Nutrition Evaluation

In vitro digestibility. The presence of sodium alginate (PAF and GPAF) greatly inhibited pepsin digestion, whereas trypsin digestion was not inhibited. When comparing the effect of germination, both GPF and TSF were hydrolyzed to the same extent by pepsin, whereas toasted soy flour (TSF) prepared from nongerminated soybeans was poorly digested with trypsin compared with germinated protein fraction (GPF). Therefore, if the combined effect of pepsin and trypsin proteolysis were taken into account, it would appear that germination may not increase protein digestibility significantly.

Trypsin inhibitor activity. TI values are given in Table IV. Compared to raw soy flour, ca. 30% of the TI activity was eliminated by the soaking process (sample SS). Comparable reduction in TI activity was obtained during germination. All of the processed protein fractions contained very little residual TI activity.

Rat Bioassay

The nutritional value of germinated soybean protein fractions, as measured by PER, are given in Table V. Protein fractions, processed with sodium alginate (GPAF and PAF), had the lowest PER values. The growth inhibitory effect of sodium alginate may be attributed to inhibition of pepsin proteolysis. Supplemental methionine increased considerably the values of all diets. The greatest supplemetary effect was obtained with diet containing GPAF and PAF proteins.

These results indicate that sodium alginate form enzyme resistant linakges with methionine residues. In comparing the PER values of diets containing GPF and PF proteins, germination improves slightly the nutritional value of soy protein.

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Effects of Soy Proteins Containing Trypsin Inhibitors in Long Term Feeding Studies in Rats

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ABSTRACT

Pancreatic hypertrophy that occurs in rats fed raw soy flour containing about 1200 mg tripsin inhibitor (TI)/100 g diet was reversed by switching the rats to control diets or to diets containing 30% toasted defatted soy flour. No pancreatic hypertrophy occurs in rats fed commercial, edible grade soy flours, concentrate or isolate from time of weaning to adulthood (ca. 300 days). TI content of the soy diets ranged from 178-420 mg/100 g. Except for pancreas enlargement in rats fed raw soy flour, gross and microscopic examination of pancreata revealed no abnormalities. The gross appearance of heart, kidney, spleen, and liver was normal. Soy flour, protein concentrate, and protein isolate in a formulated corn-soy diet provided optimum growth and maintained body weight only if supplemented with vitamin B-12 in long term feeding studies with rats. In the absence of such supplementation, rats fed soy diets initially grew at a rate equal to or greater than those fed a comparable corn-casein control diet; but, with continued feeding for ca. 300 days, body weight of rats fed the casein control was significantly greater than that of the soy flour-fed rats. Those fed soy isolate ceased to grow; and rats fed soy concentrate lost weight. No significant differences were found in organ weights between groups fed soy products and casein, except for increased kidney, liver, and testes weights relative to body weight with the group fed soy concentrate. Supplementation of the soy diets with vitamin B_{12} stimulated growth to the greatest extent, calcium pantothenate or riboflavin had an intermediate effect, other vitamins had little or no effect; whereas a complete mineral mix was detrimental. Supplementation of the soy diets with vitamin B_{12} stimulated growth to the greatest extent, calcium pantothenate or roboflavin had an intermediate effect, other vitamins had little or no effect; whereas a complete mineral mix was detrimental. Supplementation of the control diet was without effect. The dietary protein level in these diets was 20%, with casein or soy protein representing 75% of total protein. When fed continuously to rats from weaning to adulthood, properly processed soy protein products, when balanced

TABLE I

Protein and Trypsin Inhibitor (TI) Contents of Diet Ingredients

Ingredient	Protein[a] % N x 6.25	Trypsin inhibitor activity TIU/mg sample[b]	Trypsin inhibitor activity mg TI/g sample[c]
Defatted soy flour			
Raw	54.2	80.4	42.3
Toasted (average)[d]	53.1	10.5	5.6
Soy concentrate (average)	72.6	26.5	13.9
Soy protein isolate (average)	91.8	20.7	10.9
Corn meal		1.4	0.74
Casein		0.5	0.25

[a]Dry basis.

[b]TIU = Trypsin Inhibitor Units (8).

[c]Calculated on the basis that 1.9 TIU is equilvalent to 1 μg of TI (8).

[d]Averages for two different shipments.

