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Effect of Protein Source in Calf Milk Replacers on Morphology and Absorptive Ability of Small Intestine[1]

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ABSTRACT

Holstein calves were fed milk or one of four milk replacers with one-third of the total protein supplied by casein, soy protein concentrate, soy flour, or fish protein concentrate. The remainder of the protein in each replacer was from milk sources. Milk and milk replacers (13% solids) were fed at 10% of body weight daily. No dry feed or bedding was provided. Absorptive ability of small intestine was evaluated by xylose absorption test at 2-wk intervals. With calves under general anesthesia, a biopsy of small intestine was taken after each xylose test to examine morphological changes in mucosa by scanning electron microscopy.

Villi were long, tapering, and uniform in calves fed milk. Calves fed casein had greater variation in size and conformation of villi. Gradual deterioration in villous integrity was seen in calves fed soy proteins. Calves fed fish protein concentrate performed poorly and had abnormal villi.

Diets were changed to milk to test for reversal of effects after marked alterations in intestinal structure had been observed. Atrophy was reversed as villi returned toward normal size and shape within 2 wk after milk feeding began. The surgical procedure apparently did not cause harmful effects of villi. Absorption of xylose and daily gain were greater, and feces firmer, in calves fed milk than in those fed milk replacers.

INTRODUCTION

Much literature has been published concerning the substitution of soy protein for milk protein in diets for preruminant calves (9, 11, 22, 23). Although soybeans are a potential source of high quality protein, calves perform poorly when fed milk replacers in which soy products provide a large proportion of the total protein (9, 21, 23, 24). Soy protein does not form a coagulum in the abomasum and may pass more quickly through the digestive tract than milk (10, 15, 28). Associated problems include low digestibility of soy protein, low nitrogen retention, and increased diarrhea (9, 27, 31).

Utilization of soy protein by calves appears to be limited at least partially by antinutritional factors in the soy products. Raw soybeans contain trypsin inhibitor (TI), which may be significantly inactivated by heating (23). More recently, however, components of soy products have been shown to be antigenic in the young calf. Glycinin and beta-conglycinin, the major storage globulins in protein bodies of soybeans, have initiated humoral immune responses in calves sensitized to dietary soy proteins (3, 4, 14, 15, 29, 30). Benzyl isothiocyanate has been implicated as another antigenic compound causing poor performance in calves fed soy proteins (12).

Soy proteins caused lower intestinal absorption of xylose than did milk in calves at 5 wk of age (25). Villous atrophy was seen in intestinal samples from calves sacrificed after receiving milk replacers containing soy proteins for 14 wk (26) and in intestinal biopsies from calves taken after 7 d of feeding soy protein (3, 4). Scanning electron [SEM (26)] and light [LM (3, 4)] microscopy have been used to study morphological changes in the intestine of calves induced by soy protein. Digestive and absorptive disturbances were associated with these changes (3, 4, 26).

Although sacrificing an animal and taking postmortem tissue samples, or taking single

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biopsies, can provide valuable information about dietary effects at a given time, we thought more could be learned by taking repeated biopsies from the same animal at regular intervals. Intestinal biopsy is preferable to sacrificing animals for economic reasons and because it permits monitoring changes chronologically so an animal may serve as its own control (26). In this experiment, we used a multiple biopsy technique to study the effect of soy, milk, and fish proteins in milk replacers on intestinal morphology of calves.

MATERIALS AND METHODS

Animals and Treatments

Holstein bull calves were fed colostrum from birth to 3 d. They were fed whole milk (control) or one of four milk replacers containing one-third of the total protein from casein (CS), commercial soy protein concentrate (SPC), soy flour (SF), or fish protein concentrate (FPC). Composition of diets is in Table 1. There were three calves per group for treatments CS, SPC, FPC, and milk, and two calves for SF. Calves were fed liquid rations from open pails at 10% of body weight daily in two equal feedings. Solids constituted 13% of milk replacers as fed. Milk was supplemented with the same vitamin-trace mineral premix used in the milk replacers, which provided daily recommendations (20). Calves had no dry feed. Water was available ad libitum. Calves were housed in elevated metal stalls in an enclosed ventilated building with a concrete floor. Calves were weighed weekly and rations adjusted accordingly except when calves lost weight the feeding level was not decreased. General appearance of calves, changes from normal appetite, and consistency of feces (17)

