of phytic acid. De Rham and Jost (23) studied the solubility of phytate and protein in soya extracts as influenced by pH, NaCl, calcium and EDTA. Three processes for preparing low-phytate soya protein products were developed. The authors present a theory of the behavior of phytate in the presence of Ca, Mg, NaCl and soluble proteins.

The enzyme phytase exists in seeds and in many microorganisms. Soaking the seeds reduces the phytic acid content through enzymatic destruction, but the phytates in the mature dry seeds are very stable. During bread making, a considerable part of phytic acid is destroyed by enzymatic activity of the yeast. The fact that part of phytate phosphorous is available to animals shows that some phytate can be hydrolyzed in the intestinal tract, probably through the action of an alkaline phosphatase, which is activated by Mg ions (24).

Considerations for Edible Soya Products

To value the importance of the sequestering activity of phytic acid in edible soya products for human nutrition, one must consider the kind of product, the amount consumed and the kind of consumers. If the last are undernourished children or expecting or lactating mothers with low nutritional reserves and high nutritional requirements, evidently the effect may be different from that exerted on healthy, well-fed individuals. Soya products are frequently incorporated into products used for the treatment or prevention of malnutrition and are given in these cases for considerable lengths of time and in significant amounts. Possible mineral deficiency should be carefully considered, especially because Zn deficiency can produce anorexia.

Magee and Graininger (25) have recently shown that Zn added to low-protein rat diets is able to enhance growth significantly, suggesting that zinc supplementation could improve the apparent utilization of an inadequate level of dietary protein. At the same time, an antagonistic interrelationship between zinc and copper and zinc and iron at dietary zinc levels generally not considered to be toxic to young rats was evident by the marked decrease in liver copper and iron depositions in the presence of added zinc.

Whether it will be wise to supplement edible soya products with Zn or with a mixture of trace metals should be decided after careful considerations of the possible overall effects.

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Significance of Soya Trypsin Inhibitors in Nutrition

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ABSTRACT

Although recent evidence clearly indicates that trypsin inhibitors (TI) and low protein digestibility are the major factors responsible for the pancreatic hypertrophic and growth inhibitory effects of raw soybeans, there was uncertainty regarding the biological threshold level of TI at which these biological effects occur. To obtain such data, dehulled defatted flakes (10% dietary protein) containing graded levels of TI were fed to weanling rats for 4 weeks in two feeding trials. Normal pancreas weights were obtained in rats fed samples in which only 54 to 68% of the original TI of raw soya flour was inactivated. In partially toasted flakes with a nitrogen digestibility value of 77%, the average tolerance level of dietary TI activity that did not cause pancreatic hypertrophy was calculated to be 385 mg TI/100 g diet. TI tolerance level at maximum nitrogen digestibility of 85%, which did not significantly lower weight gain and reduce protein efficiencey ratios, was 260 mg TI/100 g diet. Continuous ingestion of high levels of TI (459 mg TI/100 g diet) in a 20% protein diet for 215 days did not inhibit growth nor cause pancreatic hypertrophy when compared to rats fed toasted soya flour diets. Pancreatic hypertrophy that occurs in rats fed raw soya diets containing up to about 1300 mg TI/100 g diet for 35 days was reversed by switching the rats to control diets or to 30% toasted flour. In long-term feeding studies, no pancreatic hypertrophy occurred in rats fed commercial edible-grade soya flour, concentrate, or isolate from time of weaning to adulthood (ca. 300 to 330 days). TI content of the diets ranged from 178 to 310 mg/100 g diet. Microscopic examination of the pancreas revealed no abnormalities. Gross appearances of heart, kidney, spleen and liver were normal. In long-term feeding, vitamin B-12 supplements were needed to provide optimum growth and to maintain body weight. Results of numerous chemical analyses, relatively short-term human tests and long-term animal feeding studies indicate that with proper control of manufacturing processes, soya protein products can be produced that, in mixed diets, have protein nutritional value approaching that of animal protein.

INTRODUCTION

Raw soybeans and protein products contain a number of heat-labile and heat-stable biologically active factors. The nutritional significance of these factors and their physiological and biological effects in various species of animals are reviewed in greater detail elsewhere in these proceedings.