TABLE II

Composition of Corn-Casein Control and Corn-Soy Experimental Diets

Ingredient %	Diets[a,b] Casein	Flour	Concentrate	Isolate
Corn premix[c]	70.0	70.0	70.0	70.0
Casein	16.0	—	—	—
Soy flour (toasted)	—	28.0	—	—
Soy protein concentrate	—	—	22.3	—
Soy protein isolate	—	—	—	16.3
DL-methionine[d]	—	0.18	0.18	0.30
Dextrose	14.0	1.82	7.52	13.4
Protein content	19.7	19.9	19.6	19.9
Trypsin inhibitor content mg/100 g diet	50.0	176.0	310.0	178.0

[a]Soy ingredients were substituted for casein to maintain equivalent protein level, N x 6.25.

[b]Comparable diets were also formulated with added zinc: Soy flour, 25 ppm; soy concentrate and isolate, 50 ppm.

[c]Premix: yellow corn meal 57.5; soy oil, 5.0; brewers yeast, 2.0; dehydrated alfalfa, 2.0; bone ash, 1.5; iodized salt, 0.5; vitamin A (2000 IU/g), and D_3 (200 units/g) mixture, 1.51 = 70%.

[d]Methionine supplementation calculated to achieve equivalent levels in all diets.

with essential nutrients, can provide growth comparable to corn-casein diets.

INTRODUCTION

Throughout the world, soybeans are cooked, fermented, or processed by various means before consumption. The raw soybean is not very palatable because of its texture and objectionable flavor. Also, in the raw form, nutritive value is low; but when probably processed with moist heat, a process referred to as toasting, nutritive value can be improved to nearly that of meat and milk. Precise control of heating and other processing variables ensures adequate destruction of the heat-labile, antinutritional factors to achieve optimum protein quality (1,2).

Raw soybeans contain a number of heat-labile factors that inhibit proteolytic activity of digestive enzymes, stimulate protein synthesis in the pancreas, and enhance pancreatic enzyme secretion. These effects lead to an enlargement of the pancreas and growth inhibition. Various species of animals respond differently (3).

The nutritional significance of other biologically active substances present in soy, i.e., hemagglutinins, goitrogens, estrogens, allergens, and flatulence-inducing factors, has been reviewed (3,4). Substantial evidence now indicates that both the conversion of raw soybeans into products with excellent protein quality and elimination of the growth inhibitory effects and pancreatic hypertrophy result from the simultaneous destruction of TIs and the transformation of raw protein into a more readily digestible form.

Previously (5), two short term feeding trials of 4 weeks with rats were conducted to determine the biological effects of defatted soy flour processed to contain graded levels of TI activity (71 to 1000 mg TI/100 g diet). No pancreatic hypertrophy occurred in rats fed soy flour in which 55 to 69% of TI had been destroyed. Maximum body weight, protein efficiency values (PER), and nitrogen digestibility were obtained with rats fed soy samples in which about 80% of the inhibitors were inactivated. Although it was concluded that residual TI in properly toasted soy flour has no nutritional significance in short term experiments, the question of whether the ingestion of low levels of TI activity over a prolonged period would create adverse effects remained unanswered.

In the present study initiated in 1973, edible grade, commercially manufactured soy protein products were fed to rats from weaning to adulthood (up to 300 days). The biological parameters evaluated were: body weight gain, organ weights, and histology of the pancreas. Additional work was also undertaken to investigate the reversibility of pancreatic hypertrophy when rats fed raw soy flour were switched to a toasted soy diet or to a control diet.

EXPERIMENTAL PROCEDURES

Materials and Methods

Soy protein products. Raw, dehulled, defatted soy flour (Nutrisoy-7-B), and toasted soy flour (Nutrisoy) were obtained from Archer Daniels Midland Company, Decatur, IL. Soy protein concentrate (Promosoy) and

TABLE III

Trial 1 – Growth and Pancreas Weights of Rats Fed Defatted Soy Flour up to 104 Days and Reversibility of Pancreatic Hypertrophy

Dietary group[a]	Body weight gain ± std. dev.[b] 0-33 Days[c]	33-104 Days	0-104 Days	Pancreas weight g/100 g body weight ± std. dev.
1. Wayne basal[d]	229 ± 25^{ABa}	---	---	0.47 ± 0.04^{Cc}
2. Toasted soy in Wayne basal	235 ± 20^{Aa}	---	---	0.50 ± 0.06^{Cc}
3. Toasted soy in corn-casein basal	242 ± 13^{Aa}	---	---	0.45 ± 0.04^{Cc}
4. Raw soy in Wayne basal	206 ± 21^{BCb}	---	---	0.77 ± 0.04^{Aa}
5. Raw soy in corn-casein basal	197 ± 17^{Cb}	---	---	0.63 ± 0.06^{Bb}
6. Raw soy in Wayne basal	189 ± 24^{Ab}	146 ± 30^{Bb}	335 ± 39^{Bb}	0.60 ± 0.06^{Aa}
7. Raw soy → Wayne basal[e]	201 ± 27^{Aab}	193 ± 12^{Aa}	394 ± 24^{Aa}	0.45 ± 0.05^{Bb}
8. Toasted soy in Wayne basal	221 ± 24^{Aa}	155 ± 29^{Bb}	376 ± 50^{ABa}	0.42 ± 0.05^{Bb}

[a]Level of soy in diet, 30%, TI content (mg/100 g diet) toasted soy, 189; raw soy, 1410.

