Biological and Physiological Factors in Soybeans

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ABSTRACT

There are limitations to which one is justified in drawing broad generalizations concerning the diverse biological and physiological effects of soy protein products. Nevertheless, there appear to be two distinct situations: (A.) Proper heat treatment exerts a beneficial effect upon the nutritive value of whole soybeans, full-fat and defatted meal. Associated with proper heating is inactivation of trypsin inhibitor and other heat-labile factors and conversion of raw refractory proteins to forms that are more readily digested. (B.) Moist heat also has a beneficial effect upon the nutritive value of soy protein isolates. However, a deficiency of certain essential nutrients and the interaction of phytic acid with protein, vitamins, and minerals during processing are the primary factors responsible for the poor nutritive value of soy isolates. Occasionally mineral deficiency symptoms do occur in animals fed soybean meal. It is a misnomer to refer to the growth-inhibiting and pancreatic hypertrophic properties as a "toxic" effect since both properties are reversible. Modern analytical techniques should be used to reinvestigate the relationship between phytic acid and availability of minerals and vitamins in soy protein isolate diets. Research also is needed to determine more accurately vitamin and mineral contents of soy protein isolates and the availability of vitamins and minerals in soy protein concentrates. Breeding soybean varieties genetically deficient in antinutritional and nonflatulent factors does not appear promising. More research is needed to determine whether fermentation and enzyme processes can be used to prepare flatulent-free soy products. Minor factors to be considered in assessing the nutritive value of soy products include a weak goitrogen present in soybeans, and a very low estrogenic activity.

INTRODUCTION

Soybeans, primarily in the form of fermented foods, were an ancient cornerstone of nutrition in Southeast Asia. With the development of new technology, soybeans have reemerged as one of the best sources of food to help solve the protein problems in the world today. In the raw form, soybeans contain many interrelated substances which cause differing biological and physiological responses in various species of animals (1,2). The only report on man indicates that raw soy flour can support positive nitrogen balance but not as efficiently as autoclaved flour (3). With heat treatment and processing variables properly controlled, the nutritive value of raw soy flour can be improved to ca. that of meat and milk. Heat treatment is most likely an absolute requirement if the essential nutrients in all soy protein products are to be used fully by most animals, including man. More definitive knowledge is needed to establish optimum conditions for inactivation or removal of these antinutritional factors, particularly in relation to the new technological processes now in use.

BIOLOGICAL FACTORS IN SOY PROTEIN PRODUCTS

Raw Soybean Meal

Raw full-fat and defatted soy flours inhibit growth, depress metabolizable energy and fat absorption, reduce protein digestibility, cause pancreatic hypertrophy, stimulate hyper- and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin, and mineral availability. Almost all these effects are interrelated and represent an animal's inability to use the essential nutrients fully rather than a response to toxic substances. Effects of raw meal on growth and pancreas of various animals are summarized in Table I.

Adult animals can maintain body wt when placed on a raw soy diet, although pancreatic hypertrophy still occurs. Pancreatic hypertrophy and hypersecretion of pancreatic enzymes occur in young and adult chickens, turkeys, rats, and mice, whereas normal pancreas wt and pancreatic exocrine function occur in dogs. When first placed on raw meal, there is a decrease in pancreatic juice secretion in dogs similar to that found in calves and pigs. However, dogs can adapt to a raw meal diet because pancreatic juice secretion returns to normal with continued feeding (4,5). Reduced pancreatic secretion, but no hypertrophy, occurs in pigs and calves fed raw meal; the reason for reduced exocrine function is unknown (6).

Growth depression in germ-free chicks fed raw soybean meal (7) and raw navy beans (8) is considerably less than in conventional chicks. The intestinal microflora appears to intensify the growth inhibiting properties of raw meal. The mode of interaction between the microflora and dietary constituents in raw meal is unknown; however, antibiotics have a beneficial effect on nutritive value of raw meal but not to the same extent as moist heat treatment (1). Pancreatic hypertrophy occurs in both germ-free and conventional chicks.

Breeding soybean varieties genetically deficient in antinutritional factors does not appear promising. Kakade, et al., (9) found a wide range in trypsin inhibitor (TI) and hemagglutinating activity in 108 varieties and experimental strains of soybeans (Table II). Nutritive values were increased 61-180% after autoclaving. Protein quality was evaluated in terms of protein efficiency ratio (PER, g gain/g protein eaten). Of all the parameters examined, only the wt of the pancreas showed a significant inverse correlation with PER. There was no correlation between PER and total sulfur amino acids (the first limiting essential amino acids in soybeans), TI activity, or hemagglutinating activity. Appar-

TABLE I

Biological Effects of Raw Soybean Meal in Various Animals

Species	Growth inhibition	Pancreas	
		Size	Enzyme secretion
Rat[a]	+[b]	+	+
Chicken[a]	+	+	+
Pig[a]	+	–	±
Calf	+	–	±
Dog	–	–	c
Human	d	d	d

[a]Adult animals maintain body wt, but pancreas effects still occur.

[b]+ = growth inhibition and pancreatic hypertrophy and hypersecretion; – = no effect; ± = hyposecretion.

[c]Hyposecretion initially, normal after continued feeding.

[d]Unknown. However, two adults, in a 9 day feeding trial had positive nitrogen balance for both raw and autoclaved soy flour.

[1]ARS, USDA.

TABLE II

Trypsin Inhibitor and Hemagglutinating Activities and Nutritive Value of 108 Varieties and Strains of Soybeans[a]

Parameter	Range of values, unheated
TI activity[b]	66-233[c]
Hemagglutinating activity	60-426[d]
Protein efficiency ratio	0.88-1.60[e]
Protein efficiency ratio	0.74-1.74[f]

[a]See ref. 9.

[b]TI = trypsin inhibitor.

[c]Expressed as trypsin units inhibited/mg protein.

[d]Expressed as hemagglutinating units/mg protein.

[e]Values for five selected varieties and strains corrected on the basis that protein efficiency ratio of casein control diet is 2.50.

[f]Values for 26 selected samples.

ently other factors are involved in determining nutritive value of soybean meal in which the heat-labile factors have been destroyed.

Soy By-Products

Nutritive value and presence of growth inhibiting and pancreatic hypertrophic factors in various fractions of soybeans, obtained during the processing of meal into concentrates and isolates, have been studied in detail (10-12).

Rackis, et al., (10) found that the protein quality of the by-product residue could, by toasting, be improved further to that of toasted soybean meal. Soybean whey, in which most of the antinutritional factors reside (10,13), is low in nutritive value unless subjected to moist heating. Protein quality of toasted whey protein is greater than that for meat and milk because of an excellent balance of essential amino acids (14).

A meal dialyzate and the water-insoluble residue, having relatively low TI activity, inhibit growth and cause pancreatic hypertrophy. The deleterious effects of these two fractions are not so great as those of the soy whey solids and whey protein components which contain high levels of TI activity (10). Soybean whey contains components that greatly potentiate the antinutritional properties of soybean TI in rats and chicks (1).

Protein Isolates and Concentrates

In contrast to soybean meal, a different spectrum of nutritional and biological factors is associated with the feeding of soy protein isolates. As the sole source of protein in a diet, the isolates increase the requirements for vitamins E, K, D_2, D_3, and B_{12}. Phosphorus is utilized poorly. Soy isolates also create deficiency symptoms associated with decreased availability of calcium, magnesium, manganese, molybdenum, copper, iron, and zinc. Availability of zinc is affected the most. Heat treatment; addition of natural and synthetic chelating agents, including soybean meal digests; and proper supplementation of the diet with vitamins and minerals will correct these nutritional defects. It is evident that the formation of protein-phytic acid-mineral complexes during processing may be responsible for needed mineral supplementation of soy isolate diets. Only occasionally do these deficiency symptoms occur in animals fed either raw or toasted soy flour. A soy protein isolate is more deficient in sulfur amino acids than all other soy protein products (2), and a choline deficiency lowers its nutritive value still further (15).

Soy protein concentrate, which contains ca. 70% protein, is actually a product that is a combination of water-insoluble residue and protein isolate (10). Toasted concentrates have PER values comparable to that of toasted soy flour, and their amino acid composition is similar. There is no information available on effect of soy concentrate diets upon vitamin and mineral requirements in animals.

Rachitogenic and perotic factors are concentrated in soy isolates, while growth-promoting, antiperotic, antirachitogenic, and antithyrotoxic factors are present in meal extracts. The goitrogenic factor is found in the whey. Because of differences in the biological properties of soybean meal, isolates, concentrates, and whey proteins, it is essential that investigators accurately describe the type of soy protein product they use in feeding studies and describe the processing conditions under which the product was prepared.