Of particular concern are the trypsin inhibitors (TI), which stimulate pancreatic juice secretion, cause pancreatic hypertrophy and inhibit growth (1). Because of the key role the pancreas has in the digestive process in governing the availability of nutrients from complex precursors, dietary factors such as TI's in high-protein, high-fat diets may have an important effect on endocrine and exocrine functions of the pancreas. Here we review the question of whether the ingestion of low levels of TI in soya protein products over a prolonged period of time can create adverse effects.

Soya protein products containing varying levels of TI activity were subjected to biological evaluation in short-term and long-term feeding trials in rats (2, 3). Biological threshold levels of TI were studied with respect to weight gain, protein efficiency ratio (PER), nitrogen digestibility and pancreas weight.

EXPERIMENTAL DATA

Preparation of Soy Protein Products

Raw, dehulled, defatted soya flakes were prepared at Northern Regional Research Center (NRRC) from certified seed-grade soybeans according to previously established procedures (4). Flakes containing graded levels of TI activity were prepared by treating the flakes with live steam at atmospheric pressure and 100 C (toasting) for varying lengths of time. Edible-grade, commercially manufactured soya flour, concentrate and isolate were also evaluated. The soya concentrate was manufactured by the aqueous alcohol process, and the soya isolate was in the isoelectric form (5). The official TI assay procedure of AOCS Method BC3-49, (6) as developed by Kakade et al. (7), was used to analyze TI activity. With this procedure, the activity of insoluble TI can be measured (8). Modifications of the Kakade procedure have been reported (9, 10). Protein contents and TI values of the products are given in Table I.

Effect of Toasting on TI Activity and NSI

The inactivation of TI activity and change in protein solubility (nitrogen solubility index-NSI) as a function of steaming at 100 C are given in Table II. Raw, dehulled, defatted soya flakes had an initial moisture content of 8.5%. TI activity decreased rapidly to 79% destruction within 10 min of toasting. Up to 90 and 92% of the original activity was destroyed after 20 and 30 min heating. Complete destruction of TI's by atmospheric cooking would result in degradation of certain amino acids and thereby adversely affect protein quality. In terms of NSI values, protein solubility decreased much more slowly than inactivation of TI's.

Study 1: Threshold Levels of TI in Short-Term Feeding Trials

Composition of the control diet is given in Table III. Two 28-day feeding trials were conducted to determine the relative capacity of defatted soy flours containing graded levels of TI activity to inhibit growth, reduce PER, lower nitrogen digestibility and enlarge the pancreas (2). Results of trial II are given in Table IV. As TI activity in the diet decreased, body weight, PER and nitrogen digestibility increased to a maximum with flours toasted for 9 min in which 79% of the TI had been inactivated. Additional heat treatment of 20 min (diet 7) to destroy 90% TI tended to lower nutritive value based on a comparison of body weight and PER values of diets 6 and 7.

No pancreatic hypertrophy occurred in rats fed soya flour diets containing 464 mg TI/100 g diet (diet 4) in which only 54% of the TI's were inactivated. With this amount of heat treatment, nitrogen digestibility increased from 74 to 77%. Once a minimal amount of TI activity is destroyed that no longer causes pancreatic hypertrophy (diet 4), the further increase in weight gain and PER is attributed to an increase in protein digestibility from 77 to 84% (diet 6).

An attempt to quantitate the maximum dietary level of TI that could be tolerated by the rat was made by plotting each biological parameter as a function of the TI content of the diet. Such plots were statistically evaluated with respect to the least significant difference for each parameter. Calculated tolerance levels of TI resulting in maximum body weight and PER and no pancreatic hypertrophy are summarized in Table V. The adverse effects of raw flour as

TABLE I

Protein and Trypsin Inhibitor (TI) Content of Diet Ingredients (3)

Ingredient	Protein[a]	Trypsin inhibitor activity	
	N × 6.25	TIU/mg sample[b]	mg TI/g sample[c]
Laboratory soy flour			
Raw	54.6	92.4	48.6
Toasted[d]	54.6	8.7	4.6
Commercial soy flour			
Raw	54.2	80.4	42.3
Partially toasted	53.8	29.1	15.3
Toasted	53.1	10.5	5.5
Soy protein concentrate	72.6	26.5	13.9
Soy protein isolate	91.8	20.7	10.9
Corn meal	–	1.4	0.7
Casein	86.06	0.5	0.3

[a]Dry basis.

[b]TIU = trypsin inhibitor units, as-is basis (7).

