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# Effect of Soy Protein on Calves' Intestinal Absorptive Ability and Morphology Determined by Scanning Electron Microscopy

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## ABSTRACT

Neonatal calves were fed whole milk (control) or one of three milk replacers with one-third of the total protein supplied by casein, Promocaf (a commercial soy protein concentrate), or an experimental soy flour. Xylose absorption was studied at 3 and 8 wk after a 12-h fast. Urine was collected for 5 h, and jugular blood was sampled at 0, 2.5, and 5 h after administration of xylose. Urinary excretions of xylose at 8 wk were 3.4, 5.3, 7.8, and 21.3% of xylose administered, respectively, for calves fed Promocaf, soy flour, casein, and milk. Increases in plasma xylose 2.5 h after administration were 7.7, 21.3, 31.8, and 46.5 mg/dl.

Calves were sacrificed at 12 or 14 wk and duodenal tissues sampled for scanning electron microscopy. Micrographs revealed normal intestinal morphology with long, round, tapering villi when milk was fed. Casein feeding produced shorter, broader villi than did feeding whole milk. Abnormalities included absence of villi and short, blunted, convoluted villi on mucosal surfaces of calves fed soy proteins. Reduced surface area for intestinal absorption probably resulted from villous atrophy in calves with abnormal mucosae. Impairment of absorptive ability appears to be associated with morphological changes in intestinal structure.

## INTRODUCTION

The scanning electron microscope (SEM)

with its high resolution and clarity of surface detail has proved valuable in studying small intestinal morphology, including the structure of villi, epithelial cells, and microvilli (27). Many workers, using SEM, have studied normal intestinal mucosa in various species including man (1, 2, 8, 15, 30), rat (2, 30), pig (31), and calf (17, 18, 20).

By SEM also normal intestinal structures have been compared with pathological conditions, including celiac disease in man (1, 8), transmissible gastroenteritis (TGE) in swine (31), and experimental viral infections in calves (18, 20). Histopathological changes in the small intestine associated with those conditions generally have been characterized as villous atrophy, blunting, shortening, and clumping of villi, decreased height of the brush border, and morphologic alteration of villous epithelial cells from simple columnar to cuboidal, accompanied by increased cell loss and turnover (1, 14, 19). Degeneration of intestinal mucosa was associated with impaired digestion of nutrients, diarrhea, dehydration, and weight loss in the pathological conditions mentioned (14, 19, 23).

Absorption of an oral dose of D-xylose effectively indicates absorptive ability of the small intestine and has been used to study celiac disease in man (4) as well as malabsorption syndromes in the horse (5) and dog (10). Xylose malabsorption in calves was used as an indicator of intestinal malfunction caused by viral infection (32) and by feeding milk replacers containing soy proteins (25).

Soybean products are a potential source of high quality, economical substitutes for milk protein in calf milk replacers. Young calves fed milk replacers containing a high proportion of soy protein have not consistently performed well (6, 22). Dairy calves fed milk replacers containing one-third of the total protein from soy products did not exhibit expected increases in protein digestibility with age (24). Calves fed similar rations as the only feed absorbed

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significantly less xylose at 4 and 5 wk than did control calves fed milk (25). These findings suggested that subnormal protein digestibility and xylose absorption in calves fed soy proteins may be associated with such intestinal lesions as villous atrophy. We used SEM to investigate detectable changes in small intestinal morphology of calves fed milk replacers containing soy proteins.

## MATERIALS AND METHODS

### Animals and Treatments

Four Holstein bull calves were fed colostrum shortly after birth. Beginning at 1 to 2 days of age, one calf per treatment was fed milk (control) or one of three milk replacers containing one-third of the total protein from casein (CS), Promocaf (PC) a commercial soy protein concentrate, or soy flour (SF) (Table 1). Calves were fed liquid rations once daily at 10% of body weight with dry milk replacer constituting 13% of the diet fed. Milk was supplemented

with the same vitamin-trace mineral mix in the milk replacers. No dry feed was provided. Water was available ad lib. Calves were housed in elevated metal stalls and weighed weekly with rations adjusted accordingly. General appearance of calves and consistency of feces were recorded twice daily (11). Animals were sacrificed on the same day at 12 (control) or 14 (CS, PC, SF) wk.