Many times it is difficult to resolve the conflicting data that have appeared in literature, because many investigators failed to make a distinction between full-fat and defatted soy flour, concentrate, or isolate. Some investigators refer to the many forms of soy products merely as "soy protein."

Toxicity, A Misnomer

Although toxicity or toxic factors are terms frequently used to refer to the antinutritional properties of raw soy products, such designations are unwarranted. As shown in Table III, growth inhibition and pancreatic hypertrophy are reversible. After rats had been fed for 38 days to develop maximum pancreatic hypertrophy, they were switched to a basal diet for an additional 154 days; growth wt and

TABLE III

Effect of Raw Soybean Meal on Body Wt and Pancreas Wt of Rats[a]

Diet no.	Dietary protein	Duration, days	Body wt, g		Pancreas wt, g/100 g body wt
			Start	End	
			Weanling ♀		
1	Casein	38	33	129	0.34
2	Raw meal	38	32	81[b]	0.65[b]
3	Casein	192	33	210	0.35
4	Raw meal	192	33	184[b]	0.61[b]
5	Raw meal	192[c]	33	230[b]	0.41
			Adult ♂		
6	Casein	38	298	336	0.25
7	Raw meal	38	297	297	0.46[b]
8	Casein	192	298	375	0.26
9	Raw meal	192	298	358	0.46[b]

[a]See ref. 16.

[b]Significant difference from control value, $P < 0.01$.

[c]Switched to casein control diet after 38 days.

TABLE IV

Activity of Commercial Soy Protein Products[a]

Soy product	TI activity[b]
Raw flour	96.6
Toasted flour	9.7
Protein concentrate	16.0
Protein isolate A	8.5
Protein isolate B	19.8
Flour A[c]	3.8
Flour B[c]	4.4

[a]See ref. 37.

[b]TI = trypsin inhibitor.

[c]Specially processed for calf feeding.

pancreas wt then were normal (compare diets three and five). After continuous ingestion of raw defatted meal for ca. one-fourth of the lifespan of the rat, no toxic effects were observed; and, except for the pancreas, all other organs were normal (16). Adult rats fed raw meal for 6 months maintain body wt in spite of prolonged pancreatic hypertrophy (compare diets eight and nine). No adverse histopathological effects were observed in the cellular structure of the pancreas of adult dogs fed a 15% raw soy meal diet for 16 weeks (5).

Other observations further affirm the nontoxicity of raw soybeans, as well as of other soy products. When rats are fed higher levels of raw meal or when raw meal is supplemented with additional calories and protein, growth is ca. comparable to that when toasted meal diets are fed (17). Laying hens fed raw meal maintain good egg production despite pancreatic hypertrophy (18). Good performance of swine, continuously fed through the farrow of the fourth generation of pigs, was obtained on toasted soy rations containing all plant protein and on another ration containing soybean meal and animal protein (19). There were no signs that any deleterious effects were carried over into succeeding generations. A diet of soy milk (plus vitamins and iron supplement) permitted excellent growth, reproduction, and lactation for at least three generations of rats (20).

Pirola, et al., (21) believes pancreatic hypertrophy did not aggravate pancreatitis and may have therapeutic value, since experimentally induced pancreatic damage by ethionine administration in rats already on a purified soybean TI diet was less severe than in ethionine-fed control diets.

Unidentified Growth Factors

Substances in raw soybean meal and soy protein isolates stimulate growth in turkey poults (22-25) and increase phosphorus availability (26). Soy protein isolates enhance growth of ducklings (27) and day-old coturnix quail (28). Growth factors may be essential peptides formed during digestion. Certain water and methanolic extracts of soybean meal have antiperotic activity (29,30), as well as antithyrotoxic properties (31). Soybean meal digests can promote growth and increase zinc availability in phytate-containing diets (32).

ELIMINATION OF ANTINUTRITIONAL FACTORS

Heat Treatment

By live steam treatment, a process referred to as toasting, the nutritive value of full-fat and defatted soy flours, soy protein isolates, and concentrates can be improved greatly. Since the pancreatic hypertrophic factor of the residue and whey protein fractions is relatively more heat-stable than that in the meal, Rackis, et al., (10) suggests that processing conditions required for maximum PER of these two products may differ from conditions required for soy flour. More research is needed to define manufacturing processes required to produce isolates and concentrates having optimal nutritive value. The degree of improvement in nutritive value depends upon temperature, duration of heating, and moisture conditions.

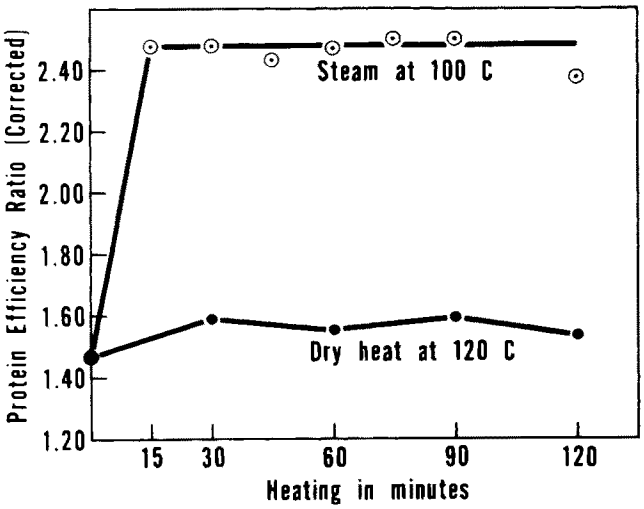

FIG. 1. Effects of type and extent of heating on nutritional value of soybean meal in rats. See ref. 33.

Effects of type and extent of heating on nutritive value of defatted soy flour is shown in Figure 1. Only 10-15 min steaming at atmospheric pressure gives maximum PER (33,34). Dry heat has no effect. Almost all investigators find that maximum protein efficiency is achieved by autoclaving at 15 lb/in.^{2} pressure for no more than 30 min (35,36). Excessive heat treatment markedly impairs nutritive value (2).

Questions concerning the accuracy and reproducibility of current TI assay methods led to an improved procedure being developed to determine TI activity (expressed as TI units/mg sample) in soy products (Table IV), particularly in heat-treated samples (37). Now minimum destruction of TI activity, to gain maximum nutritive value, has been defined more precisely (38). The relationship between TI activity and PER is shown in Figure 2. Maximum PER was reached when only 79% of TI activity was destroyed. Results were similar when body wt and nitrogen digestibility values served as indices of protein quality. With only 50-60% destruction of TI activity, pancreatic hypertrophy no longer occurs in rats. The destruction of hemagglutinin activity parallels that shown for TI in Figure 2 (39).

Some investigators (40,41) have found considerable variation in PER values of soy protein isolates and concentrates and have reported that nutritive value of some preparations can be improved by moist heat treatment, whereas others have not noted such an effect. A few isolates for infant milk formulas are processed under conditions to give maximum nutrition. Many manufacturers of isolates and concentrates employ procedures dictated primarily by their intended use as functional ingredients in food systems. In these processes, heat treatment often is not optimal or is not used at all, and there may be enough residual TI activity to cause significant pancreatic hypertrophy (10). More thorough washing of the protein curd to remove more of the occluded whey proteins would reduce TI activity. Of course further cooking of a product during preparation can provide opportunity for further inactivation of TI.

Particle size and initial moisture of soybeans are major factors influencing cooking rate, inactivation of TI, and subsequent increase in nutritive value (34,42,43). With whole soybeans, presoaking before toasting greatly accelerates TI inactivation.

Other Processing Techniques

Maximum nutritive value of soy milks is attained within

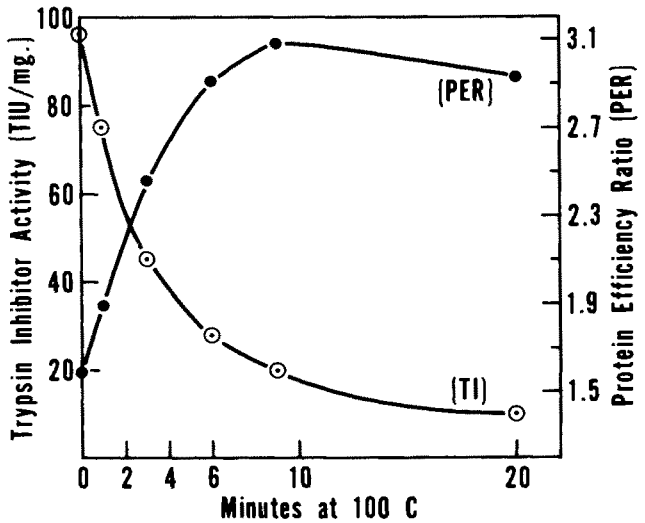

FIG. 2. Effect of atmospheric steaming on trypsin inhibitor activity and protein efficiency ratios of soybean meal fed rats. See ref. 37.