[c]Calculated on the basis that 1.9 TIU is equivalent to one μg of TI (11).

[d]Toasted 20 min at atmospheric pressure and 100 C.

TABLE II

Trypsin Inhibitor (TI) Activity and Nitrogen Solubility Index (NSI) of Heat-Treated Soya Flours (2)

Heat treatment (min)[a]	TI activity (TIU/mg sample)[b]	Destruction of TI activity (%)	NSI
0	96.6	0	97.2
1	74.9	23	78.2
3	45.0	54	69.6
6	28.0	71	56.5
9	20.5	79	51.3
20	10.1	90	37.9
30	8.0	92	28.2

[a] Live steam at 100 C.

[b] TIU = trypsin inhibitor units (7), as-is basis.

measured by weight gain, PER, and pancreas weights disappeared at different levels of TI activity in the diet.

Study 2: Threshold Levels of TI in Long-Term Feeding Trials

The objective of this study was to determine whether commercial defatted flakes containing graded levels of TI activity (Table I) inhibited growth and caused pancreatic hypertrophy when fed to weanling rats for 215 days (3). The soy flours were fed at the 30% level in a corn-casein control diet (Table VI) at the expense of casein and a portion of the dextrose, but without the addition of zinc and methionine.

As shown in Table VII, the growth of rats fed raw soy flour containing 1269 mg TI/100 g diet (diet 8) for 35, 168 and 215 days was significantly lower than that of the groups fed partially toasted (diet 10) and toasted soya flour. Pancreatic hypertrophy occurred only in rats fed raw soya flour. Of particular nutritional significance is that continuous ingestion of high levels of TI (459 mg/100 g diet) for 215 days did not inhibit growth or cause pancreatic hypertrophy (diet 10) when compared to the group fed

TABLE III

Composition of Control Diet

Ingredients[a]	Percentage of diet
Casein	11.62
Corn oil	8.00
Dextrose	49.63
Salts XIV + Zn + Co	4.73[b]
Cellulose	3.00
Vitramix	2.00
Corn starch	20.00
Water	1.02

[a] Defatted soy flours were substituted for casein, dextrose, and water to maintain a 10% protein diet (N X 6.25).

[b] Slight adjustment made in the soy diet to compensate for the greater ash content of the soya samples; diets contain 40 ppm of supplement Zn as $ZnSO_4$ and 2 ppm Co as $CoCl_2$.

TABLE IV

Effects of Feeding Defatted Soya Flour Containing Graded Levels of TI Activity on Body Weight, PER, Nitrogen Digestibility, and Pancreas Weights of Rats–Trial II (2)

Diet no.	Dietary protein[a]	TI content (mg/100 g diet)	Mean body weight (g) ± standard deviation[b]	PER[c]		Nitrogen digestibility[d] (%)	Pancreas weight ± standard deviation[b] g/100 GBW[e]
				Actual ± standard deviation[b]	Corrected		
1	Casein (0)	0	157 ± 16ab	3.51 ± 0.18a	2.50	92	0.48 ± 0.03c
2	Soy (0)	1001	84 ± 4f	1.59 ± 0.10f	1.13	74	0.68 ± 0.11a
3	Soy (1)	774	94 ± 8ef	1.89 ± 0.26e	1.35	78	0.58 ± 0.01b
4	Soy (3)	464	123 ± 5d	2.46 ± 0.14d	1.75	77	0.51 ± 0.06c
5	Soy (6)	288	141 ± 12c	2.91 ± 0.12bc	2.07	83	0.52 ± 0.04c
6	Soy (9)	212	146 ± 11bc	3.08 ± 0.15b	2.19	84	0.48 ± 0.06c
7	Soy (20)	104	139 ± 13c	2.92 ± 0.10bc	2.08	83	0.49 ± 0.06c
LSD[f]			11	0.17			0.06

[a] Time (min) of heat treatment is given in parentheses (see also Table II). Initial weight of rats, 54 g.

[b] Letters not in common denote statistical significance ($P < 0.05$), method of Duncan (12).

[c] PER = Protein efficiency ratio = weight gain/g protein consumed. Corrected value based on a PER = 2.50 for casein.

[d] $\text{Digestibility} = \frac{\text{intake nitrogen} - \text{fecal nitrogen}}{\text{intake nitrogen}} \times 100.$

[e] GBW = g body weight.