### Tissue Specimen Preparation

Immediately postmortem, duodenal tissue samples 15 cm from pylorus were taken from each calf. Sections of tubular tract 50 mm long were made by transverse cuts. Each section was sliced once longitudinally. Fixation was by immersion in 10% buffered neutral formalin, with at least 10 volumes of formalin to each volume of tissue (16). Samples were stored in formalin until dehydrated. Smaller sections (8 × 8 mm) were cut from fixed tissue and dehydrated in a graded series of aqueous ethanol solutions. Samples were immersed for 30 min

TABLE 1. Composition of milk replacers.<sup>a,b,c</sup>

Item	Soy flour	Promocaf		Casein
		(% )		
Ingredient				
Soy flour <sup>d</sup>	14.5	0		0
Promocaf <sup>e</sup>	0	10.1		0
Casein <sup>f</sup>	0	0		7.4
Dried skim milk <sup>g</sup>	17.4	16.1		13.7
Dried whey <sup>g</sup>	49.2	55.0		60.0
Fat product <sup>h</sup>	18.9	18.8		18.9
Calculated nutrient content				
Protein	20.0	20.3		20.1
Fat	12.0	12.0		12.0
Calcium	.82	.85		.85
Phosphorus	.74	.74		.70

<sup>a</sup>Dry matter basis.

<sup>b</sup>Vitamin/trace mineral premix (National Vitamin Products, Minneapolis, MN) added at .5% to provide daily National Research Council (1978) recommendations.

<sup>c</sup>Antibiotic premix TM-50D (Pfizer Co., Terre Haute, IN) added at .1% to provide terramycin at .005%.

<sup>d</sup>Treated soy flour containing 46% protein, Far-Mar-Co., Inc., Hutchinson, KS.

<sup>e</sup>Commercial soy protein concentrate containing 70% protein, Central Soya Co., Decatur IN.

<sup>f</sup>Ultra supreme sodium caseinate, Erie Casein Co., Erie, IL.

<sup>g</sup>Milk Specialties Co. Dundee, IL.

<sup>h</sup>Ho-Milk fat product containing 60% animal fat and 7% milk protein, Merrick Foods, Inc., Union Center, WI.

each in solutions of 25, 50, 70, 90, 95 (once each), and 100 (two changes) % ethanol (9). A critical-point dryer was used with carbon dioxide as the transition fluid. Dried specimens were mounted on metal stubs with conductive silver paste and double coated with carbon and 60:40 gold palladium under vacuum on a rotating oscillating stage (16). Samples were examined under SEM (ETEC U1 Model 30) with 10 kV accelerating voltage at magnifications of 50 to 250 $\times$ .

#### Xylose Absorption

During the trial, xylose absorption tests were on each calf at 3 and 8 wk with procedures and materials as in (25) except that calves were fasted for 12 h before testing and jugular blood was sampled only at 0, 2.5, and 5 h after xylose feeding. Urine was collected quantitatively for 5 h.

### RESULTS AND DISCUSSION

#### Calf Performance

During the experiment, weight gains were 14.5, 26.4, 25.0, and 24.5 kg for calves fed PC, SF, CS, and milk. The milk-fed calf was 2 wk younger than the other three. Diarrhea was common in calves fed PC and SF, especially the last several weeks of the trial. Calves fed CS and milk exhibited occasional, less severe diarrhea.

#### Xylose Absorption

Data from xylose tests are in Table 2.

Preadministration concentrations of xylose in plasma were subtracted from those taken after xylose was fed to obtain the net increase in xylose concentration (25). Absorption of xylose at 8 wk was greatest by the milk-fed calf, followed in decreasing order by calves fed CS, SF, and PC. We previously established that xylose absorption was higher in calves fed milk than in those fed soy products (25). In this study, soy proteins also appear to have depressed xylose absorption.

#### Scanning Electron Microscopy

Figures 1 through 4 show two electron micrographs of duodenum from each calf. The proximal small intestine of the normal calf contains numerous villi, mucosal evaginations of various lengths (17). Normal villi in man may be either finger-shaped and round, or else tongue-shaped and flattened in cross section. Finger-like villi range between .1 and .25 mm in diameter (15). The villous surfaces in man are interrupted by openings of mucous-secreting goblet cells 3 to 8  $\mu$  across (15). The normal situation from the milk-fed calf shows mucous in the orifices of some goblet cells (Figure 1). The bulging or corrugated appearance is from transverse furrows and individual absorptive epithelial cells on the villous surface (7). Longitudinal villous contracting may cause the furrows (8).