5-10 min when the milk is heated at 121 C, conditions which inactivate ca. 90% TI activity. Further heating causes a loss in protein value (44). Labuza (45), in converting the data of Hackler, et al., (44) into useful kinetic results, estimates that increasing drying temperature to double the rate of destruction of TI would increase the destruction of many nutrients by four- to fivefold. Since it is not necessary to destroy TI activity completely to achieve maximum nutritive value (38), one can see the need for kinetic data to maximize the destruction of antinutritional factors and minimize loss of essential nutrients.

Processing soybeans at elevated temperatures in alkaline solution accelerates inactivation of TI (46-50). Moist heating under mild alkaline conditions improves soy protein quality, but can form a toxic amino acid, lysinoalanine, in many plant and animal proteins (51). Lysinoalanine is formed when food-grade soy isolates are treated with 0.1 N sodium hydroxide for 8 hr at room temperature.

Antinutritional factors in full-fat soybeans are destroyed readily within 2 min by extrusion-cooking (52). The short cooking time in an extruder minimizes damage to nutritional properties but adequately destroys the growth inhibitors. PER values progressively improved with up to 89% inactivation of TI activity. Roasting (53), IR cooking (54), dielectric heating (55,56), and microwave processing (57,58) of soybeans improve nutritive value and destroy TI activity; however, inactivation of TI and maintenance of uniform nutritional value are both difficult to control.

INTERRELATIONSHIP BETWEEN ANTINUTRITIONAL FACTORS

Available Energy

Depression of protein digestibility in laying hens and chicks can account for the difference in fat absorption and metabolizable energy values between raw and heated soy diets (59). Purified TI preparations and soy whey fractions having high TI activity reduced fat absorption 50% in chicks up to 2 weeks old. These are the same fractions that inhibit growth, enhance pancreatic enzyme secretion, cause pancreatic hypertrophy (34), contract the gallbladder, and accelerate secretion of bile (60). Raw meal accelerates the secretion of fatty acids, lipid phosphorus, cholesterol, and other bile constituents into the duodenum and decreases their absorption in other segments of the intestinal tract (61). There is also an increase in fecal excretion of bile acids (62), which also may contribute to poor fat absorption.

In the duodenum of raw meal-fed chicks, extensive nitrogen secretion, proportional to the raw meal level in the diet, occurred (63). The 20% difference in overall protein digestion and subsequent absorption between chicks fed heated and raw meal is the result of inhibition of proteolysis in intestinal segments beyond the duodenum.

Inhibition of proteolysis, the presence of undigested protein, and the decreased release of amino acids caused by raw meal induces a compensatory pancreatic enzyme secretion and an increased secretion of water and gallbladder constituents. These effects indicate that raw meal has a general stimulatory effect on all endogenous secretions and would explain the interrelationships between depressed metabolizable energy, poor fat metabolism, and reduced protein digestion. This relationship between protein and fat absorption is consistent with the finding that extra protein and calories overcome most of the growth-inhibiting properties of raw meal in chicks (17).

TI and other heat-labile constituents of raw meal initiate these effects through the release of a hormone(s) which regulates the activity of both the gallbladder and pancreas (64). According to Melmed and Bouchier (65), the hormone is cholecystokinin-pancreozymin (CCK-PZ). The secretion of nitrogen, water, and lipids into the duodenum and subsequent reabsorption in other intestinal segments may require the expenditure of much energy and could contribute to growth inhibition (63). Defatted soy flour contains ca. 30% carbohydrate. The actual percentage of total carbohydrate which is available ranges from 14% with chicks to 40% with rats. On the basis of feeding rats, the caloric value of soy carbohydrates is 1.68 cal/g compared to a value of ca. 4.0 for highly digestible sugars (2).

TABLE V

Combined Effect of Heat and Methionine Supplementation on Nutritive Value of Soy Milk in Rats[a]

Diet and treatment	PER[b]	Increase, percent	Pancreatic hypertrophy
Raw	1.44	—	+
Raw + 0.35% methionine	2.22	54	+
Heated (10 min, 121 C)	2.23	55	−
Heated + 0.35% methionine	2.86	92	−

[a]See ref. 65.

[b]PER = protein efficiency ratio. PER corrected to casein control diet = 2.50.

Amino Acid Supplementation

Although cystine and methionine are the first limiting amino acids of raw soy protein, tyrosine, threonine, and valine exert a further supplementary effect when added to raw meal diets (66). However, supplementation of raw meal with essential amino acids will not increase its nutritive value to the same level as heated meal similarly supplemented (67). The combined effect of methionine supplementation and proper moist heat treatment on raw soy milk is illustrated in Table V.

Bielorai, et al., (68) reported that large amounts of undigested protein, which contained a high content of seven of the more important essential amino acids, were present in the intestinal tract of rats fed raw soy. The amino acid composition of the intestinal contents was similar to that of soybean meal, as reported by Rackis, et al. (14); this similarity indicated that the undigested protein is of dietary origin. Previously, Bielorai and coworkers found a correlation between TI activity and the formation of undigested protein in the intestines of chicks fed raw meal. On the basis of plasma amino acid scores, the level of essential amino acids in the blood increases when autoclaved meal replaces raw meal (69). Many workers also have found that TI creates a metabolic deficiency of sulfur amino acids, because of an enhanced conversion of methionine to cystine for protein synthesis in the pancreas (1).

Several interrelated growth-impairing factors, which function during the digestion of raw soybean meal, explain why supplementation with several essential amino acids cannot counteract completely the antinutritional effects. The factors include (A.) protein that becomes digestible only after heating of the raw meal; (B.) limited proteolytic digestion because of TI activity in raw meal; and (C.) greatly enhanced need for amino acids, especially the sulfur amino acids for resynthesis of protein in the pancreas as a consequence of enhanced pancreatic juice secretion and pancreas enlargement.

PROTEINASE INHIBITORS

Natural Occurrence

Substances having the ability to inhibit proteolytic activity of certain enzymes are referred to as proteinase inhibitors. The proteinase inhibitors of legumes, particularly soybeans, have been studied in great detail because of their role in animal nutrition (1,70). Substances that inhibit proteinase (proteolytic enzymes) are present in most organs, biological fluids, and endogenous secretions of man and animals (71). Potatoes, sweet corn, many fruits and vegetables (72), cereals (73), and peanuts (74) possess very high TI activity.

Usually several different proteinase inhibitors occur in the same plant. There may be seven-ten proteinase inhibitors in soybeans (1) that inhibit the digestive proteinases, chymotrypsin and trypsin, as well as a large number of other enzymes (1). Pancreatopeptidase E, a digestive proteinase that may have an important part in overcoming inhibition of proteolysis in chicks fed raw meal, is not inhibited by soybean TI's (75).

The several TI components in soybeans may be genetically controlled variants (76) and also may differ in their nutritional effect in animals. A soybean variety that had the TI variant (R_f 0.92), which was electrophoretically different from the TI in the usual commercial varieties, was nutritionally better than two other varieties tested (77). Nevertheless, proper heat treatment is required to obtain maximum nutritional value for all varieties (9). The TI variant (R_f 0.72) is found in high frequency in Japanese strains in the maturity groups I and II (78).

Nutritional Significance of Other Proteinase Inhibitors

Most proteinase inhibitors, like Kunitz soybean TI, specifically inhibit bovine trypsin at a molar ratio of 1:1. In their first report, Travis and Roberts (79) state that soy TI did not inhibit human trypsin based upon the measurement of esterolytic activity of human trypsin with synthetic substrates. Their statement prompted speculation that TI activity of raw meal has little, if any, relevance to human nutrition even though soybean TI's contribute significantly to the poor nutritive value of raw meal when fed to other animals.

Later, Coan and Travis (80) showed that human trypsin is inhibited by soybean TI less than is bovine trypsin. Mallory and Travis (81) now have isolated two forms of trypsin from human pancreas. The anionic form is inhibited completely by soybean TI at a molar ratio of 1:1, whereas the cationic form is inhibited 20%.

Proteinase inhibitors, which inhibit trypsin and chymotrypsin, normally are present in the human colon and the dog duodenum, jejunum, ileum, and colon (82). The nutritional significance of these endogenous TI's is not known. Melmed and Bouchier (65) suggest that the primary function of pancreatic TI is to activate the hormone CCK-PZ that stimulates enzyme synthesis in the pancreas for repletion of digestive enzymes. Soybean TI has the same effect.