[f] LSD = least significant difference at the 95% confidence level.

toasted soy flour (diet 12). Pancreatic hypertrophy was reversed when rats fed raw soy flour were switched to a toasted soy flour diet (diet 11). Final body weight also did not differ significantly when diets 11 and 12 were compared.

As in the short-term study, (Table V), long-term studies (Table VII) have established that the rat can tolerate relatively high levels of TI before the inhibitors will exert a significant effect on the pancreas.

Study 3: Reversibility of Pancreatic Hypertrophy

Earlier studies (13) had shown that maximum pancreatic hypertrophy occurs within 9 days after feeding raw soybean meal. Pancreatic hypertrophy in rats consuming raw soybean meal for 38 days was reversed when the rats were switched to a casein-control diet with no TI activity (14). As shown in Table VII (comparing diets 8 and 9), pancreatic hypertrophy in rats fed 30% raw soya flour was also reversed when the rats were switched to a toasted flour diet.

Results given in Table VIII show that diets containing 30% raw soya flour in either a Wayne basal (diet 13) or a

TABLE V

Calculated Tolerance Levels of TI Activity in Rats (2)

Biological parameter	LSD		Level of TI activity[a] (mg/100 g diet)	
	Trial I	Trial II	Trial I[b]	Trial II[c]
Body weight (g)	16	11	225	300
PER	0.21	0.17	240	270
Pancreas weight (g/100 GBW)[d]	0.06	0.06	340	430

[a] Level of TI activity that will not significantly affect each biological parameter compared to soya diets having maximum nutritive value based on the LSD values for each feeding trial.

[b] Calculations based on data given in reference 2.

[c] Calculations based on data shown in Table IV.

[d] g body weight.

TABLE VI

Composition of Corn-Casein and Corn-Soya Experimental Diets (3)

Ingredient (%)	Diets[a,b]			
	Casein	Flour	Concentrate	Isolate
Corn premix[c]	70.00	70.00	70.00	70.00
Casein	16.00	---	---	---
Soy flour (toasted)	---	28.00	---	---
Soy protein concentrate	---	---	22.30	---
Soy protein isolate	---	---	---	16.30
DL-methionine[d]	---	0.18	0.18	0.30
Dextrose	14.00	1.82	7.52	13.40
Protein content	19.70	19.90	19.60	19.90
Trypsin inhibitor content mg/100 g diet	50.00	176.00	310.00	170.00

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

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

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

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

TABLE VII

Long-Term Feeding of Defatted Soya Flour Containing Graded Levels of Trypsin Inhibitor (TI) on Body and Pancreas Weights of Rats (3)

Dietary group no.[a]	Days on soy diet			TI content (mg/100 g diet)	Body weight (± S.E.)[b] after:			Pancreas weight (± S.E.)[b] g/100 GBW
	Raw	Partly toasted	Toasted		35 days	168 days[c]	215 days	
8	215	0	0	1269	251.4 ± 4.4Bb[d]	366 ± 10Cc	432 ± 9Bc	0.509 ± 0.013Aa
9	35	0	180[d]		251.9 ± 4.8Bb	418 ± 11Bb	460 ± 13ABbc[c]	0.387 ± 0.011Bb[c]
10	0	215	0	459	289.4 ± 6.0Aa	458 ± 15ABa	498 ± 12Aa	0.367 ± 0.015Bb
11	0	35	180[d]		288.9 ± 7.3Aa	453 ± 10ABab	485 ± 9Aab	0.360 ± 0.012Bb
12	0	0	215	189	294.5 ± 6.2Aa	472 ± 13Aa	497 ± 16Aa	0.364 ± 0.012Bb

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

[b] S.E. = standard error, Duncan's Multiple Range Test (9); means without a superscript letter in common are significantly different; lower case, $P < 0.05$; upper case, $P < 0.01$.

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

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

TABLE VIII

Reversibility of Pancreatic Hypertrophy in Rats Fed Raw Soya Flour (3)

Dietary group[a]		TI content	Pancreas weight g/100 g body weight ± std dev[b]	
			Days on test	
Diet no.	Group	(mg/100 g diet)	35	104
13	Raw soya in Wayne basal	1410	0.77 ± 0.04^{Aa}	0.60 ± 0.06^{Aa}
14	Raw soya in corn-casein basal	1410	0.63 ± 0.06^{Bb}	---
15	Raw soya Wayne basal[c]	---	---	0.45 ± 0.05^{Bb}
16	Toasted soya in Wayne basal	189	0.50 ± 0.06^{Cc}	0.42 ± 0.05^{Bb}
17	Toasted soya in corn-casein basal	189	0.45 ± 0.04^{Cc}	---
18	Wayne basal[d]	---	0.47 ± 0.04^{Cc}	0.42 ± 0.05^{Bb}

[a] Level of soya in diet, 30%.