[b]Duncan's Multiple Range Test (10); means without a superscript letter in common are significantly different; $P<0.05$ = lower case; $P<0.01$ = upper case. Ten rats per group.

[c]0-35 days for groups 1-5.

[d]Commercial rat chow.

[e]Diet switched to Wayne basal after 33 days.

soy protein isolate in the isoelectric form (Promine-R) were obtained from Central Soya Company, Gibson City, IL. Protein content and trypsin inhibitor values of the products are shown in Table I. Only small differences between two shipments were found.

The official TI assay procedure of the American Oil Chemists' Society, as developed by Kakade et al. (6), was used to analyze for TI activity. Heat-treated soy products when assayed by this procedure (6) have greater TI activity, because it is now possible to measure the activity of insoluble TI (7).

Rat bioassay. Weaning male rats of the Sprague-Dawley strain, separated into groups having equal mean body weight, were housed in individual cages and fed ad libitum. Compositions of the corn-casein control and corn-soy experimental diets are given in Table II. Wayne rat chow (Allied Mills, Inc., Chicago, IL), which contained 24% protein, 4% fat, and 4.5% crude fiber, was used in some diets. Food grade casein was purchased from International Casein Corp., New Zealand. Weekly records of body weights and food intake were kept. To prevent oxidative deterioration, the diets were formulated biweekly.

Pathology studies. Pancreas tissue was fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. The stained sections were examined by light microsopy, and the following characteristics were evaluated: (a) acinar architectural organization; (b) width of apical zymogen granule zone; (c) degree of nuclear enlargement; (d) acinar epithelial cell vacuolation; (e) presence of nodules.

RESULTS

Trial 1. Reversibility of Pancreatic Hypertrophy

The purpose of this first trial was to confirm that the TI-induced pancreatic enlargement is reversible (9). Two different basal diets were employed: the first was a commercial diet (Wayne) that contained several cereal and animal protein by-products as well as some toasted soy flour and vitamins and minerals; the second was a formulated corn-casein diet (Table II). In the experimental diets, raw or toasted soy flour replaced 30% of the Wayne basal and was present at the 30% level in the casein control diet, at the expense of casein and a portion of the dextrose.

Results given in Table III show that pancreas weights of the rats fed toasted soy diets for 35 days (diets 2 and 3) were not significantly different from the Wayne basal (diet 1). Diets containing 30% raw soy flour (diets 4 and 5) caused pancreatic hypertrophy and inhibited growth.

After 33 days on test, one-half of the rats fed raw soy were switched to the basal diet for an additional 71 days (diet 7). After continuous ingestion of soy flour for 104 days, rats fed raw soy had significantly greater pancreas weights (diet 6) than the group (diet 7) that was changed at 33 days from raw soy to Wayne basal. Note that body weight gains of the raw and toasted soy groups (diets 6 and 8) were not significantly different after 104 days. These results suggest that pancreatic hypertrophy is readily and completely reversible after 33 days of feeding raw soy and that pancreatic stimulation produced by the TIs in raw soy flour is a nontoxic physiological response. Histological examination revealed no pancreatic abnormalities in either the raw or toasted fed groups.

Trial 2. Biological Threshold Levels of TI in Long Term Feeding

Short term feeding experiments (5) have established that the rat can tolerate relatively high levels of TI (340 to 430 mg/100 g diet) before the inhibitors exert a significant effect on the pancreas. The purpose of this experiment was to determine the relative capacity of defatted soy flours containing graded levels of TI activity to inhibit growth and cause pancreatic hypertrophy when fed to rats from weaning to adulthood. As in trial 1, soy flours were fed at the 30% level in the corn-casein control diet (Table II) at the expense of casein and a portion of the dextrose. Results are summarized in Table IV. Body weights of rats fed raw soy flour for 35, 168, and 215 days (diet 9) were significantly lower than the groups fed partly toasted (diet 11) and toasted soy flour (diet 13). The pancreas was also greatly enlarged in the raw soy group, although no histological abnormalities were noted in either raw partly toasted or toasted soy-fed groups.