Crypts of Lieberkuhn are intestinal glands of various lengths, which open into intervillous spaces throughout the small intestine. Crypt

TABLE 2. Results of xylose absorption tests on calves fed indicated ration.

Ration <sup>a</sup>	Calf's age (wk)	Plasma xylose (mg/dl) at:		5-h urinary xylose (% of xylose fed)
		2.5 h	5 h	
Promocaf	3	27.3	27.3	8.7
	8	7.7	17.5	3.4
Soy flour	3	14.4	20.5	7.4
	8	21.3	17.6	5.3
Casein	3	34.3	31.7	7.9
	8	31.8	26.4	7.8
Milk	3	34.8	23.8	13.2
	8	46.5	26.2	21.3

<sup>a</sup>For description of dietary treatments see Table 1.

orifices may be 100  $\mu$  or more across and often contain amorphous substance. A functional ratio of three crypts to one villus has been proposed (15). The tips of villi terminate in extrusion zones where the epithelial cells slough off after migrating up the villi (17).

In Figure 1, villi are generally long, straight, rounded, and distinct. This calf was fed milk, and its intestinal morphology resembles that of a normal gnotobiotic calf (17). As in normal pigs, the long villi tend to obscure crypts and bases of villi (31). Debris, most of which is likely mucous, is evident on and around villi. Goblet cell orifices are evident on the villi, especially at 250 $\times$ .

Villi from the calf fed CS (Figure 2) are shorter and broader. The shorter villi allow better exposure of the crypts, openings of which appear as dark areas in the intervillous

spaces. The irregular transverse furrows show clearly at 250 $\times$ . Mucous-containing material is in the intervillous spaces. Goblet cell openings are evident on villi although they are not as numerous as in Figure 1. The intestinal surface area available for absorption appears to be less than in the calf fed whole milk. Milk and CS contained only milk proteins, so the difference in response may stem from total protein consumed. A 50-kg calf consuming only liquid feed should receive 180 g of total crude protein (CP) per day (21). In this experiment, a 50-kg calf fed milk received approximately 175 g of CP daily whereas a calf of similar size fed one of the milk replacers received 130 g of CP. Thus, a dietary protein or amino acid deficiency may be at least partially responsible for differences in villous structure between calves fed milk replacers and the control.