[b] Duncan's Multiple Range test (12); means without superscript letter in common are significantly different: $P < 0.05$ = lower case; $P < 0.01$ = upper case. Ten rats per group.

[c] Diet switched to Wayne basal after 33 days feeding.

[d] Commercial rat chow.

corn-casein basal diet (diet 14) caused pancreatic hypertrophy, but the greatest enlargement of the pancreas occurred with the group fed raw soya in the Wayne basal diet. After 33 days of the test, one-half of the rats fed raw soya were switched to the Wayne basal diet for an additional 71 days (diet 15); normal pancreas weights were obtained (diet 15 vs diet 18) showing that TI-induced pancreatic hypertrophy is reversible. Pancreas weights of rats fed toasted soya flour in two types of basal diets (diets 16 and 17) were not significantly different from the Wayne basal (diet 18).

Study 4: Long-Term Feeding of Commercial Edible-Grade Soya Protein Products

In the studies reported above, pancreatic hypertrophy in rats fed raw soya flour was reversible, and residual TI activity in toasted soy flour did not create adverse biological effects or produce histological abnormalities in the pancreas of rats fed partly toasted or toasted soya flours. However, the question of whether the ingestion of edible-grade soya concentrates and isolates containing varying levels of TI activity over a prolonged period would have a deleterious effect remains unanswered. Composition and TI content of the diets are given in Table VI.

Growth Curves

As shown in Figure 1, rats fed soya diets initially grew at a rate equal to or greater than those fed a comparable corn-casein diet. With continued feeding for 237 days, body weights of rats fed the casein control were significantly greater than those of the soya product-fed rats both in the presence or absence of supplemental zinc (Table VI, footnote b). Rats fed soya concentrate exhibited the greatest difference as compared to the control. Replacement of the basic control diet (Table VI) at 237 days with a reformulated diet (Table IX) containing a complete vitamin-mineral mix brought an almost immediate stimulation of growth for all soya groups. Food consumption also increased markedly. Reformulation of the corn-casein control diet did not affect body weight and food consumption. Supplementation of the diets described in Table VI with vitamin B_{12} stimulated growth in a way similar to that observed in Figure 1 with the complete reformulated diet. Intermediate growth effects were observed with calcium pantothenate and riboflavin supplementation.

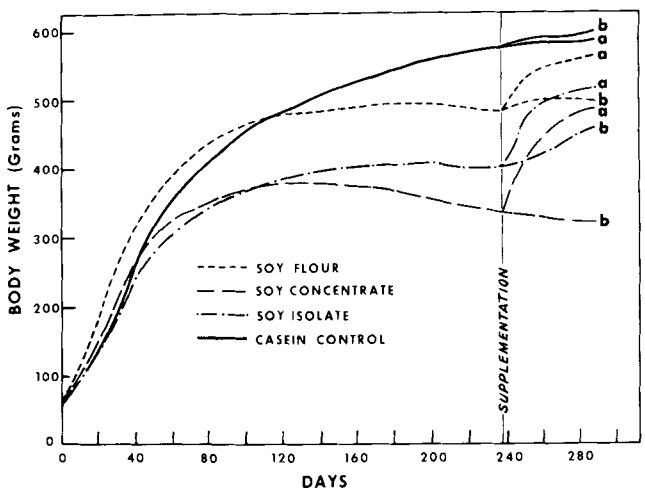

FIG. 1. Growth curves for rats fed soya products and casein diets (growth with zinc-supplemented diets not shown.) Eight rats per dietary group. Curve b represents unsupplemented diets of Table VI; curve a represents supplementation with reformulated diet of Table IX after 237 days feeding of diets of Table VI (3).

