Pseud*; ‡ Crypto	‡ Crypto
	6	*Pseud.*	Neg	Neg
Induction	7	*Pseud.*	Rota	‡ *Pseud*; D
	8	Neg	Neg	Neg
	9	Neg	Neg	Neg
	10	Neg	Neg	Neg
	11	Neg	Neg	Neg
Recovery*	12	‡ *Pseud*; D	Rota	Neg
	13	Neg	Rota	Neg; D
	14	Corona	Neg	*Pseud*; D
	15	‡ *Pseud.*	Corona	*Pseud*; D
	16	Neg	Neg	Neg; D
	17	Neg	Neg	Neg; D

Neg = no pathogenic organisms isolated
Pseud. = *Pseudomonas aeruginosa*
‡ = only small numbers present, or after enrichment
Crypto = cryptosporidia
D = diarrhoeic faeces for two or more days
* = calf E started the recovery period two weeks later
Rota = rotavirus
Corona = coronavirus

Morphology

The jejunal biopsies of the three experimental calves showed a mixture of finger-shaped and long tongue-shaped villi during the pre-induction period and the first part of the induction period. In calves H and S, from week 9 onwards, these villus forms were replaced by predominantly long to short tongue-shaped villi with a few finger- and leaf-shaped ones. In calf E, this change was observed in week 13 only. There was no essential change in villus form in the recovery period in calves H and S compared to that in the induction period.

The mean villus length and the individual values for calves E, H and S respectively are given in Figure 1. Individual values are given because each calf serves as its own control. The measurements are compared with the initial villus length in week 4, at the time of the first operation.

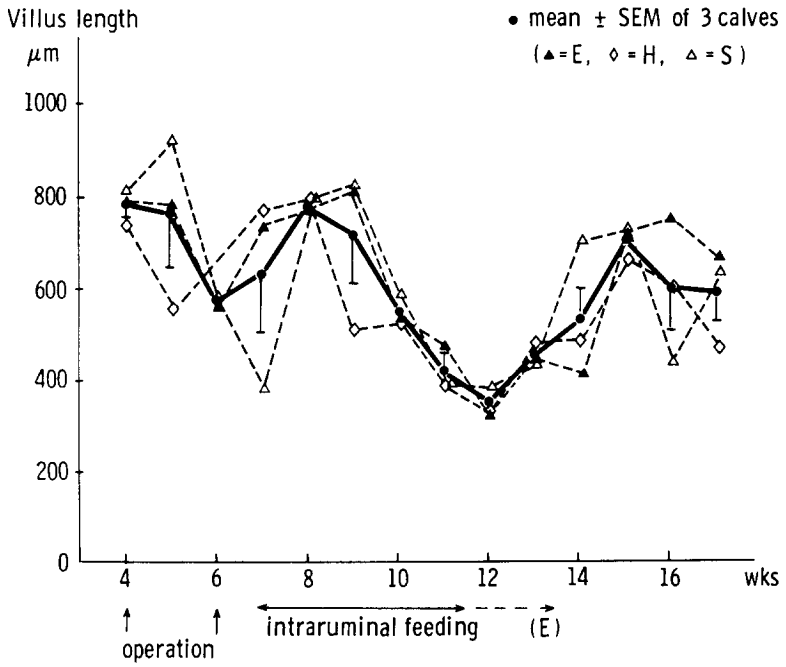

Figure 1. Villus lengths in μm measured in the jejunal biopsies of the calves E, H and S. The operations took place in weeks 4 and 6. The period of intraruminal feeding was from week 7 to week 11 for calves H and S and from week 7 to week 13 for calf E. The mean villus length (± SEM) is shown by the solid line. No SEM is given where it was too small to visualize.

In the pre-induction period, all three calves exhibited a temporary decrease in villus length to 74% of the initial length, which resolved in week 8, shortly after ruminal infusion of milk was initiated. From weeks 10 to 15 the villus length was again reduced compared to the initial values. The shortest villus length (41–47% of initial length) were observed in all three calves during week 12. In week 15, during the recovery period, all values had almost returned to their initial values.

Crypt depth did not alter after intraruminal milk administration; it ranged from 214 to 374 μm in the experimental calves.

The index of mitosis for each calf is given in Table II. Peak values ($\geqslant$ 1.00) were obtained in weeks 10–12 in calf E, weeks 13–14 in calf H and weeks 13 & 16 in calf S.