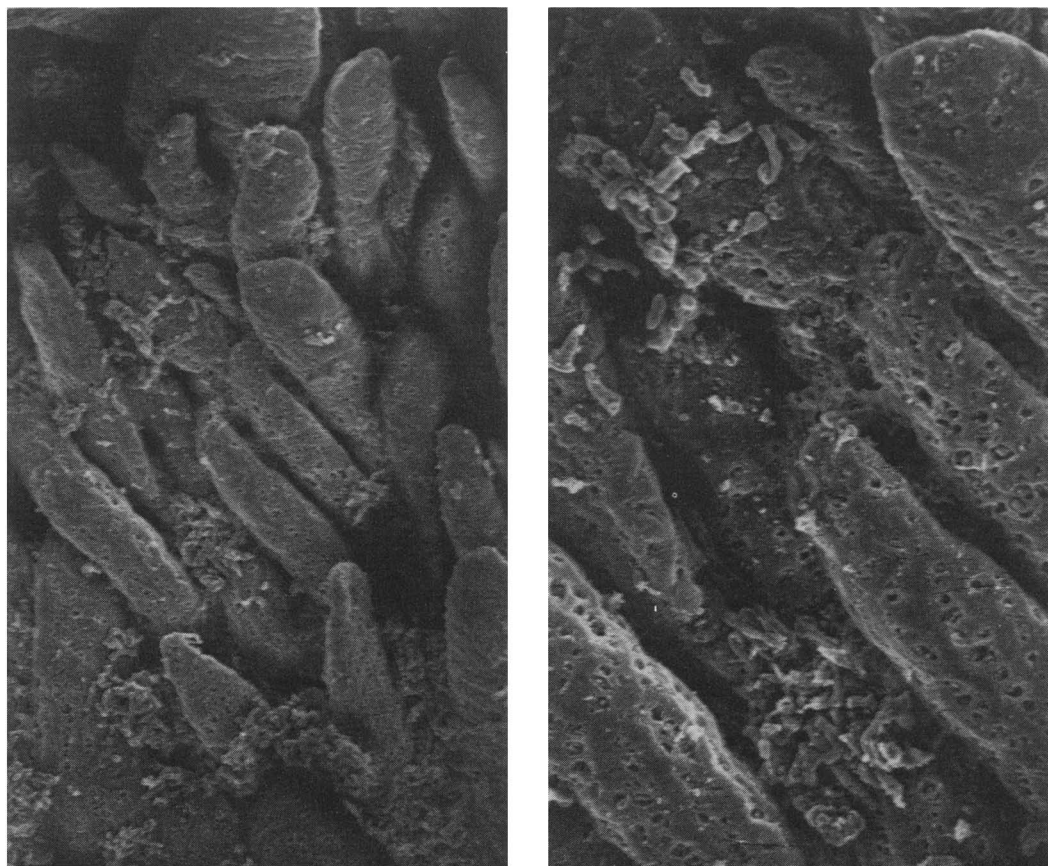


Figure 1. Scanning electron micrographs of villi in duodenum of calf fed milk. Left  $\times 100$ ; right  $\times 250$ .

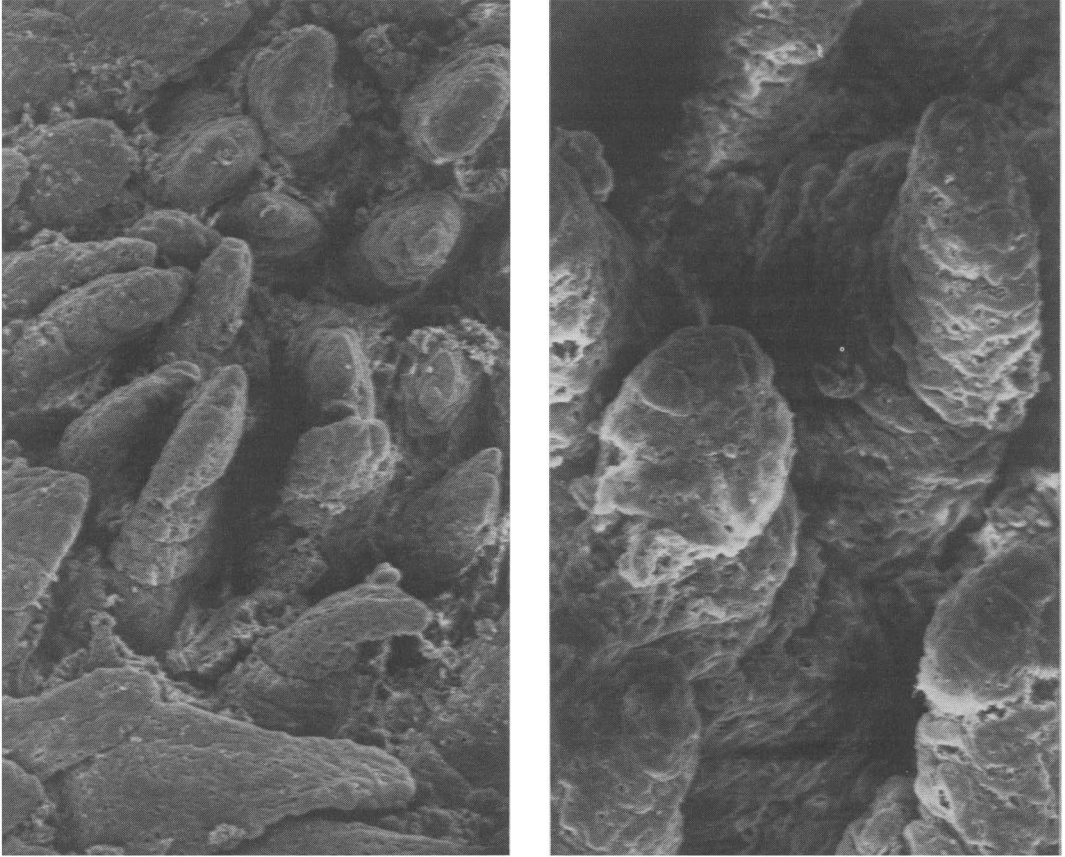


Figure 2. Scanning electron micrographs of villi in duodenum of calf fed casein (CS). Left  $\times 100$ ; right  $\times 250$ .

Figure 3 shows intestinal villi from the calf fed SF. Villi are shorter, less uniform than in Figure 1, and tend to bend. Some are convoluted, as reported in human malabsorption syndromes from celiac sprue (8). Some fusion of villi above the crypts is noticeable. The middle of Figure 3 (right) shows crypts containing openings from several intestinal glands, as reported by Asquith et al. (1). Some villous tips in Figure 3 appear blunted compared with tapered ones in normal villi (Figure 1). Shortening and blunting of villi has been reported in swine infected with TGE (29, 31) and in calves infected with calf diarrheal coronavirus (18, 19) and virus of human infantile gastroenteritis (20). The short, blunt, convoluted villi in the intestinal mucosa of this calf apparently were associated with impaired absorption as indicated by the xylose test.