Organ Weights

Additional feeding studies indicated that no deleterious effects occurred in the organs of rats fed soya products for 285 days with the diets described in Table VI. The organ weights are summarized in Table X. No TI-induced pancreatic hypertrophic effects were observed in rats fed all of the soya products for 285 days, even though TI content ranged from 310 mg TI/100 g concentrate diet to 176 mg TI for both the soya flour and soy isolate diets. Microscopic examination of pancreata revealed no abnormalities.

Except for some variability in test weights, there were no significant differences in relative organ weights among groups fed soya products and casein. Gross appearances of all of organs were normal.

DISCUSSION OF NUTRITIONAL SIGNIFICANCE

TIs and dietary protein stimulate pancreatic activity by a common mechanism—only the extent of stimulation differs. Rat tests show that pancreatic enzyme secretion is suppressed by negative feedback inhibition, resulting from the presence

TABLE IX

Composition of Reformulated Diets[a] (3)

Ingredient (%)	Casein	Soya flour	Soya concentrate	Soya isolate
Casein	16.0	---	---	---
Soya flour	---	28.00	---	---
Soya concentrate	---	---	22.30	---
Soya isolate	---	---	---	16.30
Corn meal	57.5	57.50	57.50	57.50
Corn oil	5.0	5.00	5.00	5.00
Salts[b]	4.0	4.00	4.00	4.00
Vitamins[c]	2.2	2.20	2.20	2.20
Cellulose	2.2	2.00	2.00	2.00
DL-methionine	---	0.18	0.18	0.30
Dextrose	13.1	1.12	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 (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, 0.06; 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; riboflavin, 22 mg/kg; pyridoxine, HCl, 22 mg/kg; thiamine HCl 22 mg/kg; calcium pantothenate, 66-μg/kg; D-biotin, 0.44 mg/kg; folic acid, 1.98 mg/kg; B_{12} crystalline, 29.7 μg/kg; inositol, 110 mg/kg; p-aminobenzoic acid, 110 mg/kg.

TABLE X

Organ Weights of Rats Fed Soya Protein Products for 285 Days (3)

	Organ, % of body weight					
Dietary group[a]	Pancreas	Liver	Kidney	Spleen	Heart	Testes
Soya flour	0.36^{ab}	2.50^{de}	0.56^{fg}	0.14^{a}	0.26^{ab}	0.64^{bcde}
Soya concentrate	0.37^{ab}	2.70^{cde}	0.65^{defg}	0.15^{a}	0.26^{ab}	0.76^{bc}
Soya isolate	0.39^{ab}	2.40^{e}	0.64^{defg}	0.14^{a}	0.26^{ab}	0.71^{bd}
Casein control	0.32^{a}	2.70^{cde}	0.53^{g}	0.14^{a}	0.23^{ab}	0.61^{cde}
SE[b]	0.03	0.14	0.04	0.01	0.01	0.05

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

[b] SE = standard error, four rats per group, differences among means compared using Duncan's multiple range test (12); means without a superscript letter in common are significantly different $P < 0.05$.

of trypsin in the intestinal tract (1). At high levels of activity, such as in raw soya 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. Poorly digested protein, such as in raw flour and high-protein diets, forms trypsin-protein complexes that also accelerate pancreatic stimulation.

The feedback concept (15) reinforces the evidence in this report that residual TI in toasted edible-grade soy products may have little or no nutritional significance as long as the TI level remains below the biological threshold level at which pancreatic hypertrophy occurs. With the rats fed diets containing up to 20% protein, tolerance levels of TI at which no pancreatic hypertrophy occurs range from 340 to 459 mg TI/100 g of diet (Tables V and VII, diet 10) in both short-term and long-term feeding studies. Since pancreatic hypertrophy is reversible and appearances of other organs are normal, these are important biological findings because they suggest that soya proteins do not pose a hazard to humans.

The significance of TIs with respect to human nutrition is speculative. Since pancreatic feedback inhibition also occurs in humans, the rat may be a good model for assessing the question of whether ingestion of low levels of TI activity over a prolonged period could create adverse effects in humans.

Liener (16) reported that animals whose pancreas weights are greater than 0.3% relative to body weight exhibit hypertrophy when fed raw soybeans, whereas those whose relative pancreas weight is less than 0.3% do not. In man, the pancreas is only 0.09 to 0.12% relative to body weight. If size of the pancreas reflects its functional activity (17), then the biological response to raw soybeans or TIs could in all probability be lower in man than in the rat.