TABLE 1. Composition of milk replacers.[1,2,3]

Item	Milk replacers[4]			
	SF	SPC	CS	FPC
Ingredient				
Soy flour[5]	14.5	0	0	0
Soy protein concentrate[6]	0	10.1	0	0
Casein[7]	0	0	7.4	0
Fish protein concentrate[8]	0	0	0	9.8
Dried skim milk[9]	17.4	16.1	13.7	12.2
Dried whey[9]	49.2	55.0	60.0	60.4
Fat product[10]	18.9	18.8	18.9	17.6
Total	100.0	100.0	100.0	100.0
Calculated nutrient content				
Protein	20.0	20.0	20.0	20.0
Fat	12.0	12.0	12.0	12.0
Calcium	.82	.85	.85	.77
Phosphorus	.74	.74	.74	.74

[1] Dry matter basis.

[2] Vitamin/trace mineral premix (National Vitamin Products Co., Minneapolis, MN) added at .5%, which provided daily recommendations (National Research Council, 1978).

[3] Antibiotic premix TM-50D (Pfizer Co., Terre Haute, IN) added at .1%, which provided terramycin at .005%.

[4] SF = Soy flour; SPC = soy protein concentrate; CS = casein; FPC = fish protein concentrate.

[5] Experimental soy flour containing 46% protein, Far-Mar-Co., Inc., Hutchinson, KS.

[6] Promocaf, commercial soy protein concentrate containing 70% protein, Central Soya Co., Decatur, IN.

[7] Ultra supreme sodium caseinate containing 90% protein, Erie Casein Co., Erie, IL.

[8] Spray dried condensed fish solubles containing 67% protein, Zapata Haynie Corp., Reedville, VA.

[9] Milk Specialties Co., Dundee, IL.

[10] Ho-Milc fat product containing 60% animal fat and 7% milk protein, Merrick Foods, Inc., Union Center, WI.

were recorded twice daily. Calves that died early in the experiment were replaced. Diets were changed to milk to test for reversal of effects when marked abnormalities in intestinal morphology had been observed.

Xylose Absorption

Xylose absorption tests were performed on each calf at 2-wk intervals following a 12-h fast and prior to each intestinal biopsy. Procedures and materials were as described in (25, 26) except that jugular blood was sampled at 0, 2, and 5 h after xylose feeding. Urine was collected for 5 h.

Intestinal Biopsy and Tissue Specimen Preparation

Biopsies were at 2-wk intervals following a 12-h fast and 5-h xylose absorption test. Calves were sedated via intramuscular injection with xylazine (.22 mg/kg) and positioned in left lateral recumbency for surgery. The right flank was clipped and prepared for sterile surgical procedure. Anesthesia was induced and maintained via inhalation through a nose mask of methoxyflurane, nitrous oxide, and oxygen.

The small intestine was identified and partially isolated following incisions through the skin, subcutaneous tissue, abdominal muscle layers, and peritoneum. Biopsies of the small intestines (approximately 2.5 cm × 3.0 cm) were excised from the antimesenteric border without disrupting the mesentery. Blood supply to the biopsy site was not compromised. Samples were pinned to cardboard with mucosal surface up and immediately fixed in 10% buffered neutral formalin. The remainder of the processing for SEM was as described in (26). Loops of nonabsorbable suture, the number equal to the number of biopsies per calf, were placed in the mesentery to identify the location of each biopsy. Intestine was sutured using 2-0 or 3-0 gut in a simple interrupted pattern. A solution of 500 mg oxytetracycline in 500 ml sterile saline was poured into the peritoneal cavity. The abdominal wall was closed in two layers with 0 or 1 gut in a simple continuous pattern. Skin was closed with nylon and sutures were removed after 14 d.

Calves were sacrificed after completion of the experiment. The small intestine was removed and measured, and the location of each biopsy was identified. Intestinal tracts from calves that died during the study were handled in the same manner.

Biopsy samples were examined under SEM (ETEC UI Model 30) with 2.5 to 5 kV accelerating voltage at a magnification of 100×.

