

Protein Quality of a Soybean Protein Textured Food in Experimental Animals and Children ^{1,2}

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ABSTRACT Studies were carried out in experimental animals and children to evaluate the protein quality of a textured food simulating ground beef, and made from isolated soybean protein with added egg albumin and wheat gluten. On the basis of a PER value of 2.50 for casein, the PER of the textured food and of natural dehydrated beef was 2.30 and 2.34, respectively. Highest weight gain was obtained with diets containing 16.7 and 16.3% protein, from the soybean protein textured food and casein, respectively. NPU values were 62.6 for casein and 59.1% for the soybean protein textured food. Heating of the soybean protein textured food increased weight gain but not the PER. Heating of the protein isolate and of the fiber made from it improved both. Apparently this treatment caused the elimination of adverse physiological factors inherent in soybean, or the removal of substances in the product derived from the preparation process. At the 10% protein level, supplementation with lysine and methionine added together, but not alone, improved protein quality. Growth and nitrogen balance studies with dogs indicated that the soybean protein textured food had essentially the same protein quality as that of dehydrated beef. True protein digestibility and biological value were 92.3 and 65.3%, respectively, for the soybean protein textured food, and 87.0 and 67.4% for the dehydrated beef. The results in children show that, at a protein intake level of 2 g/kg/day, no difference in quality was evident between skim milk and the soybean protein textured food. Nitrogen equilibrium was obtained when the children received approximately 138 mg of nitrogen from the soybean protein textured food, as compared with 97 mg from milk. The true protein digestibility and biological value was 92.3 and 65.3%, respectively. It was concluded that the protein quality of the soybean protein textured food was about 80% of that from milk. It was readily accepted by the children and free of adverse physiological effects.

Highly purified proteins are being isolated now from oil-free, food-grade protein concentrates such as soybean, cottonseed and sesame. Among these, protein isolates prepared from oil-free soybean flakes have received the greatest attention. The protein content of the isolate runs as high as 95%, and the products are bland in taste and have none of the flavors normally associated with the flours and other similar products (1-3).

The protein isolates are available in monofilament, granular or powder forms, which make them suitable for a wide range of functional uses, such as whipping, emulsifying, gelling, stabilizing, thickening and moisture-binding. Thus, the number of food products which can be made from them is practically unlimited.³⁻⁵

The essential amino acid pattern present in such isolates is, in the majority of cases, essentially the same as that in the material

from which it was prepared. However, the process of isolation, eliminating certain protein fractions, as well as the use of variable temperatures, treatment with chemicals and pH changes, may alter the nutritive value of such products. Information on the nutritive value of the protein of these isolates is not very extensive, and

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² INCAP Publication I-428.

³ Boyer, R. A. 1954 High protein food product and process for its preparation. Cincinnati, Ohio. U. S. Patent 2,682,466 (issued June 29).

⁴ Boyer, R. A. and H. E. Saewert 1956 Method of preparing imitation meat products (pH control). Assignors to Swift and Company, Chicago, U. S. Patent 2,730,448 (issued January 10).

⁵ Anson, M. L. and M. Pader 1959 Method of preparing a meat-like product. Assignors to Lever Brothers Company, New York, U. S. Patent 2,879,163 (issued March 24).

⁶ Westeen, R. W. and S. Kuramoto 1964 Preparation of shaped protein products. Assignors to General Mills, Inc., Minneapolis, U. S. Patent 3,118,959 (issued January 21).

there is even less material or possibly none at all on textured foods prepared from them.

The purpose of the work reported in this paper was, therefore, to evaluate the protein quality of a ground meat-like soybean protein textured food, in experimental animals and children.

MATERIALS AND METHODS

*Soybean protein textured food.*⁷ The soybean protein textured food used in the biological studies to be described was obtained from General Mills, Inc. Commercially available protein isolates prepared from soybean meal, when dispersed in aqueous alkali, display pseudo-plastic characteristics. Such a viscous "dope," when forced through textile spinnerets into a suitable coagulation bath, is converted into continuous multiple monofilaments. Absence of hardening or chemical cross-linking agents permits their use as digestible structural elements for a new class of foods that are chewed. Suitable choice of flavoring, binding and other materials can give rise to products analogous to meat or other textured natural foods.