Brush border enzymes

The mean and individual lactase activities for the three experimental calves during the three experimental periods are presented in Figure 2. The first measurements are from week 5, when the first biopsies were taken. A substantial variation in lactase activity was observed during the experimental period, although the activity in all three calves fell in weeks 7 and 12, to 28% and 19% respectively of their values in week 5.

TABLE II

Index of mitosis in the jejunal biopsies of the three experimental calves

Period	Week	Calf E	Calf H	Calf S
Pre-induction	4	0.96	0.39	0.32
	5	0.65	0.66	0.58
	6	0.78	nd	0.75
Induction	7	0.64	0.60	0.72
	8	0.62	0.77	0.47
	9	0.64	0.53	0.79
	10	1.05	0.87	0.73
	11	1.26	0.81	0.92
Recovery*	12	1.18	0.84	0.74
	13	0.57	1.04	1.00
	14	0.98	1.02	0.75
	15	0.78	0.92	0.70
	16	0.76	0.82	1.05
	17	0.41	0.58	0.62

nd = not determined
* = two weeks later for calf E

In Figure 3 the mean and individual AP activities for the three experimental calves are presented. Variation is less than with lactase activity but the low points in activity for both lactase and AP in weeks 7 and 12 were essentially the same. AP activity was reduced to 44% in week 7 and to 34% in week 12.

DISCUSSION

The use of an intestinal fistula, by which repeated biopsies of the jejunal mucosa can be taken, made it possible to compare within each calf the data obtained in the different periods. This compensates for the individual morphological and functional variations.

The clinical signs of RD in this calf model were less obvious than in RD patients. The typical clay-like faeces were only observed for a short period in the three experimental calves. This may be the consequence of more rapid clearance of rumen contents in this calf model than in chronic RD patients (van Weeren-Keverling Buisman *et al.*, 1990).

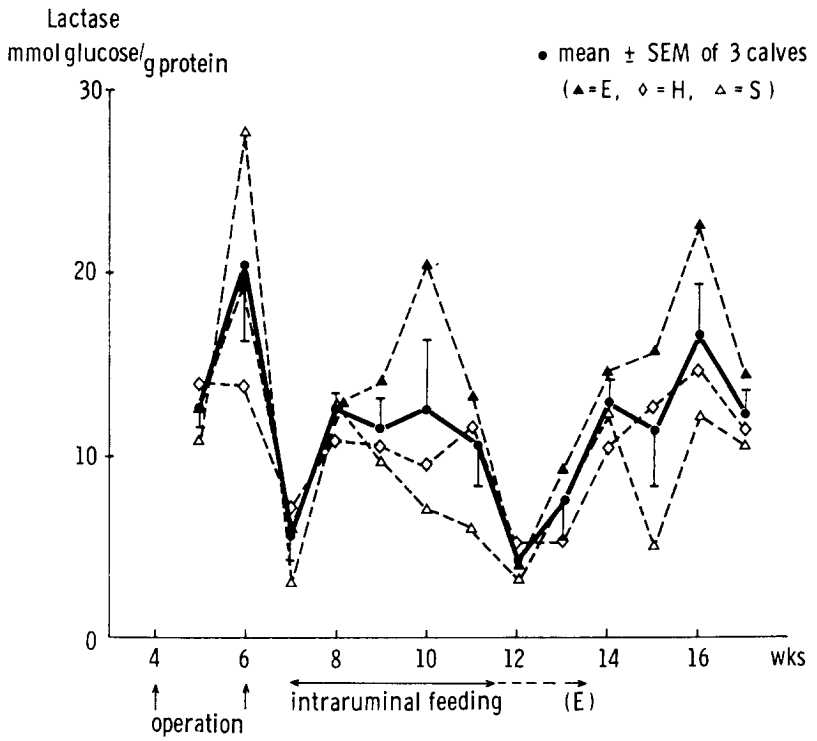

Figure 2. Lactase activity in mmol glucose/g protein of the jejunal biopsies of calves E, H and S. The mean values (± SEM) are shown by the solid line. No SEM is given where it was too small to visualize