RESULTS AND DISCUSSION

Calf Performance and Xylose Absorption

Data from xylose absorption tests and calf performance are in Table 2. Calves fed FPC lost

TABLE 2. Performance data and results of xylose absorption tests on calves fed indicated rations.

Ration[1]	Number of calves	Daily gain	Fecal[2] score	Plasma xylose (mg/dl) at: 2 h	Plasma xylose (mg/dl) at: 5 h	5-h Urinary xylose (% of xylose fed)
		(kg)				
Milk	3	.42^{a}	1.93^{a}	46.7^{a}	27.5^{a}	15.7^{a}
Casein	3	.20^{b}	2.19^{a}	36.2^{b}	24.0^{abc}	11.9^{ab}
Soy flour	2	.13^{b}	2.19^{a}	29.0^{b}	26.0^{ab}	8.9^{b}
Soy protein concentrate	3	.09^{b}	2.77^{b}	30.5^{b}	19.1^{bc}	9.0^{b}
Fish protein concentrate	3	−.07^{c}	3.49^{c}	32.9^{b}	16.7^{c}	9.2^{b}

^{a,b,c}Means in a column with different superscripts differ ($P<.05$).

[1] For description of diets see Table 1.

[2] Range of scores from 1 = normal to 4 = watery (17).

weight, whereas calves fed CS gained less than half, and those fed soy proteins (SPC or SF) less than one-third, of control animals. Higher fecal scores, indicating more problems with diarrhea, corresponded with poorer growth. Two calves fed FPC and one fed SPC died during the first few weeks of the study and were replaced. Problems with feeding FPC may be associated with poor digestibility and low retention of digested nitrogen (13). Fish protein at 43% of total protein caused poor growth, high incidence of scours, and high mortality when no dry feed was provided (8), but FPC yielded satisfactory performance at up to 70% of total protein when starter and hay were fed also (13). Calves fed FPC performed poorly in this study.

Results of xylose absorption tests correspond with those found previously, as milk-fed calves absorbed more xylose than those fed milk replacers (25, 26). Preadministration concentrations of xylose in plasma were subtracted from those taken after xylose was fed to obtain the net increase in xylose concentration, as done previously (25, 26). Ability to absorb xylose increased more with age in young calves fed milk than in those fed milk replacers containing soy proteins (7, 25, 26).

Scanning Electron Microscopy

Figures 1 through 5 show two electron micrographs of small intestine from one calf fed each diet. Pictures are representative of the intestinal morphology seen in samples from each treatment group. Age of calf at sampling and location of biopsy are given for each micrograph. Biopsies were designated as originating from one of the following five locations: duodenum; proximal, middle, or distal jejunum; and ileum. The method of tagging the mesentery with nonabsorbable suture worked well to enable identification of each biopsy site on postmortem examination. Location of biopsy in small intestine did not appear to be a major factor in the appearance of the mucosal surface.

Calves fed milk had intestinal villi that were long, round, and uniform (Figure 1), similar to those seen previously with milk feeding (26) and in normal gnotobiotic calves (19). The