Protein suspensions for spinning were prepared using 16% of isolate solids in aqueous alkali of pH 12.0 at room temperature, with total alkaline exposure time of less than 10 minutes. Monofilament production was achieved by extrusion of the alkali suspension through platinum-rhodium alloy spinnerets into an aqueous bath of acetic acid, hydrochloric acid and sodium chloride, maintained at pH 3.5. The fibrils were subsequently rinsed free of precipitant and squeeze-dried before compounding. After thorough admixture of fibrils with all other formula ingredients, plus added water, the resultant mass was heated briefly to coagulate the albumin. The "beef" analogue so obtained was ground to granules and dried in hot air to less than 3% moisture content.

In this nutritional study, a simulated beef granule of the following dry weight percentage composition was used as test material: soybean protein fibrils, 28.8; vegetable fat,⁸ 21.5; egg albumin, 12.3; wheat gluten, 11.8; toasted soybean meal, 9.6; vegetable protein hydrolysate, 1.7; brown sugar, 4.9; and non-nutritives (as

flavoring, coloring), 9.4. In addition to the final product, the soybean protein isolate and spun fibrils made from it were also tested for protein quality per se in experimental animals. Samples were taken for chemical analysis before the feeding tests were begun. Aliquots of the isolate used and the resultant fibrils were withdrawn from the process stream and freeze-dried. Amino acid chromatography was carried out on the isolate, the fibrils and the dried finished product. The results are shown in table 1. Other proteins used for comparative purposes were vitamin-free casein and dehydrated beef. The dehydrated beef was round steak which was cut into small pieces and dried in an air convection oven at 70°. It was then ground to a powder, and at least 10 samples were

TABLE 1
Proximate composition and amino acid content (%)^{1,2}

	Soybean protein		
	Isolate	Fibrils	Textured food
	%	%	%
Moisture	2.1	4.3	2.8
Ether extract	0.6	0.4	22.2
Crude fiber	0.5	0.7	1.3
Nitrogen	15.00	15.04	8.68
Protein (N × 6.25)	93.7	96.2	54.2
Ash	1.9	0.6	2.4
Lysine	5.41	5.12	3.22
Histidine	2.95	2.64	1.70
Ammonia	1.48	1.44	1.21
Arginine	7.67	7.85	4.54
Aspartic acid	9.85	10.51	6.12
Threonine	3.46	3.22	2.48
Serine	4.84	4.20	3.47
Glutamic acid	10.15	6.18	13.95
Proline	5.54	4.49	4.26
Glycine	3.08	2.93	2.10
Alanine	3.47	3.38	2.58
½ Cystine	0.84	0.72	0.96
Valine	3.98	4.22	3.16
Methionine	1.19	1.04	0.89
Isoleucine	4.58	4.46	3.28
Leucine	7.60	6.95	5.12
Tyrosine	3.62	3.62	3.08
Phenylalanine	5.45	5.12	3.97
Protein (N × 6.25)	95.3	96.9	56.4

¹ Proximate composition from INCAP laboratories on samples used for biological tests.

² Amino acid chromatographic analysis performed by the Department of Biochemistry, University of Minnesota, Minneapolis.

⁷ Registered as "Bontrae," a generic name for General Mills, Inc., specialties of the spun protein class.

⁸ Percentage fatty acid spectrum: myristic, 0.3; palmitic, 15.3; palmitoleic, 0.1; stearic, 9.9; oleic, 56.5; linoleic, 16.6; and linolenic, 1.4.

analyzed for proximate composition. All materials were kept under refrigeration at all times.

Biological tests with rats

The protein quality of the soybean protein textured food, of the isolate and of the protein fibers, was evaluated by several procedures, using rats. In all experiments weanling white rats of the Wistar strain, from the INCAP colony, were used. The number of animals used per experiment varied as indicated in the tables presenting the results. Each group consisted of equal number of female and male rats, distributed by weight so that the average initial weight was the same for all groups within an experiment. The animals were placed in individual all-wire screen cages with raised screen bottoms. They were fed ad libitum, and water was available at all times. For growth and PER studies, the changes in weight and in the amount of food consumed were recorded every week for a total of 28 days. All diets were analyzed for nitrogen content. In some experiments the animals were killed at the end of the growth period, and blood and liver collected for analysis. Protein evaluation with rats was carried out by a) feeding increasing levels of protein from the textured food, and calculation of PER; b) by the net protein utilization (NPU) assay, and c) by amino acid supplementation and PER calculation at a 10% protein level in the diet.