Soya protein products, properly processed, are an excellent source of low-cost, high-quality protein for human needs (18, 19); but in all of these studies, the testing period was relatively short. The products are reasonably well-tolerated and are of good protein value for humans of all ages, based on nitrogen balance and clinical-biochemical evidence (19, 20). However, more data on pancreatic function and histology are needed to establish the effects of long-term ingestion of residual levels of TI in soy products in humans.

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Lysinoalanine: Production, Significance and Control in Preparation and Use of Soya and Other Food Proteins[1]

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ABSTRACT

Formation of lysinoalanine (LAL) in proteins in response to alkali treatment is a well-known phenomenon. The quantity of LAL formed depends on temperature, the time of exposure to alkali, the type of protein, the concentration of protein and alkali in some instances, and probably the type of cation in the alkaline solution. Higher temperatures, longer exposure times, and higher pH's generally result in more LAL formation. The addition of mercaptoethanol or cysteine to an alkaline protein solution decreases LAL formation markedly; lanthionine is apparently a major product formed when cysteine is added to an alkaline protein solution. Some LAL is likely to be formed in any protein-containing product that is subjected to alkaline treatment, and has been shown to be formed in some protein products under extreme heat conditions. Proper control of temperature and pH in processing can reduce or eliminate LAL formation. LAL has not been shown to present a toxicological hazard to any species other than the rat. Its presence in large quantities in any protein indicates destruction of cysteine and lysine; the nutritional inferiority of severely alkali- or heat-treated proteins due to LAL formation, amino acid degradation and isomerization, Maillard product formation, and so on is well documented. The small quantities of dietary LAL in food products currently on the market seem to represent no health hazard; the reduced nutritional quality of protein products that contain relatively high levels of LAL should be considered when these products are major sources of dietary protein.

Lysinoalanine (LAL) is formed by the dehydration of either cystine or serine to dehydroalanine, followed by reaction of the epsilon-amino group of lysine with the double bond of dehydroalanine (1). Its formation in proteins exposed to alkali treatment is a well-known phenomenon (2, 3). The quantity of LAL formed is dependent on several factors, including temperature, time of exposure to alkali, type of protein, concentration of alkali and type of cation in the solution.

[1] All values of LAL have been converted to ppm for purposes of comparison in this review. The alkali concentrations in another paper were likewise converted, from moles/kg to molarity (14). Some of the LAL values were estimated from graphs and are only approximate (7).

EFFECTS OF TIME AND TEMPERATURE

DeRham (4) followed cysteine/cystine (cys) destruction by alkali at pH 12.5 at periods of 25–80 min at several temperatures. At 25 C little cys loss was observed. At 75 C, 40% of the cys in soya protein was destroyed within 45 sec, and up to 65% within 15 min. Losses of cys in whey protein were 15 and 50% under the same conditions. In soya protein exposed to pH 12.5 for 1.5 min, 1000 ppm LAL was formed at 55 C, and 1800 ppm at 65 C. Whey protein (which has a much higher cys content than soy) was subjected to similar treatment, and 4500 and 5400 ppm LAL were formed at 55 C and 65 C respectively.

Using 0.1 N NaOH at 80 C, Hasegawa and Okamoto (5) reported no apparent major difference in the quantity of LAL formed at 1 and 5 hr in a soybean protein preparation, but found 34% more LAL after a 16 hr alkali treatment than after 1 or 5 hr. When 0.2 N NaOH was used, a 15% increase in LAL was found in 5 hr compared to that found in 1 hr. The lack of increase from 1 to 5 hr in .1 N NaOH, and the small magnitude of the increase in 0.2 N NaOH is not surprising. DeRham's results indicate that the majority of easily formed LAL would be present long before the 1 hr measurement was made.

Provansal et al. (6) observed a different effect of extended alkali exposure on LAL concentration. Using 0.2 N NaOH at 80 C, and a sunflower protein isolate solution, 11,275 ppm LAL was measured after 1 hr. After 5 hr, LAL was present at 8200 ppm, and after 16 hr, at only 6150 ppm. The loss of LAL with time in strong alkaline solutions has also been observed in different proteins by other authors (7,8,9).

EFFECT OF pH AND ALKALI CONCENTRATION

As would be expected, LAL formation is usually increased by increasing pH or increasing alkali concentration. Provansal et al. (6) extracted sunflower protein with 0.05 M NaOH for 30 min, and found 4264 ppm LAL in the protein