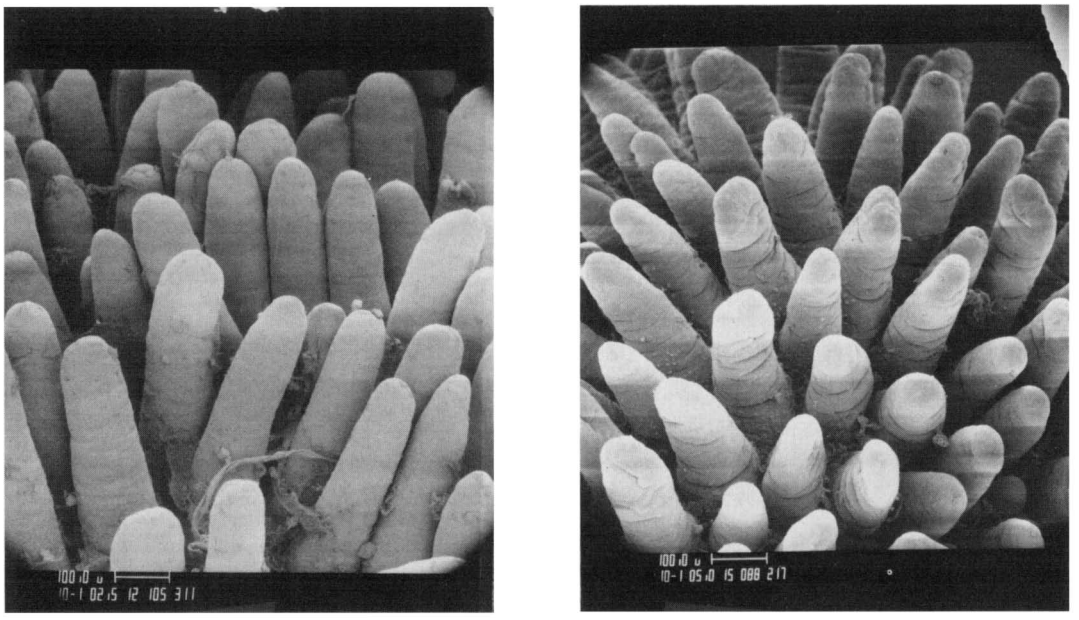


Figure 1. Scanning electron micrographs of villi in intestine of calf fed milk. Left: middle jejunum, 4 wk X100; right: proximal jejunum, 10 wk, X100.

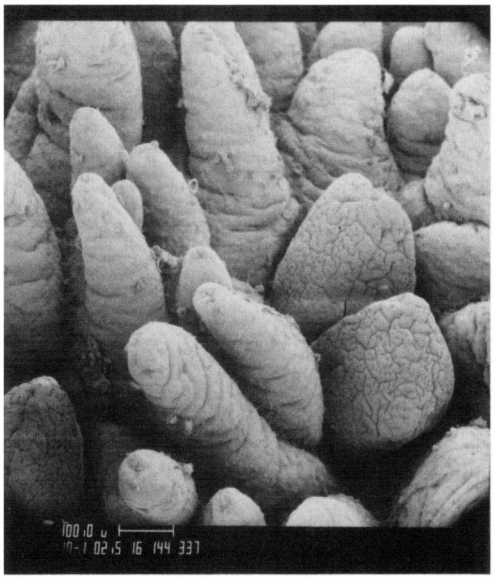
surgical procedure apparently had no detrimental effect on morphology of intestinal mucosa, because milk-fed calves had up to nine biopsies, all with normal villi.

Villi from calves fed CS were less uniform after 6 wk and became shorter and broader by 10 wk (Figure 2). Shorter villi allow improved observation of the intervillous crypts on micrographs. Milk and CS contained only milk proteins, and differences between the two in this study may be due to lower protein intake with CS than with milk, as noted previously (26).

Calves were fed assigned milk replacer diets until 8 wk of age for SPC and FPC, and 10 wk of age for CS and SF, after which rations were changed to milk. Calves fed SF had villi that were short and blunt at 6 and 10 wk with increased mucus production (Figure 3). Feeding SPC caused villi to become very broad and blunt as early as 4 wk, progressing to severe villous atrophy with intervillous bridging by 8 wk (Figure 4). These changes are similar to those found previously by SEM when soy proteins were fed to calves (26). Using LM, Barratt et al. found that villi became shorter and broader when calves were fed soy proteins for 7 d or more (3, 4), and these changes occurred simultaneously with increased titers to soy antigens in serum (4).

Degeneration of villi associated with feeding soy proteins to calves appears to occur by a similar progression of stages to that described for intestinal disease in man (18). The process begins with epithelial damage, which is followed by broadening and shortening of villi with fusion initiating at the bases and progresses to complete villous atrophy and a flat intestinal mucosa (18, 26). The more severe the degeneration, the more reduced is the surface area for absorption of nutrients. The process need not progress all the way to complete villous atrophy but may be seen at any intermediate point. In this study, normal villi are in Figure 1, intermediate stages of degeneration in Figures 2, 3, and 5, and more severe atrophy, approaching a flat mucosa, in Figure 4.