The NPU assay was carried out by feeding weanling rats for a 10-day period with the protein under study added to provide a 10% protein level in the diet. A group of rats was fed a nitrogen-free diet to correct for endogenous body nitrogen. At the end of the experimental period, the animals were weighed, killed, opened and placed in a hot-air oven at 80° for drying to constant weight. The whole dry carcass was ground in a micro-Wiley mill to pass 20 mesh. Duplicate samples were then taken for nitrogen analysis by the Kjeldahl method. NPU was calculated from the following formula (4):

$$\text{NPU} = \frac{\text{Carcass N test group} - \text{carcass N (N-free diet) group}}{\text{Dietary N intake}}$$

The composition of the basal diet (nitrogen-free diet) was: (in %) cottonseed oil, 11.72; cod liver oil, 1.00; mineral mixture (5), 4.00; dextrose, 25.00, and cornstarch, 58.28. All diets were supplemented with 5 ml of a complete B-vitamin mixture, per 100 g of diet (6). For the studies in which several levels of protein were fed, the soybean protein textured food, casein⁹ and the dehydrated beef, replaced the cornstarch, and oil was added to keep the level of total calories constant (431 kcal/100 g). When amino acids were added, these also replaced part of the cornstarch. When the protein isolate and the fiber were tested in an experiment, these were included in the basal diet in amounts supplying 10% protein in the diet.

In some studies, the materials were cooked, even though the soybean protein textured food received had already been cooked. In this process the dried material was treated in the autoclave with an amount of water equal in weight, for 5 minutes, at 122.5°. After cooling all samples were freeze-dried, ground and stored under refrigeration until they were incorporated into the diets.

Biological tests with dogs

Growth studies. Twelve 2-month-old mongrel dogs, six of each sex, weighing between 1.6 to 2.7 kg, were used in these studies. The dogs were distributed into two equal groups according to weight and sex, one of which was fed the soybean protein textured food and the other dehydrated beef. The average initial weights of the 2 groups were practically the same (2.156 kg vs. 2.191 kg). The dogs from each group were fed approximately 9 g of protein/kg/day and 168 kcal/kg/day for 60 days. The soybean protein textured food and the dehydrated beef were incorporated into a diet containing: (in %) soybean protein textured food, 50.0 or dehydrated beef, 50.0; hydrogenated vegetable oil, 10.0; cod liver oil, 1.0; mineral mixture (5), 2.0; dextrose, 0.8; and dextrin, 29.0. The diet was also supplemented with 5 ml of a complete vitamin solution per 100 g (6). Each day, the amount of food to be offered the dogs was weighed

⁹ Nutritional Biochemicals Corporation, Cleveland.

and suspended in water warmed at 38°. Records of weight changes were kept every 2 days.

Nitrogen balance. After completion of the experiment described above, the protein quality of the soybean protein textured food was studied by the nitrogen balance method. The animals were fed decreasing levels of protein per kilogram of body weight, from approximately 6 g to zero. During the last 12 days of the study, a nitrogen-free diet of the same composition described above was fed, except that the protein source was replaced by dextrin. The animals were weighed every 4 days to adjust for protein and calorie intake. The intake of calories was kept constant at 168 kcal/kg/day. Each level of protein was fed for 8 days divided into two 4-day balance periods. Feces and urine were collected daily and, at the end of each 4-day period, they were weighed or measured, homogenized and analyzed for total nitrogen content. The urine was collected in dark bottles containing 1 ml of concentrated acetic acid.

Nitrogen balance studies in children

Eight children between 22 and 72 months of age, and weighing between 9.03 and 16.25 kg, were used in these studies. These children entered the INCAP metabolism unit with protein-calorie malnutrition, but were in good health and completely recovered by the time they were placed on the protein evaluation study.