Rats fed raw soy for 35 days and then switched to a toasted soy diet showed improved growth and reversed pancreatic hypertrophy (comparing diets 9 and 10). These results show that high levels of toasted soy flour are as effective as a Wayne basal diet (see Table III) in reversing pancreatic hypertrophy. Of special significance is the finding that continuous ingestion of high levels of TI (459 mg/100 g diet) did not inhibit growth nor cause pancreatic hypertrophy (diet 11) when compared to the group fed toasted soy flour (diet 13). These results, in agreement with previously reported data (5), show that relatively high levels of TI activity can be tolerated by the rat in short and long term feeding before the pancreatic hypertrophic properties of the inhibitors will exert a significant biological effect.

Examination of weekly records of body weight data revealed that the initial rapid increase in weight gain of

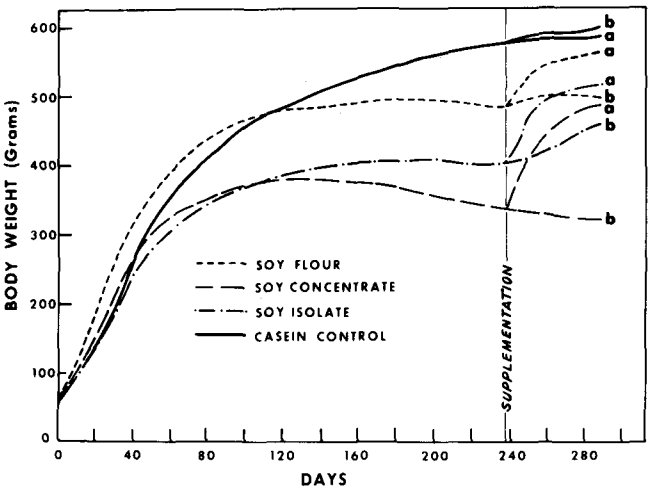

FIG. 1. Growth curves for rats fed soy and casein diets of Table II. (Growth with zinc-supplemented diets not shown.) Eight rats per dietary group. Curve b, unsupplemented diets, Series B; Curve a, supplementation with complete vitamin-mineral mix after 237 days feeding of Series A diets. See text and Tables V and VI for details.

groups 11-13 slowed considerably by the 100th day. By 133 days, all groups (diets 9-13) began to lose weight and showed considerable loss after 167 days. At this point, diets 9–13 were modified by the addition of 5% nonfat milk powder, 2% complete vitamin mixture (Table V, footnote c) and U.S.P. salts mixture XIV containing added zinc, 125 mg/kg; and cobalt, 5.7 mg/kg. All groups responded very quickly, and substantial recoveries in growth occurred in the final 47 days of Trial 2. These data indicated that a nutritional deficiency developed with continued ingestion of soy flour.

Trial 3. Long Term Feeding of Soy Flour, Concentrate, and Isolate

A third feeding trial was conducted to determine whether the slowdown in weight gain noted with soy flour was associated with other soy protein products. A corn-casein diet was used as a control. Composition of the diets is given in Table II.

As shown in Figure 1, the ability of soy flour, concentrate, and isolate to provide optimum growth in young rats and to maintain body weight in adults varied widely, in spite of the fact that the soy diets were supplemented with methionine (See Table II), calculated to provide a sulfur amino acid level approximately equivalent to the corn-casein control diet.

Initially, by 21 days the gain in body weight of rats fed toasted soy flour was significantly greater than the group fed casin control ($P<0.01$); by 125 days the casein control group overtook the rats fed soy flour. At 167 days, the differences in weight were significant ($P<0.05$). On the other hand, by 84 days on test, the growth rate of the groups fed soy concentrate and isolate was considerably lower than that for soy flour and casein ($P<0.01$).

An interesting pattern developed upon continued ingestion of soy protein products. At around 146 days, rats fed soy flour and isolate leveled off in growth, and body weight remained constant thereafter, whereas the casein group continued to gain weight. The group fed soy concentrate began to lose weight by the 174th day.

Anticipating that zinc availability may be a limiting growth factor in soy protein diets (11,12), a second series of feeding tests were carried simultaneously in Trial 3, in which the three soy diets were supplemented with zinc (Table II, footnote 2). The results indicated that zinc supplementation had no significant effect on weight gain throughout the entire period regardless of the soy protein product fed; therefore, the zinc data was not shown. Analysis of variance showed that the major variation in body weight at 237 days was related only to differences in the soy products.