The extent of pancreatic hypertrophy and release of CCK-PZ depend upon the type of proteinase inhibitor and animal species (1,83,84). TI from soybeans, lima beans, navy beans, eggs, potatoes, and other legumes cause pancreatic hypertrophy (64). Although there are some exceptions, it is probably reasonable to assume that any food that contains proteinase inhibitors at a high enough level would elicit the same responses observed with soybean TI.

Melmed and Bouchier (65) found that growth rate of rats was inversely proportional to the pancreas response. At the dosage levels used in their tests, growth of soybean TI-fed rats was the lowest, intermediate with ovomucoid TI, and growth of the bovine TI-fed group was equal to the control group. Bowman-Birk chymotrypsin inhibitor of soybeans had a greater effect on the pancreas than did the potato chymotrypsin inhibitor.

Soybean and egg-white TI affect the pancreas and exocrine enzyme profile, but their relative potency depends upon the dietary nitrogen level (85). Soybean TI greatly stimulated chymotrypsinogen secretion and depressed amylase biosynthesis, whereas the egg-white inhibitor stimulated trypsinogen secretion and increased amylase synthesis (85-87). The Bowman-Birk inhibitor from soybeans, which inhibits chymotrypsin to a much greater extent than trypsin, enhances trypsin secretion but has no effect upon chymotrypsin secretion (88,89).

In comparative rat feeding tests, Rackis (34) found that highly purified Kunitz soybean TI accounted for 100% pancreatic hypertrophic effect and for ca. 30-60% growth-inhibiting properties of raw meal in rats. In chicks, supplementation of heated soybean meal diets with the Bowman-Birk inhibitor and the Kunitz TI of soybeans resulted in maximum pancreatic enlargement, and growth was inhibited ca. 30% compared to that of a raw meal diet (75). In a different approach, Kakade, et al., (90) used affinity chromatography to remove all the different forms of TI from water extracts of raw meal. Ca. 40% growth-inhibiting effect, as well as 40% pancreatic hypertrophic effect of the original raw extract, could be accounted for by the TI's. He believes that resistance of the raw undenatured protein to proteolysis by digestive enzymes was responsible for the remaining 60% growth-inhibiting and pancreatic hypertrophic effects of the raw extract.

Green, et al., (91) also has postulated that undenatured proteins in raw meal may form trypsin-protein complexes that resist proteolysis and, in effect, simulate a trypsin-TI complex. Since Kakade, et al., (9) found no correlation between TI activity (as measured in vitro) and PER, he suggested that raw soybeans may contain a factor which causes pancreatic hypertrophy but has no TI activity.

Pancreatic Hypertrophy and Enzyme Secretion

Rackis (1) has summarized the complex changes that occur in the pancreas when either raw meal, raw whey proteins, or soybean TI is fed to various animals. Substantial evidence has accumulated to show that the primary biological and physiological effects produced by feeding raw soybeans or isolated TI are pancreatic hypertrophy, enhanced protein synthesis in the acinar cells of the pancreas, and excessive secretion of pancreatic juice enzymes.

Recent evidence suggests that soybean TI affects the pancreas indirectly by stimulating the release of excess CCK-PZ, a hormone in the duodenal mucosa that regulates protein digestion (64,65,92). A general stimulatory effect on other digestive secretions also may occur (61,63) as a result of a release of CCK-PZ. Repeated administration of CCK-PZ also causes pancreatic hypertrophy, stimulates pancreatic juice secretion, and can inhibit growth of rats (93). Also, CCK-PZ is a TI (94). The mechanisms proposed to explain the growth-inhibiting properties of raw meal are complex.

The liver, like the pancreas, is an organ having an active

protein-synthesizing system; but, unlike the pancreas, raw meal has no physiological effect upon the liver (65).

Considerable changes occur in the enzyme profile both in the pancreas tissue and in the intestinal tract of chicks fed raw soybean meal and TI's from soybeans and egg white (75,95). Dietary conditions also can change pancreatic enzyme activity. Many investigators regard the responses to raw meal or soybean TI as an adaptive mechanism similar to that which occurs with a protein-rich diet (1). Additional pertinent information on the effect of raw meal and other factors on pancreas and release of CCK-PZ are listed (75,83,85,96-98).

In rats, maximum hypertrophy occurs within 9 days while feeding raw meal (34). Maximum enlargement of the pancreas in chicks occurs after 21 days of feeding (75). Increased pancreatic secretion occurs almost immediately in rats, whereas in chicks pancreatic hypertrophy and juice secretion can be delayed. Pancreatic hypertrophy and increased enzyme secretion also occur in rats fed a protein-free diet containing soybean TI. Adult rats fed raw meal maintain body wt, but exhibit pancreatic hypertrophy. Since many naturally occurring and synthetic proteinase inhibitors have similar physiological effects, these TI's may have beneficial nutritional properties. TI might be used to stimulate the repletion of enzymes in the pancreas since pancreatic atrophy occurs in patients suffering from kwashiorkor (99) and from protein malnutrition (100). Soy TI administered intravenously has no effect upon the pancreas (4,5).

Proposed Mechanisms

Much more research is needed to propose a definitive mechanism(s) whereby raw soybean meal inhibits growth in most young animals. Soybean TI's have a significant effect on fat absorption, carbohydrate and amino acid metabolism, protein digestibility, and pancreatic hypertrophy. All these effects appear to be interrelated and, in combination, determine the growth-inhibiting capacity of raw soybean meal.

At least three inhibitory mechanisms may be in operation. These are discussed below.

TI stimulates biosynthesis of enzymes in the pancreas and thereby increases the essential amino acid requirements of the animal, because of an enhanced secretion of pancreatic enzymes into the intestinal tract. The first limiting amino acids in soy protein are cystine plus methionine. Synthesis of cystine in the pancreas is increased from seven- to tenfold, and there is a marked increase in the conversion of methionine to cystine (1). As a result, the greater need for sulfur amino acids cannot be compensated for by dietary proteins. An imbalance in the metabolic amino acid pool would enhance the growth-inhibiting effect of raw meal. In the rat, the pancreatic response is rapid. For the chick, because there is a delay in the synthesis and secretion of pancreatic enzymes, proteolytic activity in the intestinal tract initially is lower than with the rat. Because protein digestion is depressed more in the chick, this condition lowers the concentration of essential amino acids necessary for optimal growth. Some investigators have suggested that pancreatic hypertrophy leads to an excessive fecal loss of the endogenous protein secreted by the pancreas. The resulting net loss of sulfur amino acids from the body would further exaggerate the methionine deficiency of soy protein and result in growth inhibition. Although more reliable quantitative data are needed, it does not appear that significant amounts of pancreatic protein are lost to the feces.

Raw meal contains protein components refractive to proteolysis, and denaturation by heat treatment is required to increase their digestibility (90). Lepkovsky, et al., (101) suggests that raw soy diets have a lower nutritive value, because TI's inhibit proteolysis of the dietary proteins, not by decreasing proteolytic activity in the intestinal tract, but rather by forming TI-dietary protein complexes which resist digestion even in the presence of high concentrations of digestive enzymes. Growth inhibition occurs, because of the loss of protein into the feces. To account for pancreatic hypertrophy and growth inhibition in rats fed hydrolyzed protein containing TI, Lepkovsky, et al., (101) believes that endogenous protein of intestinal origin also forms resistant complexes with TI.

Recent evidence indicates that trypsin or chymotrypsin in the intestine suppresses and controls pancreatic enzyme secretion by feed-back inhibition and that the dietary TI's initiate increased enzyme secretion by counteracting the suppression induced by trypsin (64,87). TI would overcome the feed-back response by formation of a TI-trypsin complex, which would reduce proteolytic activity in the intestine and enhance protein synthesis in the pancreas.

A possible relationship between the resistance of undenatured protein to tryptic hydrolysis and pancreatic hypertrophy is suggested by Green, et al., (91). Like TI's, undigested protein also can increase pancreatic enzyme secretion by forming a trypsin-dietary protein complex similar to the TI-trypsin complex; this dietary complex would remove feed-back inhibition of pancreatic enzyme secretion by trypsin. Regardless of the type of complex formed, reducing proteolytic activity in the intestinal tract enlarges the pancreas and inhibits growth. In turn, inhibition of protein digestion induces a general stimulating effect on other endogenous secretions (64). As a result, a decrease in metabolizable energy and poor fat absorption of raw meal diets also would cause growth inhibition (61,63).