Calves fed FPC developed abnormal intestinal villi as early as 4 wk, and villi were broad, blunt, and short by 8 wk (Figure 5). Calves in this group lost weight and had more severe diarrhea and higher mortality than those fed other diets. They also had low absorption of xylose (Table 2). Roy et al. (24) found thickened intestinal walls in calves fed FPC or



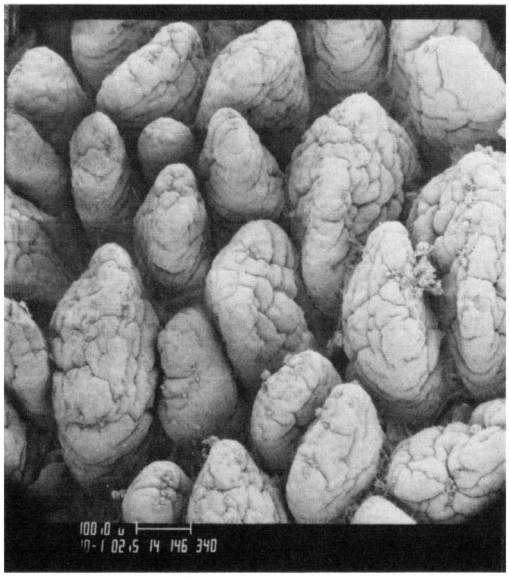


Figure 2. Scanning electron micrographs of villi in intestine of calf fed casein. Left: middle jejunum, 6wk, ×100; right: middle jejunum, 10 wk, ×100.

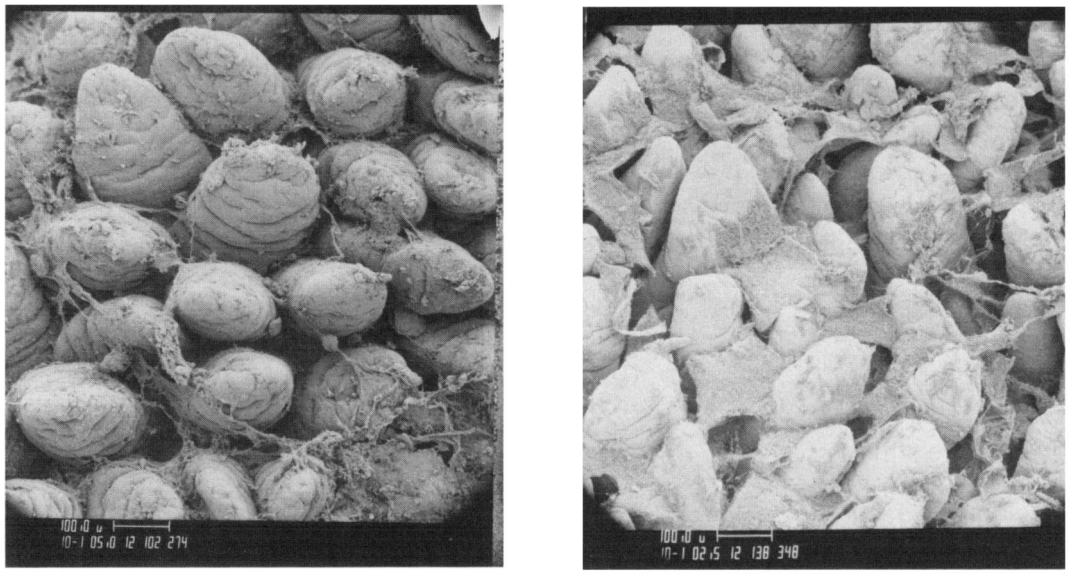


Figure 3. Scanning electron micrographs of villi in intestine of calf fed soy flour. Left: middle jejunum, 6 wk, ×100; right: proximal jejunum, 10 wk, ×100.

SF. Digestibilities of FPC have been 10 to 30% lower than that of milk protein, and there may be deficiencies of certain essential amino acids in fish protein (13).

