Pancreatic Proteolytic Enzymes and Growth in Rats Fed Soybean or Milk Proteins[1] (34236)

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Several factors including trypsin inhibitors, hemagglutinins and saponins present in raw or minimally processed soybeans have been implicated as causing poor growth, pancreatic hypertrophy, and altered intestinal proteolysis (11). Gorrill and Thomas (5) and Gorrill *et al.* (6) observed poor growth, reduced trypsin and chymotrypsin output, and reduced intestinal proteolysis and enzyme stabilities when calves were fed milk replacers containing certain soyflours as part of the protein source. These soyflours contained high levels of soybean trypsin inhibitor (SBTI), implying that SBTI may be a possible growth inhibitor. Feeding a pure trypsin inhibitor to calves seemed unfeasible and too costly. Therefore, we studied the effects of the diets previously used by Gorrill (5, 6) and some other similar diets on growth, pancreatic hypertrophy and proteolytic enzyme activity, and intestinal proteolysis in rats.

Experimental Procedures. Groups of five or six individually housed female weanling rats were fed each of the seven diets in Table I for 21 days. The ingredients in diets 2, 3, 4, and 7 are given in Table II. Diet 2 was formulated as a lactose-free diet since diet 1 caused diarrhea in rats. Diets 1, 3, and 5 had been fed previously to calves (5). Diets 1, 3, 4, 5, and 6 were fed simultaneously while diets 2 and 7 were fed at a later date.

TABLE I. Dietary Treatments.

Diet	Protein (%)	SBTI[g] (units/g)
1. All milk replacer[a]	19.2	0
2. Casein replacer	20.5	0
3. Soy protein conc replacer[b]	20.6	0
4. All milk + ground soybeans[c]	24.2	241
5. High soy replacer[d]	24.2	243
6. All soy replacer[e]	24.2	258
7. High inhibitor replacer[f]	21.3	271

[a] Skim milk and whey powder.

[b] Promosoy, Central Soya, Decatur, Indiana; percentage composition (air dried basis): protein, 71; fat, 0.5; fiber, 3.7; ash, 6.3; and carbohydrate, 18.1.

[c] Protein: 60% supplied by ground soybeans, and 40% by skim milk and whey powder.

[d] Protein: 60% supplied by a 50% crude protein soybean flour, and 40% by skim milk and whey powder.

[e] Protein: 100% supplied by a 50% crude protein soybean flour.

[f] Protein: 100% supplied by Centex, Central Soya, Decatur, Indiana, a 50% crude protein soybean flour known to contain trypsin inhibitor.

[g] Soybean trypsin inhibitor activity; 1 unit equals inhibition of the hydrolysis of 1 μmole of TAME/min.

The rats were killed by decapitation. Pancreases were removed, freed from adhering tissue by blotting on cheese cloth, weighed, and stored at −20° until analyzed for trypsin and chymotrypsin activity using *p*-toluenesulphonyl-L-arginine-methyl ester (TAME) and *N*-benzoyl-L-tyrosine ethyl ester (BT EE), respectively, as assay substrates according to the method of Gorrill and Thomas (4).

The small intestine was equally divided into upper and lower sections. Weight of contents from each section and pH of stomach

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TABLE II. Ingredient Composition of Diets 2, 3, 4, and 7.

Ingredient	Diet:	(g) 2	3	4	7
Casein		20.5	—	—	—
All milk replacer		—	—	50.8	—
Promosoy[a]		—	25.0	—	—
Centex[a]		—	—	—	43.0
Ground soybeans		—	—	38.2	—
Vitamin–mineral premix[b]		2.5	4.0	1.2	2.5
B vitamin complex[c]		2.0	2.0	0.9	2.0
Trace mineral salt		2.0	2.0	—	2.0
DL-Methionine		—	0.5	—	0.5
Aurofac-10[d]		0.25	0.25	0.1	0.25
Corn oil		10.0	—	—	10.0
Fat premix[e]		—	33.0	—	—
Glucose monohydrate[f]		62.75	33.25	8.8	39.75
Total		100.00	100.00	100.00	100.00

[a] Central Soya, Decatur, Indiana.