When considering the detection of pathogenic micro-organisms in the faeces, two periods of excretion can be distinguished. The first period (weeks 5–7) was directly post-operative, when pathogenic micro-organisms were found in 7 out of 9 samples of the faeces, but only one calf (S) was diarrhoeic in week 7. This may be attributed to increased susceptibility in the surgically stressed animals, post-operative antibiotic treatment, or both. Ampicillin, for instance, is known to reduce colonization resistance maintained by the normal gut flora (van der Waay, 1979), as a result of which pathogenic micro-organisms may start to colonize the intestine. The second period of shedding of micro-organisms was from weeks 12–15, during the recovery period; *Pseudomonas aeruginosa*, rotavirus and coronavirus were isolated. Calf S suffered from slight diarrhoea during the larger part of the recovery period; *Pseudomonas* was present in 2 of the 5 faeces samples. This is similar to observations in naturally occurring RD calves, in which a period of diarrhoea is observed after initiation of reconditioning therapy. In more than half of these cases, *Pseudomonas* were found in the faeces (van Weeren-Keverling Buisman *et al.*, 1988a). *Pseudomonas* usually shows a multiresistant pattern towards antibiotics (Batra and Garg, 1987).

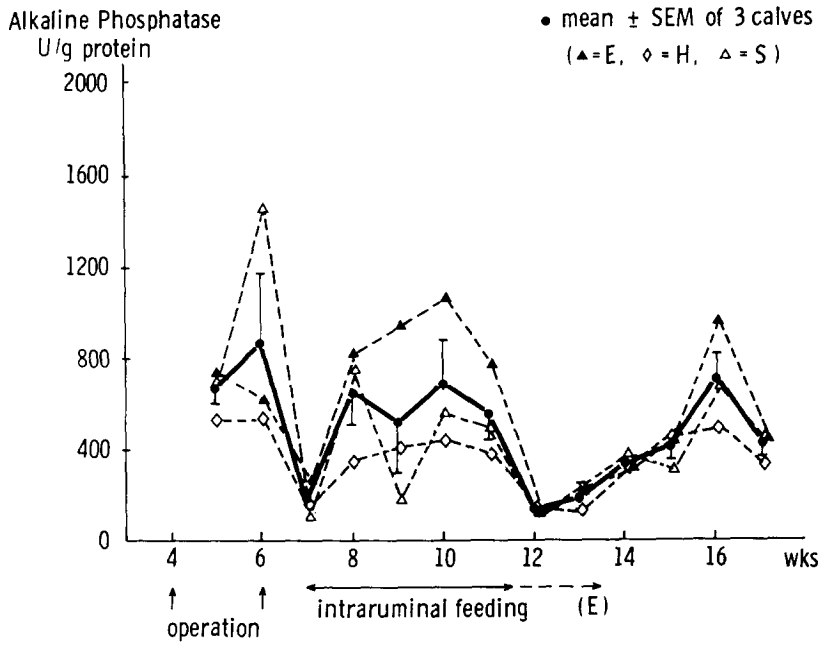

Figure 3. Alkaline phosphatase activity in U/g protein of the jejunal biopsies of calves E, H and S. The mean values (± SEM) are shown by the solid line. No SEM is given where it was too small to visualize.

Pseudomonas aeruginosa is able to cause a haemorrhagic gastroenteritis in calves (Nilsson and Thörne, 1962) but clinically healthy intestinal carriers have also been reported in dairy calves and cows (Matthews and Fitzsimmons, 1964; Hoadley and McCoy, 1968; Batra and Garg, 1987). The incidence of *Pseudomonas* intestinal carriage in veal calves is probably even higher, due to frequent antibiotic treatment or to the subtherapeutic doses of antibiotics which are frequently added to the milk replacer (Hamstra and van Haeringen, 1977). No information is available about the pathological changes in the intestine in cases of subclinical *Pseudomonas* infection.

Rotavirus was detected in calf H in weeks 7, 12 and 13 by the latex agglutination test but was not accompanied by diarrhoea. Coronavirus was detected only in the recovery period, in calf E in week 14 and in calf H in week 15. Rotavirus and coronavirus can both cause diarrhoea and villus atrophy in the jejunum of young calves under three weeks of age (Torres Medina *et al.*, 1985). However, in this experiment no pathogenic micro-organisms were shed during the period of intraruminal milk administration, while the villus length was decreasing gradually. Thus, the observed villus atrophy during the induction period is probably caused by another (non-infectious) factor, resulting from the intraruminal administration of the milk. The transitory decline in villus length shortly after the operations may be associated with the well-known relationship between surgery, stress and the shedding of pathogens.

