Morphological Alterations of Small Intestinal Epithelium of Calves Caused by Feeding Soybean Protein[1]

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ABSTRACT

Sixteen male Holstein calves were fed milk replacer (14% solids) containing 23% crude protein from: a) 100% milk protein; or b) 66% soybean protein concentrate plus 34% milk protein as the only nutrients at 8, 9, 10, 11, 12, 12, and 12% body weight from 1 to 7 wk of age, respectively. Eight calves were sensitized to soybean by feeding 66% soybean protein concentrate plus 34% milk protein for 21 d and eight were not sensitized by feeding 100% milk protein. Afterward, each calf received one of the diets for 10 d followed by the other diet for an additional 10 d. During the 2nd wk of the initial period each calf was surgically fitted with a duodenal cannula for biopsy of intestinal mucosa. Biopsies were taken at surgery and on the last day of each period. Feeding 66% soybean protein concentrate plus 34% milk protein resulted in lower body weight gain, decreased feed efficiency, higher rectal temperatures, increased diarrhea, and villus atrophy. Diminished villi size supports other studies reporting allergic reaction to soybean protein and was associated with the poor performance observed in calves fed soybean protein.

INTRODUCTION

The low digestibility of soybean protein and poor growth of calves fed milk replacers high in soybean protein have been attributed to the presence of soybean trypsin inhibitor (4, 5); to the failure of the milk replacers to form large, firm clots of coagulated protein in the calf abomasum resulting in a diminished opportunity for protease action (6); and to the lower hydrolytic activity of pepsin and pancreatic proteases on soybean proteins than on milk protein (7). Others attributed poor utilization of soybean protein by calves to a gastrointestinal allergy (8, 9, 15, 19, 20) and reported serum antibodies specific for soybean proteins (2, 8), villus atrophy, and crypt elongation (11, 18). The objective of this experiment was to detect alterations of small intestinal mucosa due to incorporation of large amounts of soybean protein in milk replacers and to relate these alterations to calf performance.

MATERIALS AND METHODS

Sixteen male Holstein calves purchased from a commercial dairy farm were removed from their dams immediately after birth and transported to the Michigan State University Dairy Cattle Center. Upon arrival, each calf was injected with vitamins A, D, E, and selenium; fed colostrum obtained from their dams for one feeding; and fed colostrum from the herd for three subsequent feedings. Colostrum was followed by whole milk for 2 d. From 5 to 46 d of age each calf was fed its designated milk replacer with 23% crude protein as the only source of nutrients at 8, 9, 10, 11, 12, 12, and 12% body weight from 1 to 7 wk, respectively. Solids content of all milk replacers was 14%.

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Replacers were prepared just before feeding and fed twice daily (at 12-h intervals) from an open pail. Fresh, clean water was available at all times and calves were kept in tie stalls bedded with straw. Despite the precautions taken to avoid infectious diseases, calves developed rotavirus infection at 6 to 12 d of age. The diarrhea induced by this infection abated in less than 1 wk and thus was not present during sampling. Milk replacers differed in protein source and were as follows: treatment M, 100% milk protein; and treatment S, 66% soybean protein concentrate plus 34% milk protein. Ingredient and chemical composition of replacers are in Table 1. All chemical components were similar in the two rations even though ether extract was about 20% lower than estimated from ingredients used.

Half the calves were sensitized to soybean protein by feeding milk replacer S for 21 d; the others received milk replacer M and were not sensitized. After the initial 21-d the calves from each group were randomly assigned to one of the replacers for treatment periods of 10 d (period 1) and then changed to the other replacer for 10 additional d (period 2).