[b] The premix contained (g): thiamine, 55; menadione, 9.9; vitamin A (30,000 IU/g) + D (2800 IU/g) + E (82 IU/g), 1100; K citrate, 3438; Na_2SeO_3, 0.624; $Al_2(SO_4)_3 \cdot 18H_2O$, 300; H_3BO_3, 10.5; $Na_2MO_4 \cdot 2H_2O$, 10.5; pyridoxine HCl, 11.6; NaBr, 20.9; ascorbic acid, 57.2; inositol, 286; folic acid, 1.1; *p*-aminobenzoic acid, 28.6; biotin, 5.5; vitamin B_{12} (0.1% trituration of cobalamin), 31.4; (kg): K_2HPO_4, 12.48; MgO, 6.24; and cerelose, 21.3.

[c] Dawes Lab., Inc., Chicago, Illinois, containing (g/lb): riboflavin, 2; pantothenic acid, 4; niacin, 9; and choline chloride, 90.

[d] American Cyanamide Co., Princeton, New Jersey, containing 10 mg of chlortetracycline per 1 lb.

[e] Consists of 30% fat premix (mixture of dried whey and fat), supplied by Milk Specialties, Inc., Dundee, Illinois.

[f] Cerelose, Corn Products Company, Argo, Illinois.

contents was recorded. Intestinal contents were then frozen and stored until later analysis. The activities and stabilities of intestinal trypsin and chymotrypsin and *in vitro* protein digestion were determined in a 0.5–1-g sample of intestinal contents. Each sample was diluted to 3 ml with 0.15 *N* NaCl, mixed with an equal volume of glycerol and centrifuged at 1300*g* for 30 min. One portion of the supernatant fluid was incubated at 37° for 2 hr while the other portion was stored at 4°. Trypsin and chymotrypsin activities were determined in both portions.

In vitro protein digestion was calculated from the disappearance of protein during incubation of intestinal contents for 2 hr at 37°. Protein hydrolyzed during this 2-hr period was assumed to be influenced by intestinal proteases and not by sources of dietary protein. Protein was precipitated with 4 vol of 10% trichloroacetic acid, washed two times with acetone, once with ether, and redissolved in 5 vol of 0.1 *N* NaOH. Protein was determined spectrophotometrically by methods of Waddell (16) and Tombs *et al.* (15).

Results and Discussion. Body weight gain, feed intake, and size and enzyme content of the pancreas of rats fed seven different diets are listed in Table III. Of the four soybean-source diets containing soybean trypsin inhibitor (nos. 4–7) only two (nos. 5 and 6) reduced weight gains significantly ($p < 0.005$) below that of the diet (no. 3) containing a noninhibitor soy protein concentrate. Pancreases of rats fed these four diets (nos. 4–7) were significantly enlarged ($p < 0.005$), averaging 620 mg/100 g of body wt. The comparable average for pancreases from rats on the noninhibitor diets was 430 mg/100 g of body wt. Pancreatic trypsin and chymotrypsin activity per animal also was increased ($p < 0.005$), but activity per gram of wet pancreatic tissue was not changed by diet. Pancreas size and proteolytic enzyme content were similar on all diets devoid of trypsin inhibitor activity (nos. 1–3) regardless of protein source. The unsatisfactory weight gains by rats fed the milk diet was apparently due to diarrhea, probably caused by the high lactose content of the diet. Body weight gains on the casein diet, which contained cerelose in place of lactose, were also unsatisfactory apparently due to reduced feed intake.

Stomach pH as well as trypsin and chymotrypsin activities in the contents of the small intestine are shown in Table IV. The differences in pH of stomach contents due to diet were not related to protein source or presence of trypsin inhibitor in the soy protein. Tryp-

TABLE III. Body Weight Changes, Feed Intakes, Size and Enzyme Content of Pancreases of Rats Fed Milk- or Soybean-Based Diets.

			Pancreas					
				Trypsin activity		Chymotrypsin activity		
Diet	Body wt change (g/day)	Feed intake (g/day)	Size (mg /100 g of body wt)	Conc (units/g of wet wt)	Total (units/ animal)	Conc (units/g of wet wt)	Total (units/ animal)	ChT/T
1. All milk	2.02[de]	13.6[ab]	438[b]	588	290[b]	2079	1095[ab]	3.78
2. Casein	3.18[c]	11.7[b]	460[b]	461	258[b]	1271	699[b]	2.71
3. Soy protein conc	5.48[a]	14.4[ab]	455[b]	558	442[ab]	1670	1323[ab]	2.99
4. All milk + soybeans	5.02[ab]	14.8[ab]	547[ab]	550	519[ab]	1740	1731[a]	3.34
5. High soy	3.12[c]	13.8[ab]	545[ab]	515	395[ab]	1790	1380[ab]	3.49
6. All soy	4.60[b]	13.8[ab]	657[a]	447	495[ab]	1733	1869[a]	3.78
7. High inhibitor	5.30[a]	15.5[a]	719[a]	679	743[a]	1832	1968[a]	2.70
$S_{\bar{x}}$[g]	0.14	0.7	49	82	100	281	310	0.42
p value[h]	<0.005	<0.005	<0.005	ns[f]	<0.05	ns	<0.05	ns

[e] Means with same superscript (*a*, *b*, *c*, or *d*) are not significantly different at $p < 0.01$ using Duncan's multiple range test (2).