Reformulation of diets in Trial 3. In an attempt to determine why growth rate of rats fed the soy products and casein in trial 3 varied so widely (See Figure 1), all diets, including those containing added zinc, were reformulated or supplemented at 237 days to give a Series A and B. The eight rats of each dietary group were divided into two subgroups of four rats each. The compositions of the Series A diets are shown in Table V. The Series B diets had the composition of the original diets shown in Table II and were supplemented with 75 mg/kg manganese as manganese citrate and 0.1% choline chloride. The design of the Series B diets was based on the fact that soy isolate contained 3 ppm manganese compared to values of 33, 36, and 0 ppm for soy flour, soy concentrate, and casein, respectively (unpublished data); whereas choline supplementation in combination with methionine greatly enhanced growth of chicks fed soy isolate (13).

Results of the continuation of the feeding trial after diet reformulation at 237 days are given in Figure 1. There was little effect on growth with the casein control diet. The reformulated diets, Series A, brought about an immediate stimulation of growth for all soy groups accompanied by a marked increase in feed consumption. The greatest increase in weight gain was obtained with the group fed soy protein concentrate, the group that was actually the most depressed at 237 days. A large increase in weight gain also occurred with the isolate group, whereas reformulation had little effect on the soy flour group. The only Series B group that appeared to benefit from choline and manganese addition was the rats fed soy isolate without zinc. Analysis

TABLE IV

Trial 2 – Long Term Feeding of Defatted Soy Flour Containing Graded Levels Trypsin Inhibitor (TI) on Body and Pancreas Weights of Rats

Dietary group No.	Days on soy diet[a]			TI content, mg/100 g diet	Body weights (± S.E.)[b] after			Pancreas weight (± S.E.)[b] G/100 G.B.W.
	Raw	Partly toasted	Toasted		35 Days	168 Days[c]	215 Days	
9	215	0	0	1269	251.4 ± 4.4^{Bbd}	366 ± 10^{Cc}	432 ± 9^{Bc}	0.509 ± 0.013^{Aa}
10	35	0	180[d]	--	251.9 ± 4.8^{Bb}	418 ± 11^{Bb}	460 ± 13^{ABbcc}	0.387 ± 0.011^{Bbc}
11	0	215	0	459	289.4 ± 6.0^{Aa}	458 ± 15^{ABa}	498 ± 12^{Aa}	0.367 ± 0.015^{Bb}
12	0	35	180[d]		288.9 ± 7.3^{Aa}	453 ± 10^{ABab}	485 ± 9^{Aab}	0.360 ± 0.012^{Bb}
13	0	0	215	189	294.5 ± 6.2^{Aa}	472 ± 13^{Aa}	497 ± 16^{Aa}	0.364 ± 0.012^{Bb}

[a]Ten weanling male rats/group, level of soy in diet, 30%.

[b]S.E. = standard error, Duncan's Multiple Range Test (10): Means without a superscript letter in common are significantly different; lower case, $P<0.05$; upper case, $P<0.01$.

[c]N = 9. Animal, number PAN 17, died at 142 days.

[d]Diet switched to toasted soy diet after 35 days.

TABLE V

Trial 3 – Composition of Reformulated Diets, Series A[a]

Ingredient, %	Casein	Soy flour	Soy concentrate	Soy isolate
Casein	16.0	—	—	—
Soy flour	—	28.0	—	—
Soy concentrate	—	—	22.3	—
Soy isolate	—	—	—	16.3
Corn meal	57.5	57.5	57.5	57.5
Corn oil	5.0	5.0	5.0	5.0
Salts[b]	4.0	4.0	4.0	4.0
Vitamins[c]	2.2	2.2	2.2	2.2
Cellulose	2.2	2.0	2.0	2.0
DL-methionine	—	0.18	0.18	0.30
Dextrose	13.1	1.12o	6.82	12.70

[a]Fed from 237 days to end of feeding trial; brewers yeast, alfalfa meal, bone ash, sodium chloride, vitamin A, and D_3 mix were eliminated from original diets (see Table III) and replaced with a complete salt and mineral mix.

[b]Bernhart and Tomerelli salt mixture (g/kg diet): calcium, 9.0; chlorine, 0.74; copper, 0.0074; iodine, 0.00022; iron, 0.037; magnesium, 0.60; manganese, 0.074; phosphorus, 7.4; potassium, 2.7; sodium, 0.74; sulfur (SO_4), 5.0, zinc, 0.02.