The feed-back inhibition mechanism can contribute significantly to the growth-inhibiting capacity of raw meal by a somewhat alternative process. The gallbladder responds to the presence of natural and synthetic TI in a manner analogous to the pancreas. Activity of both organs is mediated through the same hormone CCK-PZ (64). Dietary TI may decrease the level of trypsin and chymotrypsin activity indirectly by accelerating release of CCK-PZ from its receptor site in the duodenal mucosa (102). Since CCK-PZ also can inhibit trypsin (94), excessive release of the hormone would form CCK-PZ-trypsin complexes to counteract feed-back inhibition and accelerate activity of the pancreas. Alternatively, CCK-PZ would be able to form complexes with dietary protein which are resistant to proteolysis, even in the presence of high concentrations of proteolytic enzymes (94).

PHYTOHEMAGGLUTININS

Proteins that have the unique property of being able to agglutinate red blood cells are distributed widely in the plant kingdom (101,103,104). Liener (105) isolated phytohemagglutinin from soybeans and reported that it can account for 25% growth inhibition which raw soybeans produce in rats. Aside from his report, there is no substantial data to indicate that the hemagglutinins have antinutritional properties. However, phytohemagglutinins present in other legumes, particularly those which constitute an important source of protein in some of the lesser developed countries, inhibit growth at levels as low as 0.5% diet (106).

The growth-inhibiting properties of the legume hemagglutinins can be eliminated by proper heat treatment. The increase in nutritive value of raw soybean meal parallels the destruction of hemagglutinating activity, the same conditions which inactivate TI (Fig. 2). The nutritional significance of the phytohemagglutinins in soybeans is still a matter of speculation.

ANTIVITAMIN AND MINERAL FACTORS

General Considerations

In a mixed diet, soybeans are not considered to be an

TABLE VI

Zinc Availability in Various Foodstuffs[a]

Foodstuff	Availability, percent	
	Chick assay	Rat assay
Wheat	59	38
Corn	63	57
Rice	62	39
Soybean meal	67	—
Fish meal	75	84
Nonfat dry milk	82	79

[a]See ref. 123.

important source of vitamins, although the B vitamins are present in good amounts. Toasting destroys a good portion of the vitamins, with more than half of the thiamine lost. Soybean protein products are devoid of vitamin D and B_{12}. Concentrations of vitamin C are high in immature soybeans and increase markedly in soybean sprouts. There is no vitamin C in mature soybeans; β-carotene and choline are present in minute amounts. Vitamins E and K are found only in whole soybeans and full-fat soy flour.

Vitamin and mineral contents of soybean protein products have been reported by Liener (2). A close examination of compositional data compiled by manufacturers of soy products reveals wide variation in vitamin and mineral contents. Since soy protein isolates contain constituents that interfere with the availability of most minerals and some vitamins, it is questionable whether any nutritional significance should be attached to commercial analytical data. Flavor and functionality characteristics are the primary criteria that determine the processing conditions to be used in the manufacture of soy isolates. Not only is there considerable variability in the PER values of commercial soy protein isolates, but large differences also occur in mineral availability. Lease (32) reported that some isolates contain modified protein components that increase availability of phosphorus, zinc, and other minerals. These components have been referred to as "carrier" components. Only occasionally do vitamin-mineral deficiencies occur in diets containing soy flour because of the presence of carrier components.

Effect of Phytic Acid

It is evident that phytic acid has a major role in influencing the availability of vitamins and minerals. Development of improved methods for analysis of phytate phosphorus in wheat products (107) and navy beans (108) has been reported. Several good reviews on certain aspects of mineral availability have appeared (109-113). Phosphorus content of soy products and the interaction of phytic acid with protein during processing has been reviewed (114).

Phytic acid in its natural form as a phytate-mineral-protein complex decreases the availability of zinc, manganese, copper, molybdenum, calcium, magnesium, and iron. Phytate decreases the absorption of chromium in rats (115). Most of these same effects are observed in diets containing soy protein isolates. Autoclaving and adding chelating agents can either eliminate the mineral binding properties of phytate or facilitate the absorption of the essential minerals during digestion. In chicks, some chelating agents decrease iron and cobalt absorption (116). Phosphorus availability in plant foodstuffs varies widely (117). Although most studies have been made with animals, phytic acid also interferes with calcium and iron absorption in man (118,119).

Although many dietary variables affect the availability of phytate phosphorus, the level of phytase activity in the intestinal tract is also an important factor (110). With the addition of a phytase preparation from *Aspergillus ficuum* NRRL 3135 to a chick diet, Nelson, et al., (120,121) found

TABLE VII

Effect of Phytate-Protein Complexes on the Need for Supplemental Zinc in Chick Ration[a]

Supplement	Wt at 4 weeks, g	
	Casein diet	Soy isolate diet
Basal	454	142
Zinc, 15 ppm	—	387
Zinc, 55 ppm	458	457
Phytate, complex[b]	180	96[c]
Phytate, complex + 55 ppm zinc	434	214[c]

[a]See ref. 124.
[b]5% Phytic acid mixed with casein.
[c]4% Additional phytic acid added.

that total enzymatic hydrolysis of phytate occurred in the alimentary tract and that the hydrolyzed phytate phosphorus was utilized, as well as supplemental inorganic phosphorus. Contrary to some reports, Bitar and Reinhold (122) reported that phytase activity is present in the small intestinal mucosa in man, but its role in phytate phosphorus metabolism is unknown.

Zinc

Biological availability of zinc in various foodstuffs (123) is summarized in Table VI. O'Dell (111) attempted to delineate the factors that affect zinc utilization in plant foodstuffs. Zinc deficiency occurred in Egyptian boys whose diet consisted largely of bread and beans. Other reports show that inclusion of animal protein products prevents zinc deficiency symptoms in similar diets, largely because of the presence of chelating agents that facilitate increased zinc absorption during digestion. On the other hand, addition of phytate salts to animal products decreases zinc utilization.

Ca. 84% zinc in casein diets is absorbed by the rat, compared to only 44% for soy protein isolate diets. Lease (32) found that one commercial soy protein isolate diet required 15 ppm supplemental zinc to promote good growth and to prevent zinc deficiencies in chicks, whereas a second isolate preparation required the addition of 30 ppm zinc. These differences in zinc requirements are attributed to processing conditions that promote the formation of phytate-protein complexes in protein products of plant origin (Table VII [124]).

Autoclaving soy isolates for 4 hr at 115 C destroys most of the phytic acid and increases zinc availability; however, because of the excessive heat treatment, essential amino acids also are destroyed, and nutritive value decreases markedly. Under similar conditions, only 20% phytate phosphorus in sesame meal is destroyed. Chelating agents with a stability constant for zinc in the range of 13-17 have near the optimum value, and their addition to soy isolate diets leads to practically complete utilization of the zinc. Some chelating agents also increase phytate phosphorus availability.

To be effective, chelating agents must compete with phytate and then form soluble zinc complexes that then can be readily released into the intestinal tract. The zinc in soybean meal is bound as water-soluble, dialyzable complexes and is absorbed readily under the conditions present in the intestinal mucosa. The binding agent is termed a carrier (32). Since soy protein isolates have little or no carrier components, zinc availability in soybean meal is twice that of the isolates. Lease (32) also showed that zinc in sesame and safflower meal was present as insoluble nondialyzable complexes formed at intestinal pH and that little zinc absorption occurred in the chick as measured by uptake of zinc from labeled digests. Addition of histidine, a chelator for zinc, to soy isolate diets overcomes some of the symptoms associated with zinc deficiency (125).

TABLE VIII

Goitrogenicity of Soy Products[a]

Product	Iodine content, μg/100 g diet	Thyroid wt, mg/100 g body wt
Raw soy flour	1.0-2.3	37
Toasted soy flour	0.7	19
Soy isolate	0.9	16
Soy infant food, iodized	40	7
Raw soy flour + 10 μg I_2/g protein	196	8
Casein	30	7

[a]See ref. 151.

It would appear that the nutritive value of soy protein products, particularly soy protein isolates, can be improved greatly by: (A.) selecting processing conditions which increase proteolytic digestion of the proteins in the intestinal tract, promote formation of carrier components, and greatly enhance availability of zinc and other minerals and (B.) using processes to prepare phytate-free protein products.