Each child was first fed whole milk for a 10-day period, to provide 2 g of protein/kg/day, followed by the feeding of the soybean protein textured food at decreasing levels of protein intake from 2 to zero grams of protein/kg body weight/day. Each level of protein was also fed for 10 days. The intake of calories remained constant at 100 kcal/kg/day. Vitamins¹⁰ and ferrous sulfate (0.32 g/day) were added to each child's diet, in physiological amounts. The first 4 days of each 10-day period were used as an adaptation to the dietary change, and two 3-day balance periods were obtained from the remaining 6 days, in which feces and urine were collected quantitatively. An aliquot of the food consumed was also

collected for nitrogen analysis every 3 days. Urine was collected in bottles containing 1 ml of concentrated acetic acid, which were constantly immersed in ice. The 3-day urine and fecal collections were weighed and homogenized before nitrogen analysis, which was performed by the macro-Kjeldahl method.

A representative composition of the daily food consumed both for the milk and the soybean protein textured food feeding is given in table 2 for case PC-164. To feed decreasing levels of protein, the soybean protein textured food was replaced by a mixture of dextrin, maltose and hydrogenated vegetable fat.

TABLE 2
Representative daily intake

	Milk ¹	Textured food ¹
	g	g
Whole milk	108	—
Sugar	70	80
Cornstarch	20	40
Mixture of dextrin and maltose	111	121
Margarine	2	—
Salt	1	—
Water	888	719
Soybean protein textured food	—	51
Hydrogenated vegetable fat	—	19
Tomato and onion flavoring	—	10
Salt	—	2
Water	—	1604

¹ A multivitamin preparation and FeSO₄ was given daily to the children in both diets.

RESULTS

Rats. The effect of feeding increasing levels of protein from the soybean protein textured food, from casein and from dehydrated beef is shown in table 3. At the 11.6% protein level in the diet, both protein sources gave maximum PER, with casein giving the highest, 2.66, as compared with 2.44 for the soybean product. On the basis of a PER of 2.50 for casein, the textured protein food would have a PER of 2.30, similar to that found for dehydrated beef.

As the protein content of the diet increased above 12%, PER decreased. The decrease was similar for all protein

¹⁰ Each 0.6 ml provided 5000 IU vitamin A; 1000 IU vitamin D; 1 mg thiamine; 0.4 mg riboflavin; 1 mg pyridoxine; 2 mg panthotenic acid (no salt); 5 mg nicotinamide; and 50 mg ascorbic acid.

TABLE 3
Growth performance and other parameters of rats fed decreasing protein levels from the soybean protein textured food (SPTF), casein and dehydrated beef (12 rats/group)

Protein source	Amount in diet	Protein in diet	Avg wt gained ¹	PER	Serum proteins		Liver		
					Total	Albumin	Fresh wt	Moisture	Fat ²
	%	%	g		%	%	g	%	%
SPTF	9.21	7.4	35 ± 15 ³	1.58 ± 0.53	4.74 ± 0.28	2.44 ± 0.35	4.6 ± 3.8	65.4 ± 3.8	24.1 ± 10.7
SPTF	18.43	11.6	125 ± 21	2.69 ± 0.21	5.43 ± 0.37	3.04 ± 0.26	8.3 ± 1.4	66.9 ± 0.9	16.0 ± 4.5
SPTF	27.64	16.7	158 ± 22	2.28 ± 0.20	6.09 ± 0.38	3.18 ± 0.21	10.7 ± 1.7	67.0 ± 0.4	12.5 ± 3.3
SPTF	36.85	20.4	162 ± 34	2.04 ± 0.24	6.07 ± 1.00	3.10 ± 0.29	10.8 ± 2.5	67.6 ± 0.9	11.7 ± 3.4
SPTF	55.28	30.8	141 ± 30	1.32 ± 0.20	6.12 ± 0.36	3.11 ± 0.28	10.2 ± 1.7	68.2 ± 0.5	10.2 ± 1.6
Casein	5.60	7.2	29 ± 11	1.39 ± 0.53	4.78 ± 0.48	2.79 ± 0.27	4.5 ± 1.6	65.7 ± 3.8	32.4 ± 8.9
Casein	11.20	11.7	116 ± 18	2.66 ± 0.15	5.16 ± 0.42	2.92 ± 0.18	8.1 ± 1.0	67.4 ± 1.0	16.3 ± 3.7
Casein	16.80	16.3	165 ± 40	2.14 ± 0.30	5.81 ± 0.28	3.12 ± 0.20	10.6 ± 2.7	67.8 ± 0.7	13.9 ± 2.6
Casein	22.40	22.1	169 ± 37	1.91 ± 0.31	6.03 ± 0.29	3.33 ± 0.23	10.7 ± 2.3	68.0 ± 0.7	12.2 ± 1.9
Casein	33.60	31.4	170 ± 44	1.44 ± 0.22	6.03 ± 0.30	3.18 ± 0.11	10.9 ± 2.4	67.8 ± 0.8	11.5 ± 1.2
Dehydrated beef	8.31	8.4	51 ± 17	2.02 ± 0.38	—	—	—	—	—
Dehydrated beef	16.62	12.2	123 ± 22	2.66 ± 0.30	—	—	—	—	—
Dehydrated beef	24.93	17.0	151 ± 35	2.40 ± 0.29	—	—	—	—	—
Dehydrated beef	33.24	22.0	156 ± 34	1.94 ± 0.24	—	—	—	—	—
Dehydrated beef	41.55	27.5	166 ± 35	1.58 ± 0.26	—	—	—	—	—