The flat intestinal mucosa of the calf fed PC is shown in Figure 4. In contrast to the other three figures, it has no identifiable villi. Many crypt openings and their secretions are evident. It appears similar to colonic mucosal surface (19) or to small intestinal mucosa with the villi removed (15). This animal apparently suffered from acute malabsorption from an essentially complete villous atrophy. The surface is ridged between crypts, probably the remnants of gyrated or convoluted villi. Severe malabsorption, a condition that may have worsened the last 6 wk of the experiment, was indicated by the xylose test when the calf was 8 wk of age.

If studies of abnormal mucosa are to be valid, certain assumptions must be made (1). Evidence indicates that villous length and shape are highly related to absorptive capacity.

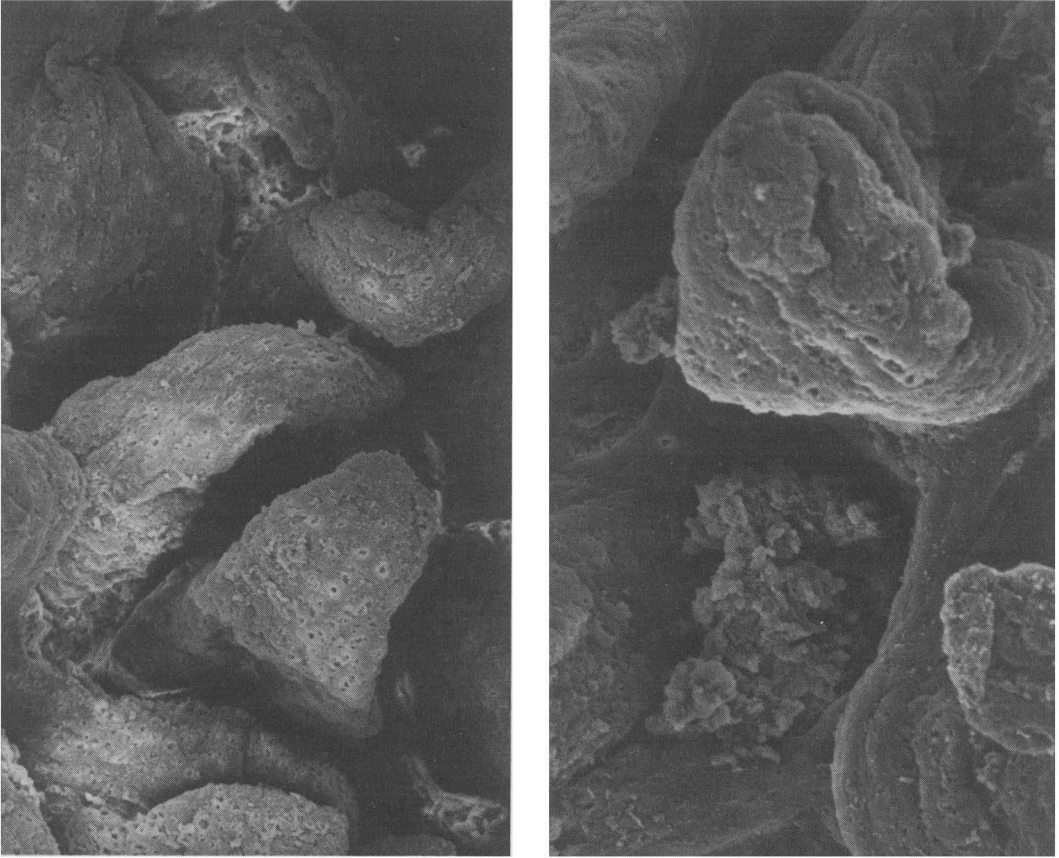


Figure 3. Scanning electron micrographs of villi in duodenum of calf fed soy flour. Left  $\times 100$ ; right  $\times 250$ .