During the 2nd wk of the sensitization, each animal was surgically fitted with a 1-cm diameter, plastic Y-shaped duodenal cannula that was positioned about 100 cm distal to the pylorus. This was to permit access to the intestinal lumen for biopsy. Biopsies were taken during cannula placement and on the last day of each period. The sample taken at surgery was from the site of cannulation. Subsequent biopsies were taken 40 to 50 cm distal to the cannula with a suction biopsy instrument at a negative pressure of 38 cm Hg. Samples were fixed immediately in 10% phosphate-buffered formalin. Approximately half of each sample was stained with aqueous new methylene blue (1%) and the individual villi separated with a dissecting needle under a sterioscopic microscope. This staining procedure stains the enterocytes but not the crypt cells. The length of 20 villi in each section was measured by an occular micrometer. The other half of each biopsy sample was sectioned at 7 μ, mounted, and stained with haematoxylon and eosin for routine histopathological examination. Samples so prepared were given a score based on the cellularity of the lamina propria with 0 being

TABLE 1. Ingredient and chemical composition of milk replacers containing milk (M) or soy (S) protein.

Item	Treatment	
	M	S
	(% of dry matter)	
Ingredients		
Nonhydroscopic edible whey	58.82	52.12
Fat-milk concentrate[1]	23.00	23.20
Casein[2]	17.40	0
Soybean protein concentrate[3]	0	23.90
Vitamin-mineral premix[4]	.78	.78
Chemical analysis[5]	96.83	97.30
Dry matter		
Crude protein	23.00	23.06
Ether extract	7.21	7.53
N-free extract	62.90	62.33
Ash	6.89	7.08

[1] Contained 40% fat as homogenized white grease, which was spray-dried with 60% whey and contained 7% protein.

[2] Was 90% protein.

[3] Trade name: Procon 2000; ethanol-extracted soy protein concentrate containing 67% crude protein.

[4] Suppled per kilogram: 44,000 USP units vitamin A; 11,000 USP units vitamin D_3; 44 USP units vitamin E; 6.6mg thiamine; 6.6 mg riboflavin; 2.6 mg niacin; 13 mg d-pantothenic acid; .11 mg biotin; 110 mg ascorbic acid; 6.6 mg pyridoxine hydrochloride; .55 mg folic acid; .07 mg vitamin B_{12}; 2650 mg choline chloride; 100 mg iron; 10 mg copper; .1 mg cobalt; 40 mg zinc; 40 mg manganese; .25 mg iodine; and .1 mg selenium.

[5] According to methods of the Association of Official Analytical Chemists (1).

normal and 3 being markedly hypercellular.

Feed intake of calves was recorded daily, and body weights were recorded on the first and last days of the experiment, at weekly intervals, and on the 1st d of each feeding period. Fecal consistency was rated daily on a scale from 1 to 4 (13). Rectal temperatures were recorded daily just before the morning feeding.

Statistical treatment of data was as described by Gill (3). Values taken at surgery and at 21 d were analyzed as a completely randomized design and those from 10-d periods as split-plots with treatments as subplots in a crossover Latin square design. The F test was used to compare means.

RESULTS AND DISCUSSION

Initial body weight of calves did not differ between treatments, but those at 21 d were higher ($P<.05$) for calves fed M than S. Similar treatment differences were observed for total weight gains and ADG (Table 2).

Treatment had no significant effect on body weights during the two subsequent 10 d when the calves were shifted from one treatment to the other; however, calves that received only milk protein during the initial 21-d had higher ($P<.005$) body weights than those sensitized with soybean protein. As expected, body weights were higher ($P<.05$) after period 2 than 1 (Table 3).

Previous sensitization to soybean protein did not significantly affect average daily gains (ADG) during periods 1 and 2, but treatments and periods did affect gains (Table 3). Calves fed M had higher ($P<.025$) ADG than S, and ADG was higher ($P<.10$) during periods 2 than 1. Due to a significant ($P<.05$) sensitization × period interaction, a comparison between periods was made for nonsensitized and sensitized calves (Table 4). No differences were observed between periods for sensitized calves, but a higher ($P<.01$) ADG was observed for nonsensitized calves during period 2 than 1, suggesting a lower capacity of sensitized calves to gain weight as age advanced.

Feed intake, which was based on body weight, was not significantly affected by treatments M or S during either period (Tables 2 and 3) because the switchover design equalized body weights for the two treatments. However, feed intake was higher ($P<.005$) for nonsensitized than sensitized calves during periods 1 and 2 and was higher ($P<.025$) during period 2 than 1 (Table 3). Feed intake differences were due to variation in body weight because calves were fed according to body weight.