[c]Vitamin mixture (in diet): A acetate, 19.8 iu/g; D_3, 2.2 iu/g; DL-α-tocopherol acetate, 0.12 iu/g; choline chloride, 1.65 g/kg; menadione, 49.6 mg/kg; nicotinic acid, 99 mg/kg; rivoflavin, 22 mg/kg; pyridoxine.HCl, 22 mg/kg; thiamine.HCl 22 mg/kg; calcium pantothenate, 66 mg/kg; D-biotin, 0.44 mg/kg ; folic acid, 1.98 mg/kg; B_{12} crystalline, 29.7 ug/kg; inositol, 110 mg/kg; p-aminobenzoic acid, 110 mg/kg.

TABLE VI

Trial 3 – Organ Weights of Rats Fed Soy Products for 285 Days[a]

Diet[c]	Organ, % of body weight (means ± S.E.)[b]					
	Pancreas	Liver	Kidney	Spleen	Heart	Testes
Soy flour						
Series A	0.36 ± 0.01^{ab}	2.5 ± 0.1^{de}	0.56 ± 0.02^{fg}	0.14 ± 0.01^{a}	0.26 ± 0.0^{ab}	0.64 ± 0.04^{bcde}
Series B	0.38 ± 0.03^{ab}	2.9 ± 0.02^{cde}	0.71 ± 0.08^{def}	0.14 ± 0.01^{a}	0.26 ± 0.01^{ab}	0.68 ± 0.05^{bcd}
Soy concentrate						
Series A	0.37 ± 0.01^{ab}	2.7 ± 0.0^{cde}	0.65 ± 0.03^{defg}	0.15 ± 0.02^{a}	0.26 ± 0.01^{ab}	0.76 ± 0.04^{bc}
Series B	0.32 ± 0.02^{b}	4.1 ± 0.2^{a}	0.99 ± 0.05^{ab}	0.17 ± 0.01^{a}	0.27 ± 0.01^{ab}	0.97 ± 0.06^{a}
Soy isolate						
Series A	0.39 ± 0.02^{ab}	2.4 ± 0.1^{e}	0.64 ± 0.02^{defg}	0.14 ± 0.01^{a}	0.26 ± 0.01^{ab}	0.71 ± 0.03^{bd}
Series B	0.44 ± 0.06^{a}	3.1 ± 0.3^{bc}	0.78 ± 0.02^{cd}	0.16 ± 0.02^{a}	0.28 ± 0.01^{a}	0.74 ± 0.03^{bc}
Casein control						
Series A	0.32 ± 0.02^{b}	2.7 ± 0.1^{cde}	0.53 ± 0.01^{g}	0.14 ± 0.01^{a}	0.23 ± 0.01^{ab}	0.61 ± 0.03^{cde}
Series B	0.36 ± 0.05^{ab}	2.7 ± 0.1^{cde}	0.60 ± 0.02^{fg}	0.16 ± 0.01^{a}	0.25 ± 0.02^{ab}	0.53 ± 0.06^{de}

[a]Animals fed for 237 days on original diets (Table II) plus 48 days on reformulated diets.

[b]S.E. = Standard error, four rats per group, differences among means compared using Duncan's multiple range test (10): means without a superscript letter in common are significantly different, $P < 0.05$.

[c]See text and Table V for reformulation of diets to give Series A and B diets.

of variance indicated that dietary supplementation and type of soy protein product fed had a significant effect on growth. Zinc supplementation was without effect.

Organ weights of rats in Trial 3. Organ weights of rats fed soy products for 285 days are summarized in Table VI. There was no indication of a pancreatic stimulation effect of TIs for either Series A or B diets for all soy protein products, even though TI content ranged from 310 mg TI/100 g concentrate diet to 176 mg TI for both the soy flour and soy isolate diets (Table II).

Significantly, increased liver weight relative to body weight was noted for the group fed soy concentrate Series B. This group of rats lost weight during the first 237 days of feeding (Fig. 1) and failed to respond to the reformulated diet as well. There were no significant differences in relative kidney weights between groups fed soy products and casein, except for an increased relative kidney weight with the group fed soy concentrate Series B. Spleen and heart weights were similar in all diets. Relative weights of the testes for the soy-fed groups were all greater than those of the controls and appeared to be inversely related to the growth rate of the rats fed the various diets. The testes of rats fed soy concentrate Series B were significantly larger compared to the other soy and casein diets. Although the data are not reported here, similar organ weights were also noted for the rats fed diets supplemented with zinc.

These feeding studies indicate that no deleterious effects occurred in the organs of rats fed soy protein products for 285 days. A microscopic examination of the pancreatic tissues revealed no abnormalities. Significant differences in relative organ weights occurred only in the group fed soy concentrate Series B. In this group, the liver, kidney, and testes exhibited significantly higher organ weights relative to body weight.