Flat mucosa, the most abnormal, represents the severest form of malabsorption, as in the calf fed PC (Figure 4). Other appearances such as convolutions and ridges are less severely abnormal (Figure 3). Nematode infestations in rats have produced flat intestinal mucosa. Infected rats demonstrated severe changes, with shortening or absence of villous projections, lengthening of crypts, and infiltration with inflammatory cells. The histological appearances resembled celiac disease in man and were associated with malabsorption and reduced activity of enzymes, including maltase and alkaline phosphatase. Other observations were increased cell production in the crypts and increased cell migration on the villi (13, 28).

Loehry and Creamer (12) suggested a progression of five stages, representing changes in the small intestinal mucosa from normal

to completely flat. Villi begin to fuse at their bases by forming intervillous ridges that grow as the villi shorten. Eventually the ridges broaden and flatten and make up the entire mucosal surface. Figure 3 may represent an intermediate step in such a process. The primary defect may result from the villous surface being injured so the epithelium and basement membrane are denuded, followed by rapid proliferation and abnormal migration of epithelial cells, and increased cell loss.

During their first 2 wk of life, calves can generate a local intestinal antibody response to antigens. Antibodies secreted are primarily IgA and IgM classes, with the former increasing in importance with maturation of lymphocytes in the lamina propria up to 3 mo (3). But normal calves secreted little or no antibodies into the lumen in response to soy protein



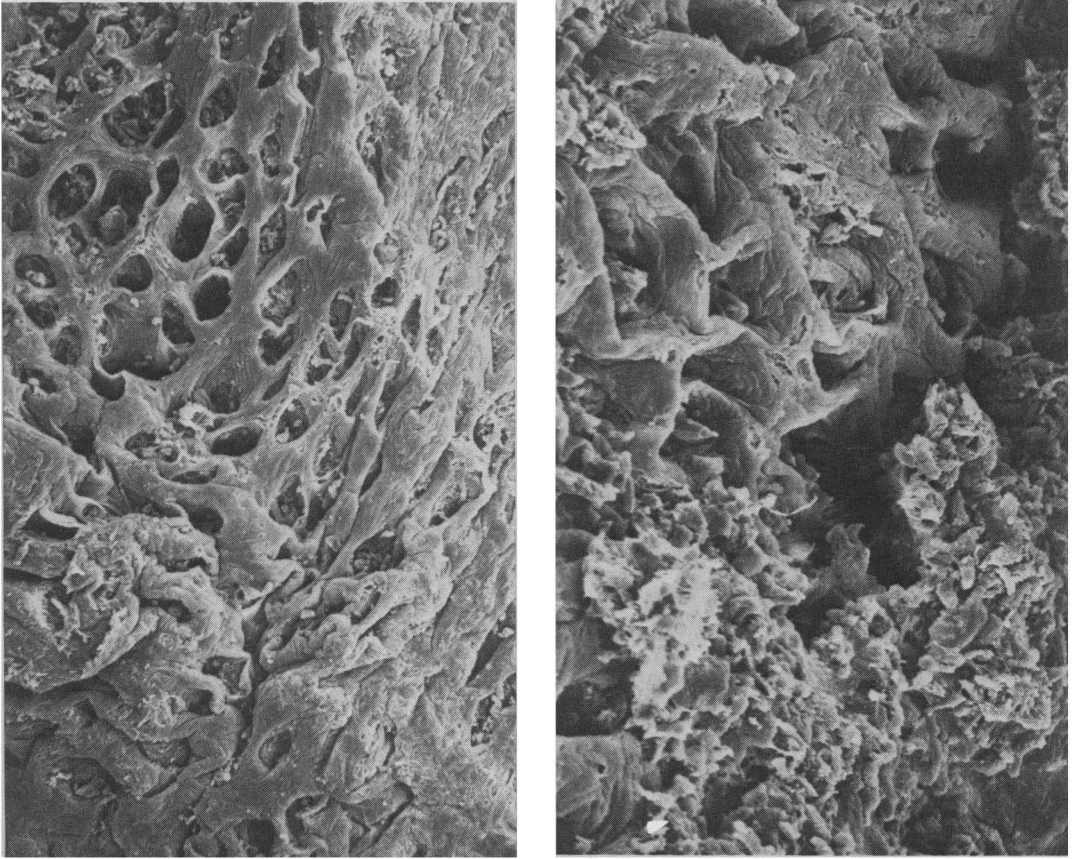


Figure 4. Scanning electron micrographs of mucosa in duodenum of calf fed Promocaf. Left  $\times 100$ ; right  $\times 250$ .

antigens, so those antigens may be able to avoid the local immune system. Barratt and Porter (3) reported an unusually high serum antibody response of IgG class to ingested soy antigens. The differences between calves fed SF and PC were not expected from previous work (25). Possibly PC, containing a more concentrated soy protein product, is more antigenic than SF in young calves. Another explanation is the possibility of a dietary amino acid deficiency. Since protein quality declines with successive steps in purification of soy protein, PC may be lower than SF in one or more essential amino acids. Further research is needed on the physiological response of calves to dietary soy proteins.