Feed efficiency (gain/intake) was higher for calves fed M than S during the 21-d sensitization ($P<.001$) and during periods 1 and 2 ($P<.005$)

TABLE 2. Influence of feeding milk protein (M) or soybean protein concentrate (S) to calves during a 21-d sensitization on body weights, body weights gains, average daily gains, feed intakes, feed efficiencies (gain/intake), rectal temperatures, fecal scores, and villi length.

Variable	M	S	SE	$P<$
Initial body weight, kg	42.1	42.3	1.2	ns[1]
Final body weight, kg	46.2	41.5	1.4	.05
Body weight gain, kg	4.1	−.7	.6	.001
Average daily gain, g	197.0	−35.0	26.0	.001
Feed intake, kg	11.2	11.1	.3	ns
Gain/intake	.4	−.1	.1	.001
Rectal temperatures, °C	38.7	38.9	.1	.05
Fecal score, 1 to 4 scale[2]	2.4	2.9	.2	.10
Surgery villus length, μm	504.0 ± 47.0	387.0 ± 41.0	...	.10
Final villus length μm	696.0	616.0	67.0	ns
Cellularity score[3]	1.062	1.37		ns

[1] ns = Nonsignificant.

[2] 1 = Normal feces; 2 = pasty consistency; 3 = slightly fluid; 4 = fluid.

[3] 0 = Normal, 3 = marked infiltration of inflammatory cells.

TABLE 3. Influence of sensitization (NS = nonsensitized; SS = sensitized), treatment (M = milk protein; S = soybean protein concentrate), and experimental period (P_1 = period 1; P_2 = period 2) on body weights (BW), average daily gains (ADG), feed intakes (FI), feed efficiencies [gain/intake (G/I)], rectal temperatures (RT), fecal scores (FS), and villi length of calves.

	Sensitization				Treatment				Period			
Variable	NS	SS	SE	$P<$	M	S	SE	$P<$	P_1	P_2	SE	$P<$
BW, kg[1]	52.8	48.5	.7	.005	51.3	50.0	1.6	ns[2]	47.9	53.5	1.6	.05
ADG, g	502.0	459.0	28.0	ns	592.0	369.0	57.0	.025	400.0	561.0	57.0	.10
FI, kg	7.9	7.2	.1	.005	7.5	7.6	.2	ns	7.0	8.1	.2	.025
G/I	.6	.6	.04	ns	.8	.5	.1	.025	.6	.7	.1	ns
RT, °C	38.8	38.9	.04	.10	38.8	38.9	.1	ns	38.8	38.9	.1	ns
FS, 1 to 4 scale[2]	2.6	2.9	.1	ns	2.4	2.9	.2	.10	2.8	2.4	.2	ns
VL, μm	611.0	576.0	24.0	ns	634.0	553.0	36.0	ns	611.0	576.0	36.0	ns
Cellularity score[3]	1.45	1.78		ns	1.5	1.7		ns	1.60	1.63		ns

[1] For sensitization, BW represent weights at the end of period 2. For treatment, the BW represent weights for each treatment at the end of each period.

[2] ns = Nonsignificant.

[3] 1 = Normal; 2 = pasty consistency; 3 = slightly fluid; 4 = fluid.

[4] 0 = Normal; 3 = marked infiltration of inflammatory cells.

(Tables 2 and 3). Effect of sensitization and periods on feed efficiency was not significant during periods 1 and 2, but the sensitization x period and sensitization x treatment interactions were significant (Tables 4 and 5). Gains and efficiencies were higher ($P<.025$) for nonsensitized calves during period 2 than 1, but differences between periods were not significant for sensitized calves (Table 4). Diet had little effect on efficiencies and villi length of nonsensitized calves, but both were higher in sensitized calves fed M than S (Table 5), again suggesting that calves fed soybean protein for 3 wk early in life were not able to gain weight as rapidly as nonsensitized calves.

These growth measurements show milk protein superior to soybean protein concentrate (SPC). Moreover, negative responses to SPC were greater for calves initially sensitized to soybean than to milk protein.