Antinutritional factors in soybeans may limit their utilization by young calves. Trypsin inhibitor is largely inactivated by heat treatment of the soy product, but it may be re-

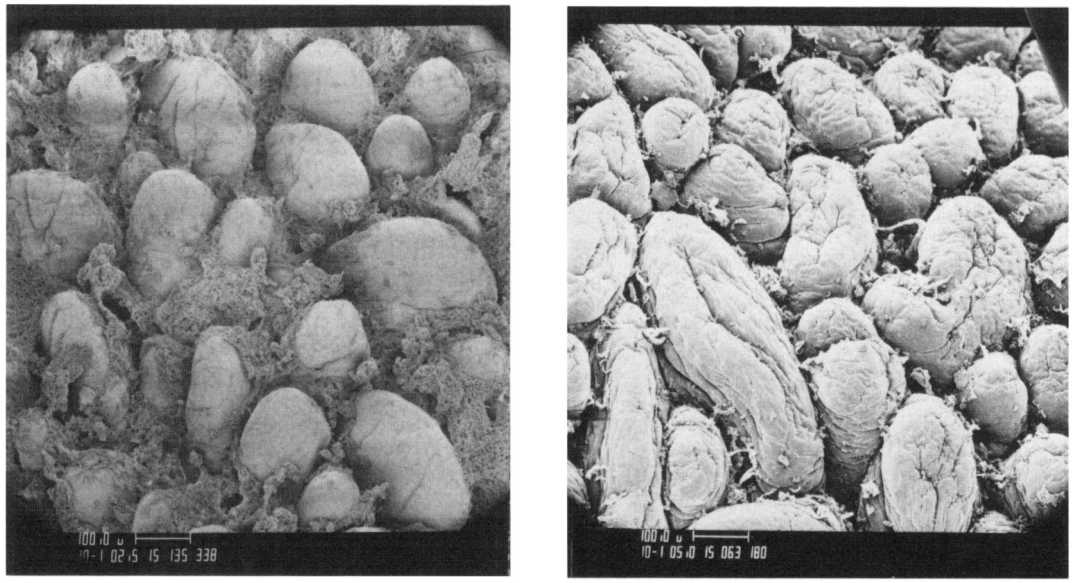


Figure 4. Scanning electron micrographs of villi in intestine of calf fed soy protein concentrate. Left: middle jejunum, 4 wk, ×100; right: distal jejunum, 8 wk, ×100.

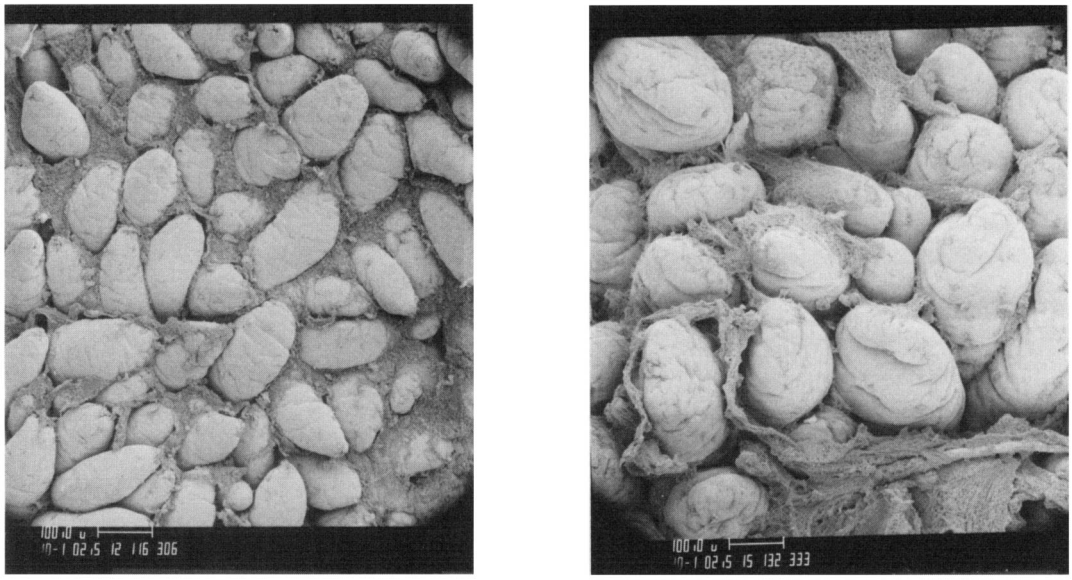


Figure 5. Scanning electron micrographs of villi in intestine of calf fed fish protein concentrate. Left: distal jejunum, 4 wk, X100; right: middle jejunum, 8 wk, X100.

activated in the alkaline pH of the proximal small intestine (23). The importance of TI in the nutrition of calves fed soy-containing milk replacers is uncertain, but it may block the activity of the pancreatic enzymes trypsin and chymotrypsin in the intestine, thus inhibiting normal proteolysis.