¹ Average initial weight, 49 g.
² Dry weight basis.
³ SD.

sources. The average weight gained per dietary protein level for each of the protein sources was similar, except in the groups fed the 30% protein level, where the soybean protein textured food showed an unexpected weight decrease. Weight gain reached a plateau with diets containing 16.7, 16.3 and 17.0% protein, for the soybean protein textured food, casein and dehydrated beef, respectively. Food intake was also very similar for all proteins within each protein level in the diet.

The table also shows values for total serum protein, albumin concentration, and the fresh weight of the liver and liver fat, with respect to protein level of intake for two of the protein sources. In both cases total serum protein increased as dietary protein level was raised in the diet. The increase was similar for both protein sources. Similar tendencies were found in albumin content, and the values found at each level of protein intake were similar, except at the lowest level, where the soybean protein textured food gave a lower value than casein and was statistically significant at the 5% level.

Fresh liver weight correlated with body weight for both protein sources. Liver fat decreased as protein in the diet increased. However, it remained essentially the same for protein levels above 16% in the diet.

Table 4 shows the PER and NPU of the protein isolate, fiber and soybean protein textured food as well as those of casein. The isolated protein had the lowest PER, followed by the protein fibers. The PER of the soybean protein textured food gave a

TABLE 4

PER and NPU values of isolate, fiber and soybean protein textured food (12 rats/group)

Protein	Avg wt gain ¹	PER	NPU
	<i>g</i>		
Isolate	5 ± 10 ²	0.33 ± 0.30	39.0 ± 9.4
Fiber	59 ± 14	1.90 ± 0.31	36.7 ± 6.5
Textured food	123 ± 26	3.01 ± 0.27	56.5 ± 8.6
Casein	114 ± 28	3.38 ± 0.32	61.1 ± 8.9

¹ Average initial weight, 44 g.

² SD.

value equivalent to 89% of the value of casein, which is similar to that found in the previous study. NPU results correlated with PER values, with the exception of the protein isolate.

The results obtained with the isolate were unexpected, since the process used to obtain the product consists of a simple extraction with an alkaline reagent, and there was no reason to believe that it could cause damage to the protein. Since it has been shown that heat treatment is beneficial for soybeans, destroying trypsin inhibitors, it was decided to repeat the previously presented study, using heat-treated products. The results are shown in table 5. It is interesting that heat treatment of the isolate and of the fiber caused a significant increase in weight gain as well as in PER. Food intake for the isolate doubled upon heat treatment. Heating of the soybean protein textured food did not increase PER but a significant increase took place in weight gain, with a higher intake of

TABLE 5

Effect of cooking on the protein quality of the isolate, fiber and soybean protein textured food (8 rats/group)

Proteins	Wt gain ¹	Avg food consumed	PER
	<i>g</i>	<i>g</i>	
Isolate	— 5 ²	159	—
Fiber	59 ± 22 ³	309	1.88 ± 0.56
Soybean protein textured food	98 ± 20	326	3.30 ± 0.25
Casein	118 ± 21	365	3.53 ± 0.30
Isolate (heat-treated)	81 ± 13	340	2.44 ± 0.32
Fiber (heat-treated)	72 ± 11	320	2.23 ± 0.34
Soybean protein textured food (heat-treated)	138 ± 20	414	3.38 ± 0.19
Casein (heat-treated)	130 ± 22	379	3.38 ± 0.28

¹ Average initial weight, 42 g.