Rectal temperatures were higher ($P<.05$) for calves fed S than M during the initial 21 d (Table 2); the calves sensitized to soy tended ($P<.10$) to have higher rectal temperatures during periods 1 and 2 than calves initially fed milk (Table 3). However, treatments and periods had no significant effect on rectal temperatures during periods 1 and 2. These data suggest part of the initial reaction of calves to soybean protein is slightly elevated rectal temperatures, which do not persist after 3 wk of age. Fecal scores tended to be higher ($P<.10$) for calves fed S than M during the initial 21 d and during periods 1 and 2 (Tables 2 and 3).

Measures of villi length from biopsy samples taken at surgery tended to be higher ($P<.10$) for calves fed M than for S, but no significant differences were observed for biopsies taken after 21 d of treatment (Table 2). No treatment, sensitization, or period effect on villi length were observed during periods 1 and 2. However, the sensitization x treatment interaction was significant (Table 5). Soy treatments did not affect villi length of nonsensitized calves; however, sensitized calves fed M had longer villi ($P<.05$) than those fed S.

TABLE 4. Interaction of sensitization X period on average daily gain (ADG) and feed efficiencies [gain/intake (G/I)] of calves fed milk or soy protein.

	Nonsensitized		Sensitized		
Variable	Period 1	Period 2	Period 1	Period 2	SE
ADG, g	329.0[a]	675.0[b]	472.0	447.0	81.0
G/I	.5[c]	.8[d]	.7	.6	.1

[a,b]Means in the same row and under the same subtitle with different superscripts differ ($P<.01$).

[c,d]Means in the same row and under the same subtitle with different superscripts differ ($P<.025$).

TABLE 5. Interaction of sensitization X treatment (M = protein; S = soybean protein concentrate) on feed efficiencies [gain/intake (G/I)] and villi length (VL) in calves fed milk or soy protein.

	Nonsensitized		Sensitized		
Variable	M	S	M	S	SE
G/I	.7	.5	.9[a]	.4[b]	.1
VL, μm	616.0	607.0	652.0[c]	500.0[d]	51.0

[a,b]Means in the same row and under the same subtitle with different superscripts differ ($P<.005$).

[c,d]Means in the same row and under the same subtitle with different superscripts differ ($P<.05$).

No differences in cellularity of the lamina propria were demonstrated, although there was a tendency for the cellularity scores to be higher in calves fed soy protein at the end of the sensitization (Table 2). The highly subjective method used for assessing cellular reaction would have detected only marked infiltrations of inflamatory cells.

Villus atrophy associated with crypt hyperplasia and an increased cell renewal rate has been associated with soybean protein intolerance and celiac disease in man (12, 16) as well as with the feeding of heated soybean flour to calves (11). Morphological disturbances to the villi and lamina propria of the intestine described by Barratt et al. (2) were caused by feeding toasted soybean flour to calves. A cell-mediated immune reaction was suggested by MacDonald and Ferguson (14) as the cause of villus atrophy, crypt hyperplasia, and malabsorption in food allergies. Kilshaw and Sissons (8, 9) found an increase in immunoglobulins (Ig) and IgE specific to soybean storage globulin glycinin and betaconglycinin in preruminant calves fed heated soybean flour. Soybean antigens are resistant to proteolysis (17) and, to a lesser degree, to microbial action of rumen fluid (2).

Some calves appear more susceptible to soybean antigens than others. Susceptible calves become sensitized after having been fed heated soybean protein and the reactions become progressively worse. Sensitivity may be retained for several weeks without reexposure to soybean antigens (10, 21). Intestinal mucosal changes were observed 24 h after feeding heated soybean flour to sensitized calves, but normal morphology was again observed after 10 d of returning to milk feeding (11).

In conclusion, data suggest that calves fed milk replacer with 66% of its protein replaced by SPC had performance inferior to calves fed milk replacer of all milk protein. The higher rectal temperatures, more liquid feces, and villus atrophy suggest an allergic reaction to SPC that was associated with the poor performance. Villus length data suggest that calves were more susceptible to villus atrophy induced by soy protein at 2 wk than at older ages, and SPC effects were more pronounced in calves previously exposed to this protein source.

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