Many nutritionists add calcium to diets to obtain a proper calcium-phosphorus ratio in foods. In vegetable protein diets, however, addition of calcium can decrease zinc absorption greatly, because of the presence of phytic acid (111). In phytate-free diets, like casein and other animal proteins, calcium does not affect zinc availability. When humans consume high and low levels of calcium in which animal products supply most of the protein, even a tenfold increase in calcium has no deleterious effect upon zinc absorption. Not all the problems associated with decreased availability of minerals in plant foodstuffs, however, can be attributed solely to phytic acid (32,126,127). Processes should be developed in which phytate-free protein can be prepared and then tested in animal diets.

Iron

Reports regarding iron availability in soybeans have ranged from 29-80%. Percentage availability of iron in soy protein isolate diets likewise has been inconclusive. With chicks, it was reported that soy protein isolates did not interfere with iron absorption for growth and hemoglobin synthesis (126,128,129). Monkeys, on the other hand, develop iron deficiency anemia when fed diets containing soy isolates (130).

Whether these conflicting reports can be explained by the presence or absence of carrier protein, as postulated for the efficient utilization of zinc, should be determined (32,126). Iron from soybean protein or supplemental iron in soybean diets was utilized better by the baby pig than the iron in casein diets. In contrast, zinc phytate binding decreases zinc absorption. Addition of ethylenedinitrilotetraacetic acid can increase iron absorption significantly and raise hemoglobin content in low-iron diets (112).

Biological availability of iron salts in processed infant formulas is high. Theuer, et al., (15,131) concludes that many iron salts, other than ferrous sulfate, can be used in liquid products on milk-based soy protein isolate with the assurance that they furnish high levels of absorbable iron. They also suggest that there is a lower dietary iron requirement with these iron-fortified products compared to iron-enriched cereals.

Iron absorption measurements have been made in 131 individuals consuming nine different foods tagged biosynthetically with radioiron (132). Iron absorption from whole soybeans was much greater than from most plant foods and was comparable to absorption from animal protein. Good absorption occurs in males with adequate iron stores and even better in individuals with either no iron stores or with iron-deficiency anemia. Ca. 20% iron in soybeans is available, which is twice as high as that normally obtained with plant foods, whereas 40-50% is absorbable from soy protein milk formulas (15). The usual assumption is that only 10% or less of the iron is utilizable from foods containing plant proteins (133,134).

Because of their high iron content and high biological availability, soybean flour products are recommended for programs oriented to prevent iron-deficiency anemia (135). Absorption of iron in healthy Jamaican children given baked soybeans was twice that for maize (136). In some infants fed fresh cows' milk, the albumin turnover and iron anemia which occurred were not corrected by iron therapy (137). Formulas based upon soy protein isolates restored normal albumin turnover and corrected the altered intestinal absorption of iron.

Vitamin B_{12}

Soybeans contain no B_{12}. Raw soy flour increases B_{12} requirements in rats, as indicated by decreased levels of the vitamin in the tissue and increased urinary metabolites associated with enzymes that require B_{12} as a coenzyme (138-140). These effects disappear with toasted soy flour. The increased requirement for B_{12} in rats fed raw flour was attributed to decreased availability of the vitamin formed by intestinal flora and to an increased turnover of the absorbed vitamin.

Although supplementation of raw soy flour diets with methionine, cystine, and choline greatly improves growth and overcomes most of the biochemical signs of vitamin B_{12} deficiency, their addition does not restore growth to levels obtained in rats fed toasted soy flour (139,140).

Patrick and Whitaker (141) also reported that supplementation of soy protein isolate diets with a combination of choline and methionine or hydroxymethionine increased chick growth. Whether the improvement reflects a correction of a vitamin B_{12} deficiency is not known. The biological values of the three soy proteins used in their study differed greatly even after supplementation, if growth response served as a criterion for nutritive value.

Vitamin D

Turkey poults fed a soy protein isolate-glucose diet grew poorly and developed rickets although the diet contained normally adequate levels of vitamin D_3, calcium, and phosphorus (142,143). Growth and tibia ash were improved with two- and fourfold increases of vitamin D_3 or by the replacement of one-third of the soy protein with heated soybean meal protein or by the direct addition of 5% raw soybean meal. Addition of vitamin D_3 to soybean meal diets had no significant beneficial effect. This work led to the conclusion that soybean meal contains a heat-stable antirachitogenic factor. A heat-labile rachitogenic factor is present in soy protein isolates (144). Jensen and Mraz (145) reported that both calcium and phosphorus were required to overcome rachitogenic activity of soy protein isolates. They suggested that rachitogenic activity of soy protein isolates might be due to phytic acid. Whether or not phytic acid is involved in the development of rachitogenicity remains to be determined.

Other Vitamins

Heated soybean oil significantly decreases urinary excretion of thiamine and riboflavin, while their content in liver is increased (146), a condition which suggests that toasted full-fat soy flour exerts a beneficial effect on the utilization of the B vitamins. Toasting destroys most of the thiamine and causes a moderate loss of pantothenic acid (147). Infant formulas based upon soy protein isolates have higher levels of vitamin K compared to cows' milk (148). Soy protein inhibits β-carotene absorption (149).

GOITROGENICITY

Iodine

Konijn, et al., (150) reviewed the older literature on goitrogenicity of soybeans. As shown in Table VIII lack of iodine is the principle cause of goiter, but heat treatment abolished most of the goitrogenic activity (151). Addition of 160 μg iodine (as potassium iodide)/100 g diet will cause the enlarged thyroid to return to normal.

A goitrogenic factor, which is either an oligopeptide of two or three amino acids or a glycopeptide, has been isolated from soybean whey (152). The soybean factor is a weak goitrogen, and addition of small amounts of iodine is sufficient to overcome the goitrogenic effect. The supplementation, however, should be high enough to overcome the goitrogenic-like properties of certain fresh and oxidized vegetable oils (153).

Iodine goiter and high levels of serum protein bound iodine are evidence for excessive intake of iodine. In a preliminary report, Landau, et al., (154) showed that a few soybean milk preparations contained too much iodine. One of the preparations contained 1400 μg/liter of milk, an amount which was 10 times higher than the content in several well-known soybean formulas. An iodine content of 34-160 μg/liter appears to represent an adequate supplementation level for soy products, since no goitrogenic effects of such formulas are being reported. Whether soy products should be supplemented at all is questionable, since there are indications that consumption of iodine in American diets is higher than recommended daily allowances (155). Little information on the retention of supplemental iodine in soy products is available; however, 50-80% iodine added to bread, potato chips, and frankfurters is retained throughout their processing and storage (156).

Antithyrotoxic Factor (ATF)

Possibly related to goitrogenicity, which increases iodine requirements, is a factor that may counteract effects of excess iodine. An ATF in soybeans counteracts growth depression in rats fed desiccated thyroid powder or iodinated casein (157). The ATF is not the same as the growth factor present in soy protein isolates. Westerfeld, et al., (31) indicated that the two different factors may be responsible for the antithyrotoxic activity and chick growth-promoting properties of soy isolates. The ATF may be a peptide, since proteolytic digestion of the isolate increased its activity (158).

ALLERGENICITY

Glaser and Johnstone (159) reported only 15% children from allergenic families fed soy milk from birth to 6 months developed allergenic diseases by 6 years of age, whereas 65% sibling controls and 52% nonrelated controls fed cows' milk developed similar illnesses. In a later study, Johnstone and Dutton (160) reported allergy occurred in 18% soy group and 50% control group in 235 children from allergenic families. In contrast, Brown, et al., (161) reported allergenic responses of 11 and 13% for the soy milk and cows' milk group, respectively.

For 7 years, Halpern, et al., (162) studied a group of 1753 infants fed breast, soy, and cows' milk from birth-6 months and found the development of allergy was similar in the three milk groups. However, in the first 6 months, allergy to soy milk occurred in 0.5% children to 1.8% for the cows' milk group. This finding agrees with other findings that soy milk is much less allergenic compared to cows' milk.

In attempting to answer the questions of why earlier workers always reported so much more allergy in babies fed cows' milk and reported soy milk to be hypoallergenic, Halpern, et al., (162) believes that with better control of processing, coupled with higher and longer heat treatment, cows' milk is now considerably less allergenic. Formulas based upon soy protein isolates alleviated symptoms of allergy and maintained an adequate nutritional state (163,164). Other investigators believe that food allergenicity is increasing (165,166). This situation implies that, with a better knowledge of processing soy products, particularly those based upon soy isolates, soybeans could find increasing use as a hypoallergenic substitute.

A weak soybean allergen has been isolated (167). The allergen preparation was quite heterogeneous and failed to exhibit any activity when injected directly into the intestinal mucosa of dogs (168). On the basis of its solubility and other properties, the allergen would be a component that normally is present in the whey solution. Almost all plant allergens are much more active compared to the soybean allergen (167).