Trial 4. Identification of the Growth Factor in Reformulated Soy Diets

Results of Trial 3 (Fig. 1) indicated that a nutrient in the reformulated diet greatly stimulated growth of rats fed soy protein products, particularly those fed soy concentrate. In Trial 4, weanling rats were fed the casein control and soy concentrate diets (composition, Table II) for 148 days until a large difference in body weight occurred; body weights were 485 and 409 g, respectively. As in Trial 3, Figure 1, rats on the casein diet outgrew those fed soy concentrate; the difference in body weight became significant at about 64 days in both Trial 3 and 4. In Trial 3, growth ceased and was followed by weight loss with continued feeding. In Trial 4, the soy concentrate-fed group exhibited a slow rate of growth compared to the casein group. This variation may

TABLE VII

Trial 4 – Weight Gain of Rats Fed Soy Concentrate Supplemented with Vitamins and Minerals after 148 days[a]

Dietary group	Mean weight gain (g)[b] 7 Days	14 Days	21 Days	28 Days
Diet 14, casein control[c]	13.2^{bcde}	25.9^{bc}	28.5^{cd}	31.4^{bcde}
Diet 15, soy concentrate[c]	8.8^{de}	18.7^{bc}	24.3^{cd}	32.0^{bcde}
Diet 16, soy concentrate[d]	28.1^{a}	43.0^{a}	50.2^{ab}	58.7^{a}
Soy concentrate Diet 23 plus supplement:[e]				
1. Complete vitamins and minerals	19.0^{abc}	29.6^{abc}	34.3^{bc}	42.7^{ab}
2. Complete vitamins	20.8^{ab}	32.1^{ab}	42.0^{abc}	46.8^{ab}
3. Complete minerals	-1.8^{f}	1.0^{d}	5.2^{e}	12.8^{e}
4. Vitamins A, D_3, E	9.0^{cde}	18.8^{bc}	23.5^{cd}	27.7^{bcde}
5. B vitamins	21.4^{ab}	40.5^{a}	53.5^{a}	56.8^{a}
6. B_{12}	22.3^{ab}	43.5^{a}	53.7^{a}	61.8^{a}
7. Ca pantothenate	16.8^{bcd}	31.1^{ab}	38.2^{abc}	41.2^{abc}
8. Riboflavin	9.7^{cde}	23.9^{bc}	32.5^{bc}	39.7^{abcd}
9. Pyridoxine	14.9^{bcde}	20.9^{bc}	23.0^{cde}	29.8^{bcde}
10. Folic acid	8.2^{cde}	19.0^{bc}	25.2^{cd}	28.3^{bcde}
11. Thiamine	4.9^{ef}	10.4^{cd}	12.2^{de}	19.7^{cde}
12. Nicotinic acid	-2.6^{f}	3.9^{d}	12.7^{de}	17.0^{de}

[a]After 148 days on test, soy groups were subdivided and then supplemented as shown.

[b]Duncan's multiple range test (10): Means, at any given time, without a superscript letter in common are significantly different, $P < 0.05$. N = 8., casein control; N = 6, all other diets. Mean starting body weight (149 days on test): Casein control, 485 g; all other diets, 408 to 409 g.

[c]Composition of diets, Table II; the casein and soy diets contained 0.6 and <0.2 mg B_{12}/100 g, analyzed by Warf Institute, Inc., Madison, Wisconsin, AOAC method (14). These values are the original diets fed to weanling rats at the start of Trial 4 to 148 days.

[d]Soy concentrate diet of Table V was substituted for that of Table II at 149 days.

[e]Concentrations of added vitamins and minerals as shown in Table V, footnotes b and c.

reflect some unknown differences in processing between soy concentrate samples, even though proximate analyses were quite similar.

After 148 days on test, the soy groups were divided into 14 subgroups of 6 rats, each subgroup having the same mean body weight. Vitamins and minerals were added as indicated in Table VII and growth rate was followed for the next 28 days.

Rats receiving diet 16, the reformulated diet supplement, responded in the first week. Rate of growth was significantly greater than with either the unsupplemented diet 15 or the casein control (diet 14). Supplements 1,2, and 5, all of which contained B_{12}, also stimulated growth similar to that observed with diet 16. B_{12} alone (supplement 6) accounted for the stimulating effects observed. An intermediate growth effect, not significant, was obtained with calcium pantothenate and riboflavin. Minerals, thiamine, and nicotinic acid appeared to inhibit growth. As in all previous trials with edible grade, heat-processed soy products, no pancreatic hypertrophy occurred.