² Three animals out of eight died.

³ SD.

food. Only weight gain was affected by heating of casein.

Soybean protein is known to be deficient in sulfur-containing amino acids, and chemical and physical treatments are applied in the conversion of soybean protein to a simulated food, which could change amino acid availability. It was, therefore, of interest to learn whether or not an improvement in the nutritive value of the protein of the soybean protein textured food could be obtained by supplementing it with methionine and lysine.

Table 6 shows the results of the study. Supplementing the soybean protein textured food with lysine has no effect on weight gain and PER. The addition of methionine caused only a slight increase in both PER and weight gain. When both amino acids were added, however, there was a significant increase in both parameters. Table 7 summarizes the results of similar studies, in which raw and cooked soybean protein textured food was supplemented with the same 2 amino acids. Examination of the corrected PER values indicated that cooking caused an increase in PER with and without amino acid supplementation. The soybean protein textured food has a lower PER than

dehydrated beef, which gave also higher values than casein.

Dogs. The growth of the two groups of dogs is shown in figure 1. The growth of the dogs fed the soybean protein textured food was essentially the same as the growth of the animals fed the natural beef diet. The average total food intake, as well as the protein ingested, is also shown in the table. Food intake was higher for the animals fed the soybean protein textured food; however, the protein content of the diet was lower resulting in a lower protein intake as compared with that of the animals fed the dehydrated beef-containing diet. PER values were calculated from the weight gained and the protein consumed. These are essentially equal. Even though the animals were fed large amounts of the soybean protein textured food, no adverse physiological effects were observed.

The nitrogen balance data are presented in table 8. Higher nitrogen retention values appear to occur for the textured food at the higher levels of protein intake. Nitrogen balance was only slightly higher for the protein of dehydrated beef than for the soybean protein textured food at the lower levels of nitrogen intake. Negative

TABLE 6
Effect of methionine and lysine supplementation on the PER of soybean protein textured food (SPTF) (18 rats/group)

Treatment	Wt gain ¹	PER
	<i>g</i>	
Soybean protein textured food	114 ± 16 ²	2.64 ± 0.21
SPTF + 0.25% L-lysine·HCl	120 ± 19	2.71 ± 0.23
SPTF + 0.30% DL-methionine	123 ± 20	2.81 ± 0.25
SPTF + 0.25% L-lysine·HCl + 0.30% DL-methionine	130 ± 24	2.95 ± 0.34

¹ Average initial weight, 49 g.

² SD.

TABLE 7
Effect of methionine and lysine supplementation to the soybean protein textured food (SPTF) (12 rats/group)

Treatment	Wt gain ¹	PER
	<i>g</i>	
Soybean protein textured food	123 ± 23 ²	2.57 ± 0.19
(SPTF) (re-cooked)	131 ± 22	2.68 ± 0.22
SPTF + lysine + methionine	123 ± 24	2.76 ± 0.30
SPTF + lysine + methionine (re-cooked)	121 ± 17	2.97 ± 0.25
Casein	122 ± 21	2.77 ± 0.18
Dehydrated beef	149 ± 26	3.07 ± 0.28

¹ Average initial weight, 45 g.

² SD.