Thermostability is a characteristic feature of most proteinaceous allergens (169,170). To destroy the allergenic activity of raw meal extracts completely requires a heating time of 180 C for 30 min (169), which is more heat than that required to inactivate the antinutritional factors in raw soy flours (34) and may be sufficient to lower nutritional quality.

OTHER FACTORS

Saponins

An informative review of saponins, including soybean saponins, has been written by Birk (171). Soybeans contain ca. 0.5% saponin. Although saponins in some plants have antinutritional properties, saponins isolated from soybean meal do not harm chicks, rats, and mice, even when fed at a level three times greater than the level in diets containing soy flour. Saponins are hydrolyzed by bacterial enzymes in the lower intestinal tract, but neither saponins nor sapogenins can be detected in blood of test animals. Enough evidence has been accumulated to remove them from the list of antinutritional factors in soybeans.

Sterols

Sterols (phytosterols) are distributed widely in the plant kingdom. The sterols are absorbed poorly through the intestinal mucosa. When present in some diets, nearly all the dietary sterols are found in the fecal matter, while in some human subjects on formula diets, only 25-58% ingested sterols was recovered in the feces (172). Small amounts of noncholesterol sterols of plant origin are found in practically all animal tissue (173). A large proportion of the total intestinal tract sterol is of plant origin, but the amount varies widely among animal species (174). The intestinal mucosa exhibits much discrimination in the uptake of sterols (174). In man, 5-10% dietary sterols is absorbed, whereas up to 32% absorption occurs in rats (175). In man, plant sterols are distributed in the tissue in a manner similar to that of cholesterol. The significance of plant sterols in cholesterol metabolism is not known. Sterol content of soybeans is quite low. Most of the sterols are found in the crude phospholipid fraction, a by-product in the refining of crude soybean oil. Only small amounts occur in defatted soybean meal and protein isolates (176).

TABLE IX

Effect of Soy Products on Flatus in Man[a]

Product[b]	Daily intake, g	Flatus volume, cc/hr	
		Average	Range
Full-fat flour	146	30	0-75
Defatted flour	146	71	0-290
Protein concentrate	146	36	0-98
Proteinate	146	2	0-20
Water-insoluble residues[c]	146	13	0-30
Whey solids[d]	48	300[e]	---
80% Ethanol extractives[d]	27	240	200-260
Navy bean meal	146	179	5-465
Basal diet	146	13	0-28

[a]See ref. 194.

[b]All soy products and navy beans were toasted with live steam at 100 C for 40 min.

[c]Fed at a level three times greater than that present in the defatted soy flour diets.

[d]Amount equal to that present in 146 g defatted soy flour.

[e]One subject. Otherwise four subjects/test.

Phenolic Compounds

The major phenolic compounds in soybeans are the isoflavones, genistein and daidzein, which occur mainly as the respective glycosides, genistin and daidzin. Several phenolic acids also are present in small amounts (1). A third isoflavone, glycitein (177), and a 6,7,4′-trihydroxy flavone (178) have been isolated from defatted soybean meal. Estrogenicity of these flavonoids has not been determined.

Defatted meal contains ca. 0.15% genistin and 0.007% daidzein. No other quantitative data are available, but small amounts also occur in soy isolates and tofu.

Substances exhibiting estrogenic activity are distributed weakly in animals and plants, as well as in many refined vegetable oils, including soybean oil (179). Genistin, which accounts for most of the estrogenic activity of soybean meal, is stable to autoclaving but is extracted readily with aqueous alcohol. Genistein is only 10^{-5} as active as diethylstilbestrol, and daidzein is one-fourth as active as genistein (180).

Commercial defatted meal is estrogenic to rats; although no information was given on the amount of meal used in the diet, ca. threefold increase in uterine wt occurred (181). However, Wong and Flux (180) found that mice consuming 5.8 mg genistein in 15 g diet over a 6 day period showed a twofold increase in uterine wt. Assuming a genistin content of 0.15% in soybean meal, Wong and Flux would have had at least 41% soybean meal in their diet for the mice to receive 9.3 mg genistin (equivalent to 5.8 mg genistein) and for uterine wt to increase twofold. After aqueous alcoholic extraction, the extracted meal had no estrogenic activity (179).

In a control diet containing 0.2% added genistin, Carter, et al., (182) found little effect on reproduction in mice. There was a decrease in the number of litters, but litter size was not affected. In the same study, a diet containing 80% soybean meal would provide a dietary level of 0.12% genistin, and yet there appeared to be no statistically significant adverse effects on reproduction.

Matrone, et al., (183) and Magee (184) found that genistin and genistein when fed to rats at high levels (0.5% of the diet) inhibited growth, increased iron content in liver and spleen, showed antiperotic properties, elevated zinc content in bones and liver, and increased deposition of calcium, phosphorus, and manganese. No significant adverse effects were noted in rats fed 0.1% genistein (equivalent to 0.16% genistin) in a 19% casein diet for 4 weeks. To obtain an effect from soybean meal equivalent to that observed in the experiments of Magee (184), soybean meal would have to be the sole constituent of the diet. The isoflavone content of soy protein isolates is extremely low.

Another aspect to be considered regarding isoflavones is their possible modification during processing. Pieterse and Andrews (185,186) found that moldy corn and alfalfa silage had increased estrogenic activity greatly as a result of enzyme action. A new isoflavone 6,7,4′-trihydroxyisoflavone is present in tempeh, a fermented soy product, during fermentation. The amount found in tempeh is very small (187) and probably would have little, if any, effect on estrogenic activity.

Griffiths and Smith (188) observed that genistein and daidzein are metabolized by the microflora in the intestinal tract of man and that the same metabolites are present in the urine after oral administration. The soybean isoflavones are not metabolized completely in the intestinal tract; only an indeterminate amount is absorbed and then excreted as glycosides (189). Most likely soybean isoflavones have no nutritional significance in man.

FLATULENCE

There are many causes for the formation of gastrointestinal gas (190,191) which may lead to nausea, cramps, diarrhea, pain, social discomfort, and uneasiness when the gas is egested rectally. In humans, flatus produced by fermentation of dietary carbohydrates in the lower intestine is composed of carbon dioxide, hydrogen, and methane. The microfloral profile will determine their relative amounts. Steggerda (192) reported that a basal diet produced, on the average, 16 ml flatus/hr in humans, with 10-12% carbon dioxide. On a navy bean diet, flatus volume increased to ca. 190 ml/hr with a content of 50% carbon dioxide. In some humans, flatus contains large amounts of hydrogen and methane (190). Analyses of orally and rectally expelled flatus in man indicate that the pattern of expelled gases reflects differences in type, location, and abundance of intestinal microorganisms (193). Incidence of flatulence in humans is unpredictable; it depends upon the psychological and physical state of the subject and the type of diet. In research over a number of years, Steggerda had some volunteers who produced less than 16 ml/hr and others 600 ml.

Human Studies

Flatus activity of various commercially manufactured, toasted, soy products is shown in Table IX (194). The gas-producing factor resides mainly in the low mol wt carbohydrate fractions, soy whey solids, and aqueous alcohol extractives. These fractions contain 60-80% water-soluble, alcohol-soluble oligosaccharides, primarily as sucrose, raffinose, and stachyose. Little or no flatus activity is present in the hulls, fat, or protein (195). The water-insoluble, high mol wt polysaccharides (residue product, Table IX) are practically devoid of flatus activity, even when consumed at a level three times that present in defatted soy flour. The type and content of carbohydrates in soybean

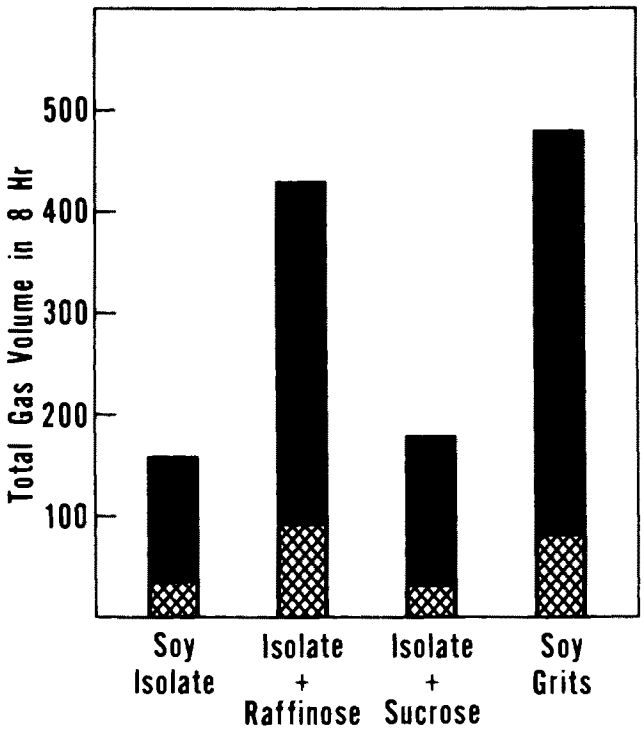

FIG. 3. Flatus activity of soybean oligosaccharides in humans. Raffinose intake 13 g/day; sucrose intake 23 g/day. See ref. 196. ▩ = 4 hr collection after lunch. ■ = 4 hr collection after dinner.

meal are given in Table X.