DISCUSSION

Rat tests show that pancreatic enzyme secretion is suppressed by negative feedback inhibition resulting from the presence of trypsin in the intestinal tract (15-17). At very high levels of activity such as that in raw soy flour, TI evokes hypersecretion of pancreatic enzymes by forming trypsin-TI complexes, thereby decreasing the suppression exerted by free trypsin. Continuous pancreatic stimulation leads to hypertrophy and growth inhibition. The secretory response of the pancreas to TIs is an indirect one that is initiated in the intestinal tract and not in the blood (18). In previous short term rat bioassays, partially processed soy flour as the sole source of dietary protein did not enlarge the pancreas. The diet contained 464 mg TI/100 g of diet and represented only ca. 54% destruction of TI activity originally present in raw flour (5). Even in the long term feeding tests described here, normal pancreatic enzyme secretion appears to be maintained with diets containing relatively high levels of TI activity.

In the present study, initially all soy diets supported growth equal to rats fed casein. With continued feeding, the growth of soy-fed rats lagged behind that for the casein control. A vitamin B_{12} deficiency may be responsible for most of this growth lag. Since growth rates of rats fed soy flour, concentrate, and isolate differed markedly, it would appear that more research will be needed to determine whether the nutrient requirements of rats change substantially during continuous consumption of soy protein.

Results of numerous chemical analyses and short term animal bioassay indicate that with precise control of manufacturing processes, soy protein products, in mixed diets, have protein nutritional value approaching that of animal protein (4). In extensive human tests, equivalent results were obtained with an intake of at least 0.6 g protein per kg of body weight per day, whether as soy isolate or as beef protein, based on nitrogen balance and chemical-biochemical parameters (19). These reports suggest it is unlikely that such soy protein products would be a health problem in man. Our long term feeding tests support this assessment, because when soy proteins were fed to rats from weaning to adulthood, no pancreatic hypertrophy was observed.

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Dry Roasting of Beans

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Dr. Irving Liener, University of Minnesota, Minneapolis, MN, and Grant Kuhn of the Grant L. Kuhn and Co., Saginaw, MI, reviewed a new process for dry-bed roasting of navy beans. The work is reported in detail in an article by Yadav and Liener, Legume Research 1 (1):17 (1977). The procedure appears applicable to many seeds including soybeans that need heat treatment prior to consumption by animals or humans. The seeds are roasted for a short time at ca. 200 C before being processed to flour or retained as seeds. By means of a screw conveyor, beans are dropped into an inclined rotating drum. It contains sand, salt or ceramic pellets as a heat transfer medium held at 196-204 C. The beans are retained for ca. 20-25 seconds. Beans are dropped into a separator which permits transfer of the salt, sand, or pellets back to the rotating drum heater. Roasted beans may be ground to flour, converted to other products or marketed as "roasted" beans. Tests showed the beans to have digestibility and PER superior to autoclaved beans. Trypsin inhibitor was reduced ca. 75% to 4 x 10^{-3} units per g and hemagglutinin units to 0.2. With added methionine, the PER was 3.1 ± 0.08 and with 50-50 corn-beans, the PER was equal or better than casein. Tests are currently being completed on soybeans.

Physiological Effects of High Soybean Diet in Man

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Two groups of 46 human volunteers were fed a control or a soy diet for 4 weeks and the diet switched for another 4 weeks. The soy diet was based on a soy concentrate of ca. 62% protein. The nutritional, medical and toxicological experts of the group involved in this study concluded unanimously that changes in physiological parameters were not abnormal. Where significant differences were noted, they fell within normal clinical limits. No undesirable effect was noted with no indication of any undesirable long term effect. Properly heat-treated soy protein products should not give any health problems with humans. See Cereal Foods World 23(5):234 (1978).

Toxicity of Cereal Protein - Derived Peptides for in Vitro Developing Intestine from Rat Fetus

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ABSTRACT

This paper describes work that shows that the PTC digest of gliadins extracted from hexaploid wheat flour have the same toxic activity of the PTC digest of gliadins extracted from hexaploid wheat gluten. Moreover, not all wheat species apparently contain the toxic components, thus suggesting that durum wheat foods may present, as compared to soft wheat foods, a lower risk for human health under particular circumstances. Similar considerations seem to apply to other cereal genera that seem to differ with respect to the presence and/or content of toxic peptides. Further experiments to test such a working hypothesis are now being made.

INTRODUCTION

Many factors, such as protein content, essential amino