The scanning electron microscope is valuable in studying intestinal morphology. Pictures

taken at higher magnifications than those accompanying this paper may help assess the status of individual epithelial cells and the brush border. Transmission electron microscopy (TEM) and light microscopy (LM) have been used effectively for this purpose (17, 20), and both may aid studies of the effects of soy proteins on calf intestinal ultrastructures.

The ability of the young calf to utilize soybean proteins may be associated with the type of soybean product fed, and its amount and duration of feeding. Areas for future study that may be affected by these factors include: absorptive ability by xylose test or other means; histological studies of intestinal mucosa; immune system responses; plasma amino acid concentrations; histochemical studies of enzyme



activities; calf weight change; and fecal consistency.

The progression of morphological changes in intestinal mucosa during disease syndromes (12) indicates that the process may develop gradually in calves fed soy proteins. We found that feeding PC produced markedly abnormal intestinal morphology in a calf sacrificed at 9 wk. Scanning electron microscopy revealed shortened, convoluted, and twisted villi with an apparently reduced surface area for absorption (Seegraber and Morrill, unpublished). We also found that intestinal biopsies on calves fed PC have shown progressive villous atrophy, which was reversed by changing rations to whole milk. Intestinal biopsy is preferable to sacrificing animals both for economic reasons and because it permits monitoring changes chronologically, so an animal may serve as its own control. An intestinal biopsy technique for calves has been described (26). We now are using SEM and LM with intestinal biopsies at regular intervals to study calves fed soy proteins. Other measurements include xylose absorption and serum immunoglobulin, and the regenerative ability of intestinal mucosa after a diet is changed.

#### CONCLUSIONS

Impaired intestinal absorptive ability, shown by xylose tests, appeared to be associated with morphological changes in intestinal structure seen via SEM in calves fed soy proteins. Structural changes similar to those reported from TGE in swine, celiac disease in man, viral infections in calves, and nematode infestation in rats were observed. Perhaps the intestinal mucosa responds via a similar progression of degenerative stages to insults or injuries of widely differing origins. Feeding whole milk produced intestinal villi similar to those reported in normal calves and other species. Normal villi were associated with increased ability to absorb xylose. When more is known about soy proteins, their antigenicity, and their effects in calves, methods to eliminate problems with soy proteins may lead to increased use of soy products by preruminant calves.

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#### REFERENCES

- 1 Asquith, P., A. G. Johnson, and W. T. Cooke. 1970. Scanning electron microscopy of normal and celiac jejunal mucosa. *Am. J. Dig. Dis.* 15:511.
- 2 Balcerzak, S. P., W. C. Lane, and J. W. Bullard. 1970. Surface structure of intestinal epithelium. *Gastroenterology* 58:49.
- 3 Barratt, M.E.J., and P. Porter. 1979. Immunoglobulin classes implicated in intestinal disturbances of calves associated with soya protein antigens. *J. Immunol.* 123:676.
- 4 Benson, J. A., Jr., P. J. Culver, S. Ragland, C. M. Jones, G. D. Drummey, and E. Bougas. 1957. The D-xylose absorption test in malabsorption syndromes. *New England J. Med.* 256:335.
- 5 Bolton, J. R., A. M. Merritt, R. E. Cimprich, C. F. Ramberg, and W. Streett. 1976. Normal and abnormal xylose absorption in the horse. *Cornell Vet.* 66:183.
- 6 Coblentz, E., J. L. Morrill, D. B. Parrish, and A. D. Dayton. 1976. Nutritive value of thermoalkali-processed soy materials for young calves and rats. *J. Dairy Sci.* 59:481.
- 7 Dellmann, H., and E. M. Brown. 1976. Textbook of veterinary histology. Lea & Febiger, Philadelphia, PA.
- 8 Demling, L., V. Becker, and M. Classen. 1969. Examinations of the mucosa of the small intestine with the scanning electron microscope. *Digestion* 2:51.
- 9 Hayat, M. A. 1978. Introduction to biological scanning electron microscopy. University Park Press, Baltimore, MD.
- 10 Hill, F.W.G., D. E. Kidder, and J. Frew. 1970. A xylose absorption test for the dog. *Vet. Rec.* 87:250.
- 11 Larson, L. L., F. G. Owen, J. L. Albright, R. D. Appleman, R. C. Lamb, and L. D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. *J. Dairy Sci.* 60:989.
- 12 Loehry, C. A., and B. Creamer. 1969. Three-dimensional structure of the human small intestinal mucosa in health and disease. *Gut* 10:6.
- 13 Loehry, C. A., D. N. Croft, A. K. Singh, and B. Creamer. 1969. Cell turnover in the rat small intestinal mucosa: An appraisal of cell loss. *Gut* 10:13.
- 14 Maronpot, R. R., and C. K. Whitehair. 1967. Experimental sprue-like small intestinal lesions in pigs. *Can. J. Comp. Med. Vet. Sci.* 31:309.
- 15 Marsh, M. N., and J. A. Swift. 1969. A study of the small intestinal mucosa using the scanning electron microscope. *Gut* 10:940.
- 16 McGavin, M. D., and J. L. Morrill. 1976. Dissection technique for examination of the bovine rumino-reticulum. *J. Anim. Sci.* 42:535.