Raffinose, when added to a soy protein isolate diet at a level equivalent to the total amount of raffinose plus stachyose in a defatted soy flour diet, produced just as much flatus as the control diet of soy grits (Fig. 3 [196]). A sucrose diet, consumed at a level twice that of the soy flour diet, is devoid of flatulence. Cristofaro and Wuhrmann (197) showed that the flatus activity of stachyose in rats is much greater than with raffinose. Hellendoorn (198) showed that the flatulent effect of beans is caused by fermentation of undigested starch granules in the lower intestine. Mature soybeans do not contain starch.

Textured soy flour caused flatulence in five of six human subjects consuming 50 g or more (199). Althoff's (200) textured soy flour had flatus activity, but no data were given. In contrast, Kies and Fox (201) had no complaints of flatulence in subjects consuming 25 and 50 g textured soy flour. These results are not contradictory but point out the aleatory nature of human subjects. As shown in Table IX, flatulence susceptibility varies widely. The range in flatus volume for a navy bean diet was 5-465 ml/hr. When flatulent problems arise in human feeding programs, extruded soy protein concentrates in place of extruded soy flour would be an effective way to solve them.

Murphy, et al., (202) reported that fractions rich in carbohydrates obtained from navy beans produced the largest amounts of carbon dioxide, but the amounts produced 4-7 hr after ingestion of raffinose and stachyose were not greater than the control diet. Hickey, et al., (203) found that flatulent effects were greatest with commercial wheat cereal and milling fractions containing the highest levels of raffinose and stachyose.

Much of the contradiction regarding the influence of raffinose and stachyose in human flatulence may be the motility rate since more time is needed by intestinal bacteria to digest these oligosaccharides completely (204). Gitzelmann and Auricchio (205) found no α-galactosidase activity in the human intestinal mucosa. They showed that after consumption of raffinose and stachyose by a normal child and a galactosemic child, there was no absorption of

TABLE X

Carbohydrate Constituents of Dehulled, Defatted Soybean Meal[a]

Constituent	Meal, percent
Polysaccharide content, total	15-18
Acidic polysaccharides	8-10
Arabinogalactan	5
Cellulosic material	1-2
Oligosaccharide content, total	15
Sucrose	6-8
Stachyose	4-5
Raffinose	1-2
Verbascose	Trace

[a]See ref. 194.

galactose into the blood. Only trace amounts of raffinose and stachyose in a soybean powder were found in the urine of these children. Raffinose and stachyose can pass into the fecal matter completely unchanged or can be metabolized completely (205); these findings were reviewed (197,206). It is not surprising, therefore, to find that in some humans a normally flatulent diet would not necessarily result in flatus.

Animal and in vitro Studies

Richards and Steggerda (207) and Rackis, et al., (204) have shown that 80% gas produced in surgically prepared intestinal segments of dogs incubated with navy bean and soybean meal homogenates occurred in the ileum and colon. Gas production also occurs in fecal matter. Antibiotics will completely inhibit flatus activity (207) which effectively inhibit *Clostridia*. Bacteriostatic agents, Vioform and Mexaform, inhibit flatus production in humans (208).

An in vitro technique previously developed by Richards, et al., (209) was used by Rackis, et al., (204) to show that soybean meal contains both a growth-promoting and a growth-inhibiting factor(s) which are soluble in aqueous alcohol. Some of the soybean phenolic acids, syringic and ferulic, are effective inhibitors in vitro and in intestinal segments of dogs. The in vitro technique also was used to show that the same soy products that caused flatulence in humans also produced the greatest amount of gas with anaerobic cultures from the dog ileum and colon mucosa. Only the complex carbohydrates that can be metabolized microbially to monosaccharides act as substrates to produce flatus (204). Rockland, et al., (210) and Kurtzman and Holbrook (211) demonstrated that carbohydrate constituents in beans stimulate growth of *Clostridia*.

Rats can be used to study flatus production (212). In a series of detailed experiments with rats, Cristofaro and Wuhrmann (197) investigated flatus activity of soybean oligosaccharides. Raffinose produced little gas, whereas a marked increase in volume of intestinal gas containing high concentrations of carbon dioxide was produced with 2 and 4% stachyose in a diet based upon soy milk or casein.

In a repeat experiment, they tested the relative effects of verbascose, stachyose, raffinose, and lactose on flatus activity at levels calculated to give the same level of galactose in the diet. The diet containing stachyose and verbascose produced the highest volume of flatus. Little was produced with raffinose and lactose. These data indicate that an oligosaccharide with at least two α-galactose units will produce the highest amount of flatus. Verbascose, a pentasaccharide, with three such units produced the same volume of gas as did stachyose.

Elimination of Flatulence

Carbohydrate content of several varieties and strains of soybeans (Table XI) was determined by Hymowitz, et al., (213); he indicates that removal of soybean oligosaccharides by breeding does not look promising. Products devoid of flatulence can be prepared by various processing

TABLE XI

Strain, Maturity Groups, and Average Wt of Total Sugar, Sucrose, Raffinose, and Stachyose in Seed[a]

Strain	Maturity groups	Wt in g/100 g seed			
		Total sugar	Sucrose	Raffinose	Stachyose
Ada	00	9.68	6.33	0.54	2.80
Flambeau	00	9.86	6.43	0.74	2.69
Morsoy	00	10.11	6.38	0.95	2.78
Clay	0	10.04	6.78	0.77	2.49
Merit	0	10.07	6.33	0.69	3.05
M60-92	0	10.22	6.76	0.78	2.68
Chippewa 64	I	9.29	6.15	0.86	2.28
M59-120	I	9.33	5.71	0.60	3.02
Hark	I	9.41	6.11	0.68	2.62
Beeson	II	9.04	5.64	0.72	2.68
Corsoy	II	9.25	5.76	0.59	2.89
Amsoy	II	9.79	6.28	0.65	2.86
SL-9	III	8.57	5.52	0.82	2.23
Wayne	III	9.08	5.71	0.88	2.48
Calland	III	9.44	5.90	0.94	2.60
L66-1359	IV	8.30	5.06	0.80	2.46
Kent	IV	8.37	5.10	0.76	2.48
Cutler	IV	8.65	5.28	0.78	2.59
Mean		9.36	5.96	0.75	2.65
Range of means		8.30-10.11	5.06-6.78	0.54-0.95	2.23-3.05

[a]See ref. 213.

[b]For maturity groups 00, 0, I, II, III, and IV, average values are for 10, 12, 23, 33, 27, and 24 locations, respectively.

techniques. Because only small amounts of raffinose and stachyose are present in soy protein concentrates and isolates, these products produce little flatus.

Tempeh, a fermented soy food, has very little activity (214), because the hot water extraction used to prepare the soybeans removes most of the oligosaccharides.

Enzyme processes that hydrolyze soybean oligosaccharides have been developed (215-217). Although Calloway, et al., (214) was not able to demonstrate significant reduction in flatus activity of enzyme-treated soybeans, Steggerda and Rackis (unpublished data) found that in some subjects flatus activity was reduced greatly with a product prepared by Rohm and Haas (215). In these latter experiments, analytical analyses showed that the oligosaccharides were almost completely hydrolyzed, although small amounts of melibiose were present. No analyses were made in the Calloway study. Sprouting also appeared to have little effect on flatus activity of soybeans and mung beans (217).

A gas chromatographic technique was published by Delente and Landenberg (218) to determine oligosaccharides in soybean meal and enzymatically hydrolyzed meal.

In vitro and in vivo experiments (204,208) have shown that antibiotics and certain phenolic acids, syringic and ferulic acid, can inhibit flatus activity; however, it is unlikely that such additives would be approved for human consumption. More data are needed to determine whether long-term use of soy flour products at practical levels of consumption will create flatus problems. Textured soy concentrates and isolates are devoid of flatus activity, although their use will increase food costs.

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