[f] No difference between treatment means, $p < 0.05$.

[g] Standard error of the mean.

[h] Probability of a difference between means arising by chance rather than due to treatment.

sin and chymotrypsin concentrations tended to be higher in the lower half than in the upper half of the small intestine, but no change due to dietary treatment occurred in one segment that did not occur in the other. Therefore, when the casein and high inhibitor diets were fed, the total intestinal contents were pooled. Apparent total trypsin activity was not significantly affected ($p > 0.10$) by dietary treatments, averaging 137 and 171 units/animal on the SBTI (nos. 4–7) and non-SBTI diets (nos. 1–3), respectively. However, chymotrypsin activity was 5 times higher (767 vs 147 units/animal) on the SBTI diets. These divergent changes indicated that either (a) the SBTI-containing diets inhibited trypsin secretion, or (b) trypsin secretion was increased in the same proportion as chymotrypsin but the enzyme assay procedure used measured only trypsin which was not bound to SBTI. The increase in both trypsin and chymotrypsin observed in the pancreas was evidence against the first possibility. If the second situation was true, then the rat apparently compensated for the SBTI by secreting a sufficient excess of trypsin so as to maintain a relatively constant level of free trypsin in the intestinal contents. Other studies (1, 8, 9, 14) indicated that such a compensatory capacity exists in the rat.

Protein content of intestinal contents as well as the amount of protein digested *in vitro* and trypsin and chymotrypsin *in vitro* stabilities are shown in Table V. *In vitro* protein digestion per gram of intestinal content was not affected by dietary treatment. The non-SBTI diets averaged 3.22 mg/g as opposed to 3.14 mg/g for the SBTI diets. The SBTI fed rats tended to have a larger quantity of material remaining in the tract. Therefore, when protein digestion was corrected to a per animal basis, the averages were 6.0 and 10.4 mg protein digested for the total intestinal contents of an animal on the non-SBTI and SBTI diets, respectively. This difference ($p > 0.10$) tended to correlate with total proteolytic activity in the intestinal con-

TABLE IV. Stomach pH and Intestinal Content Trypsin and Chymotrypsin Activities of Rats Fed Milk- or Soybean-Based Diets.

Diet	Stomach pH	Intestinal contents: Trypsin activity: Section (units/g of contents) Upper	Lower	Total (units/animal)	Chymotrypsin activity: Section (units/g of contents) Upper	Lower	Total (units/animal)	ChT/T
1. All milk	3.8[c]	26.5	57.4	144.8	38.1	71.2	183.8[bc]	1.26
2. Casein	6.0[a]	229.7		194.8	136.4		117.0[b]	0.59
3. Soy protein conc	4.3[ab]	38.1	87.5	172.0	70.7	43.7	142.5[b]	0.83
4. All milk + soybeans	4.7[ab]	26.1	29.9	141.0	113.6	121.3	592.0[a]	4.20
5. High soy	4.4[ab]	38.8	26.4	108.3	95.5	174.9	636.7[a]	5.88
6. All soy	3.5[b]	40.9	70.2	146.8	200.4	438.9	922.4[a]	6.28
7. High inhibitor	4.9[ab]	61.6		151.0	363.9		918.6[a]	5.91
$S_{\bar{x}}$[e]	0.4			19.0			119.4	
p value[f]	<0.01			ns[d]			<0.005	

[c] Means with the same superscript (*a* or *b*) are not significantly different at $p<0.01$ using Duncan's multiple range test (2).

[d] No significant difference between treatment means, $p<0.05$.

[e] See footnote *g*, Table III.

[f] See footnote *h*, Table III.

tents (see Table IV), and indicated that intestinal protein digestion was not limiting growth. These results support observations by Scow (14) that the pancreas has the capacity to secrete greater levels of enzymes when SBTI is fed.