- 17 Mebus, C. A., L. E. Newman, and E. L. Stair. 1975. Scanning electron, light, and transmission electron microscopy of intestine of gnotobiotic calf. *Am. J. Vet. Res.* 36:985.
- 18 Mebus, C. A., L. E. Newman, and E. L. Stair. 1975. Scanning electron, light, and immunofluorescent microscopy of intestine of gnotobiotic calf infected with calf diarrheal coronavirus. *Am. J. Vet. Res.* 36:1719.
- 19 Mebus, C. A., E. L. Stair, M. B. Rhodes, and M. J. Twiehaus. 1973. Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. *Vet. Pathol.* 10:45.
- 20 Mebus, C. A., R. G. Wyatt, and A. Z. Kapikian. 1977. Intestinal lesions induced in gnotobiotic calves by the virus of human infantile gastroenteritis. *Vet. Pathol.* 14:273.
- 21 National Research Council. 1978. Nutrient requirements of dairy cattle. 5th ed. Natl. Acad. Sci., Washington, DC.
- 22 Nitsan, Z., R. Volcani, A. Hasdai, and S. Gordon. 1972. Soybean protein substitute for milk protein in milk replacers for suckling calves. *J. Dairy Sci.* 55:811.
- 23 Schenk, E. A., I. M. Samloff, and F. A. Klipstein. 1965. Morphologic characteristics of jejunal biopsy in celiac disease and tropical sprue. *Am. J. Pathol.* 47:765.
- 24 Seegraber, F. J. 1978. The effect of soy protein on digestibility of calf milk replacers and on intestinal absorptive ability as determined by the xylose absorption test. M. S. thesis, Kansas State Univ., Manhattan.
- 25 Seegraber, F. J., and J. L. Morrill. 1979. Effect of soy protein on intestinal absorptive ability of calves by the xylose absorption test. *J. Dairy Sci.* 62:972.
- 26 Sterzing, P. R., A. D. McGilliard, and R. S. Allen. 1971. Application of a biopsy technique to the study of lipid absorption and transport by bovine small intestine. *J. Anim. Sci.* 32:774.
- 27 Swift, J. A., and M. N. Marsh. 1968. Scanning electron microscopy of rat intestinal microvilli. *Lancet* 2:915.
- 28 Symons, L.E.A., and D. Fairbairn. 1962. Pathology, absorption, transport, and activity of digestive enzymes in rat jejunum parasitized by the nematode *Nippostrongylus brasiliensis*. *Fed. Proc.* 21:913.
- 29 Thake, D. C. 1968. Jejunal epithelium in transmissible gastroenteritis of swine. *Am. J. Pathol.* 53:149.
- 30 Toner, P. G., and K. E. Carr. 1969. The use of scanning electron microscopy in the study of the intestinal villi. *J. Pathol.* 97:611.
- 31 Waxler, G. L. 1972. Lesions of transmissible gastroenteritis in the pig as determined by scanning electron microscopy. *Am. J. Vet. Res.* 33:1323.
- 32 Woode, G. N., C. Smith, and M. J. Dennis. 1978. Intestinal damage in rotavirus infected calves assessed by D-xylose malabsorption. *Vet. Rec.* 102:340.