Recent investigation has been concerned with antigenic components in soy products fed to calves. Calves appeared to become sensitized to dietary soy proteins, exhibiting altered gastrointestinal flow rates and lowered nitrogen retention (15, 27, 31). Increased permeability of the gut to macromolecules was associated with feeding soy proteins (16).

High serum antibody titers were found to be specific for glycinin and beta-conglycinin (3, 4, 14, 15, 29). An in vitro immunochemical technique to detect these antigens (30) has been suggested to predict the suitability of soy products for feeding to calves. Extraction of soy materials with hot aqueous ethanol has reduced the antigenicity of these products in some studies (14, 15, 29), but not in others (3, 4).

Morphological changes in intestine associated with feeding soy proteins to calves (3, 4, 26) or human infants (1, 6) and with feeding wheat gluten in man (2), have been attributed to gastrointestinal allergy. Antigen-induced intestinal damage may be associated with a lack of protective emigration of neutrophils from the lamina propria into the lumen, where antigens are either inactivated or destroyed. This process is mediated by antibody and is specific for antigen (5, 6). Soybean proteins appear to have an ability to avoid the local immune mechanism in the small intestine (3).

Similar morphological changes to those seen with feeding soy proteins have been seen with enteric viral infections in calves (19) and pigs (32). Rotavirus infection in calves caused decreased ability to absorb xylose (33). The possible interaction of viruses and dietary antigens in this atrophic process in the intestine has not been reported but may be worthy of investigation.

When marked intestinal lesions were seen in calves fed milk replacers, the diet was changed to whole milk to study reversal of effects. Within 2 wk of change to milk, intestinal biopsies showed regeneration of villi in calves that had been fed soy proteins previously (Figure 6). Similar reversal of structural damage has been shown in humans with allergy to soy protein when the antigenic stimulus was removed (1). Villi in calves fed CS or FPC also

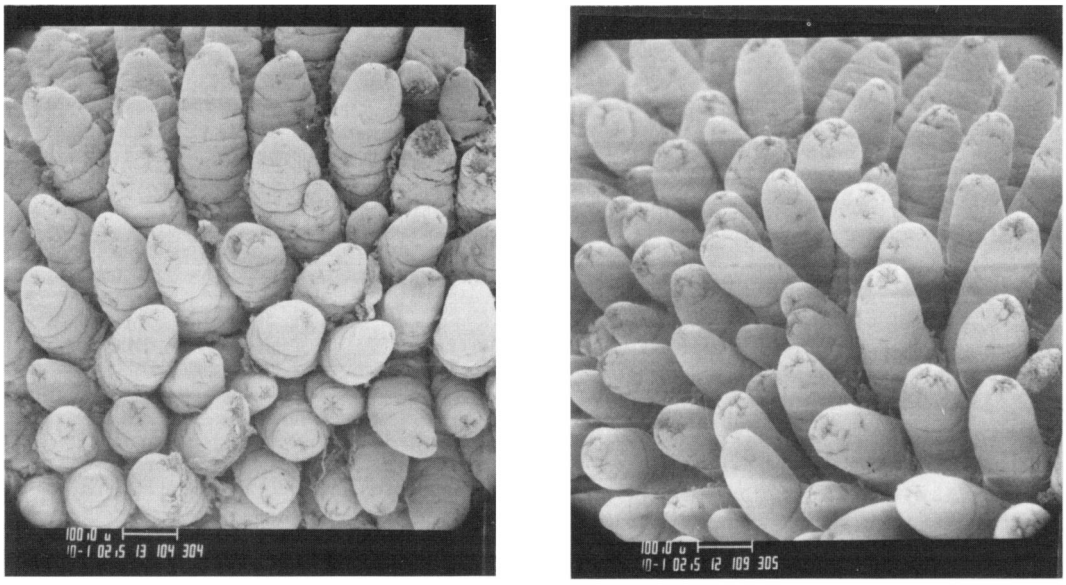


Figure 6. Scanning electron micrographs of villi in intestines of calves fed milk for 2 wk after receiving soy protein concentrate (left) or soy flour (right). Left: same calf as in Figure 4, middle jejunum, X 100; right: same calf as in Figure 3, middle jejunum, X 100.

regenerated within 2 wk after the diet was changed to milk (Figure 7).