Trypsin stability decreased in intestinal contents of rats fed SBTI diets ($p < 0.005$), averaging 0.88 on diets 4–7 as opposed to 0.99 on non-SBTI diets (nos. 1–3). The opposite occurred with chymotrypsin stabilities which averaged 0.90 and 0.80, ($p < 0.005$), respectively. These data support the hypothesis that SBTI binding reduced the amount of trypsin available for substrate hydrolysis (7, 10). As a result, chymotrypsin was degraded less rapidly especially in the lower half of the small intestine and thus appeared to be more stable on SBTI diets. Intestinal trypsin was more stable than chymotrypsin particularly in the contents of the lower small intestine of calves, sheep, and rats fed normal diets (3, 12, 13), but chymotrypsin was more stable than trypsin in human intestinal juice (17).

Growth depression apparently was not caused by SBTI since growth of rats was reduced on only two (nos. 5 and 6) of the four (nos. 4–7) SBTI-containing diets. The increased pancreatic size and accompanying increased trypsin and chymotrypsin synthesis and secretion associated with all SBTI-containing diets indicated a possible compensatory mechanism for combatting SBTI effects, but appeared to be unrelated to growth depression. *In vitro* protein digestion in intestinal contents was unrelated to growth of rats. Thus, impaired protein digestion also can be eliminated from consideration as a mechanism by which soyflour diets 5 and 6 depressed growth.

Summary. Growth, pancreatic function, and intestinal protein digestion were studied in weanling rats fed milk protein or soybean protein with or without soybean trypsin inhibitor (SBTI). Diets containing SBTI caused pancreatic enlargement with corresponding increases in total trypsin and chymotrypsin activities. Enzyme activities per milligram of pancreatic tissue remained constant during all dietary treatments. These same SBTI-containing diets caused increased chymotryp-

TABLE V. Protein Content and *in Vitro* Digestion, and Trypsin and Chymotrypsin *in Vitro* Stabilities of Intestinal Contents from Rats Fed Milk- or Soybean-Based Diets.

Interaction	Protein (mg/g)		*In vitro* enzyme stability[e]	
	Nonincubated conc	*In vitro* digestion[d]	Trypsin	Chymotrypsin
Diet X intestinal section				
1. All milk				
upper	30.7	3.20	0.90	0.89
lower	22.1	1.61	1.01	0.80
total	25.4	1.75	0.99[af]	0.82[bc]
total (mg/animal)		6.99		
2. Casein, total	22.0	5.86	0.99[a]	0.76[c]
total (mg/animal)		6.83		
3. Soy protein concentrate				
upper	61.2	11.86	1.05	0.85
lower	23.3	—2.14	0.96	0.80
total	37.7	2.04	0.98[a]	0.83[bc]
total (mg/animal)		4.26		
4. AM + soybeans				
upper	38.9	5.64	0.80	0.76
lower	22.7	1.01	0.90	0.82
total	26.9	2.12	0.88[bc]	0.80
total (mg/animal)		10.53		
5. High soy				
upper	27.9	4.49	0.79	0.92
lower	18.9	—1.09	0.80	0.91
total	23.0	1.99	0.80[c]	0.91[ab]
total (mg/animal)		7.44		
6. All soy				
upper	50.7	11.24	0.91	0.86
lower	38.1	—2.14	0.91	0.92
total	42.9	2.04	0.92[ab]	0.92[ab]
total (mg/animal)		4.26		
7. High inhibitor				
total	30.9	4.33	0.94[ab]	0.98[ab]
(mg/animal)		11.38		
p value[g]	ns	ns	<0.005	<0.025

[d] Protein digested during a 2-hr incubation of diluted intestinal contents at 37°.

[e] Ratio of enzyme activity of incubated-to-nonincubated intestinal contents.

[f] Means of total contents in the same column followed by the same superscript (*a, b,* or *c*) are not significantly different at $p < 0.05$ using Duncan's multiple range test (2).

[g] See footnote *h*, Table III.

sin activity and stability, decreased trypsin stability, but did not change free trypsin activity in the intestinal contents. Intestinal protein digestion, measured in an *in vitro* system, was not impaired in the intestinal contents of SBTI-fed rats. Growth rates were depressed from normal values of 5.0–5.3 g/day to subnormal values of 3.1–4.6 by two of the four SBTI diets indicating that the growth depression exerted by raw or minimally processed soybean products is not caused by SBTI alone and apparently occurs by some mechanism other than by interference with protein digestion.

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