The observed villus forms in the pre-induction period, a mixture of finger-shaped and long tongue-shaped villi, is the normal stereomicroscopic appearance for veal

calves (van Weeren *et al.*, 1986). In the induction period, these villus forms are replaced by long to short tongue-shaped villi with few finger- and leaf-shaped ones. The index of mitosis was increased at the end of the induction period and the beginning of the recovery period, although the crypt depth in the experimental calves did not change. This indicates that the villus atrophy induced by intraruminal feeding is one of the hyperplastic type. Villus atrophy with hyper-regenerative crypt epithelium in chronic RD calves was also found in previous studies (Breukink *et al.*, 1988; van Weeren-Keverling Buisman *et al.*, 1988a).

A reduced lactase activity compared to the initial values was found in all three experimental calves in week 12, at the end of the induction period. A similar decrease in lactase activity was found in week 7, shortly post-operatively. These changes in enzyme activity were accompanied by parallel changes in villus length and indicate a reduction in functional intestinal epithelium, caused by immaturity or damage to the epithelial cells (Landsverk, 1981).

Lactase is localized mainly in the brush border of the epithelium of the villi and the apical portions of the crypts in the proximal small intestine (Landsverk, 1980). Its function is the hydrolysis of lactose into glucose and galactose, which are absorbed in the small intestine (Coombe and Smith, 1973). Normally, lactase activity is highest in very young calves (Huber *et al.*, 1961) and decreases with age. Toofanian *et al.* (1974) observed a gradual decrease in activity during the first four weeks of life; after that the lactase activity remained constant in calves up to 6 months old. Lactase activity is also influenced by the type of diet offered: continuation of a liquid lactose-containing diet and prevention of the functional development of the rumen prevented the normal post-weaning decline in lactase activity (Toofanian *et al.*, 1974). Supplementation of milk diets with extra lactose resulted in an even higher lactase activity (Huber *et al.*, 1964). The lactase activity in our study was therefore probably not only influenced by the observed villus atrophy but was also partly the result of a reduced amount of substrate. In the rumen, an unknown amount of lactose is converted into lactate and volatile fatty acids (van Weeren-Keverling Buisman *et al.*, 1990).

Comparison of the absolute values of lactase activity in our study with those found in the literature is difficult because of the differences in determination of enzyme activity. A reduced lactase activity was reported in diarrhoeic calves between 10 and 30 days of age, which also showed villus atrophy and crypt hyperplasia in the proximal jejunum (Landsverk, 1981). Youanes and Herdt (1987) reported similar results. Dargel and Hartmann (1984) also found that lactase activity in diarrhoeic calves from 7 to 11 days of age was about half that of healthy calves of the same age.

A reduction in AP activity was observed at the same times as the reduction in lactase activity, i.e. during weeks 7 and 12. Restoration of the depressed activity of this enzyme after the induction period was somewhat delayed when compared with the lactase activity. At the end of the recovery period, in week 16, the AP activities of the experimental calves had almost completely regained their initial values. This suggests that AP activity may also be influenced by villus atrophy.

Alkaline phosphatase (AP) is a brush border enzyme with slight additional activity in the Golgi apparatus (Landsverk, 1980). It is mainly localized in the duodenum and proximal and mid jejunum (Landsverk, 1980), where it is involved in energy-consuming sugar and lipid absorption (Stiglmair-Herb *et al.*, 1986). It is not known if diet or age influence AP activity. However, in diarrhoeic calves up to 4 days of age with experimental *Escherichia coli* and rotavirus infections, AP activity is reduced

(Stiglmair-Herb *et al.*, 1986). This reduction was also found in 12-week-old calves with parasitic gastroenteritis (Benz and Ernst, 1986). In dogs with small intestinal bacterial overgrowth, caused by aerobic flora such as enterococci and *Escherichia coli*, AP activity was reduced, while lactase activity and the activities of other brush border enzymes were not altered significantly. This suggests that bacterial secretions or metabolites also specifically act on AP activity (Batt and Hall, 1989).

CONCLUSION

It can be concluded that intraruminal administration of half the milk ration to normal veal calves results in clinical symptoms of RD and villus atrophy in the proximal jejunum. This hyperplastic villus atrophy is similar to that found in chronic RD patients and is accompanied by a reduction in brush border enzyme activities. Clinical recovery is fast after returning to the normal feeding regimen, with restoration of villus length and brush border enzyme activities in three to four weeks.

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