Regeneration of villi occurred rather rapidly after calves drank milk, but some calves did not readily accept milk after consuming milk replacers for many weeks. This reluctance tended to last a few days, after which calves drank milk normally. Mixing milk with the pre-

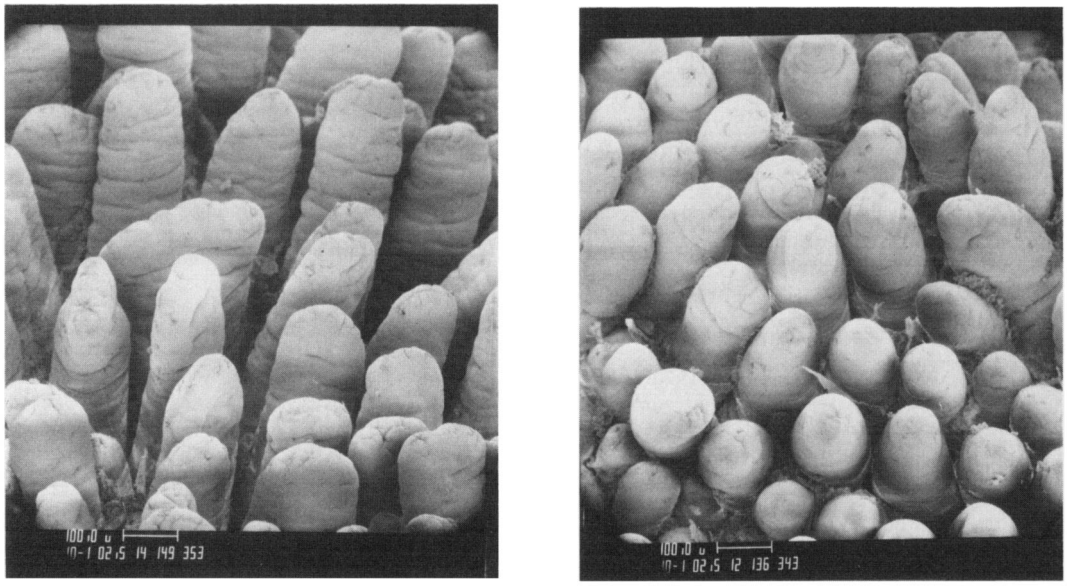


Figure 7. Scanning electron micrographs of villi in intestines of calves fed milk for 2 wk after receiving casein (left) or fish protein concentrate (right). Left: same calf as in Figure 2, middle jejunum, X 100; right: same calf as in Figure 5, proximal jejunum, X 100.

vious diet for a short period seemed to speed the transition.

CONCLUSIONS

Feeding milk replacers containing soy proteins, FPC, or CS caused varying degrees of reversible abnormalities in the morphology of small intestines of calves. Degeneration of mucosa was more rapid and more severe with SPC than with SF. Problems with feeding soy protein were due at least partially to antigens contained in the soy products, and TI content may have been a significant factor. Calves fed FPC performed poorly, had high mortality, and those that survived were weak. Calves fed milk replacers consumed less protein than those fed milk and this may partially explain the differences observed.

Further research is needed to develop processing methods for soy products, which will enhance their utilization by preruminant calves. There is also much more to be learned about the pathophysiological processes, especially those involved in immunological reactions related to feeding soy proteins to calves.

A biopsy technique has been described by general anesthesia and abdominal surgery on young calves at 2-wk intervals. Calves generally recovered well and were alert and hungry the morning after surgery. Several hundred biopsies have been performed and calves seem able to tolerate the procedure quite well.

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