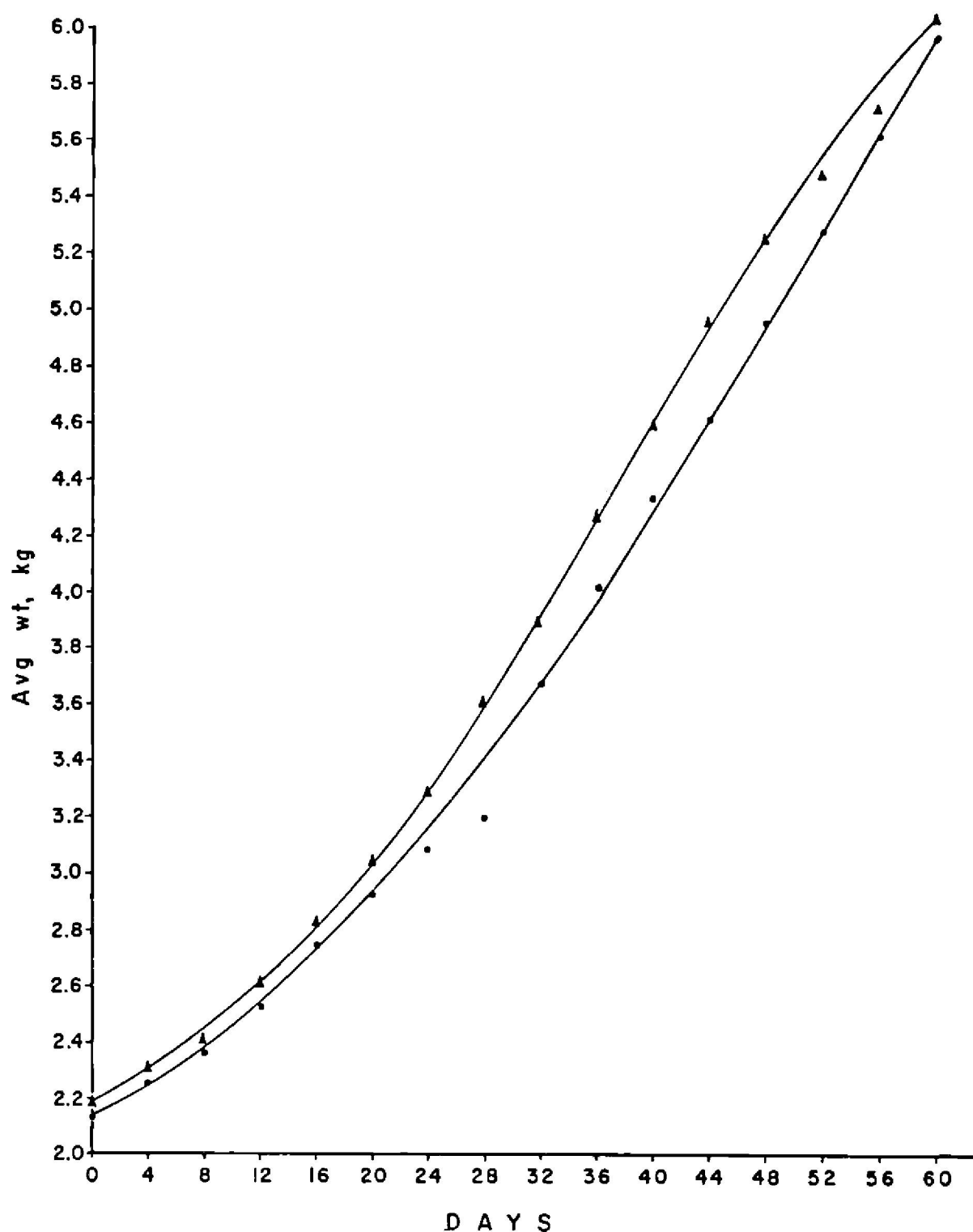


Fig. 1 Growth of young dogs fed soybean protein textured food and dehydrated beef; Average 6 dogs/group. ▲, dehydrated beef; ●, soybean protein textured food.

	SPTF	Dehydrated beef
Wt gain, g/day	62.7	63.8
Food consumed, g	6325	5835
Protein intake, g	1714	1921
PER	2.20	2.00

balances were obtained at intakes of nitrogen under 200 mg N/kg/day. Despite these differences, the results indicate that the nutritive value of the two protein sources is essentially the same. Weight gains were higher for dogs fed dehydrated beef. The animals started losing weight at nitrogen intakes below 100 mg/kg/day.

True protein digestibility and biological value were 90.0 and 68.3%, respectively, for the soybean protein textured food, calculated at a nitrogen intake of 261 mg/kg body weight/day. For dehydrated beef, true protein digestibility was 87.0% and its biological value 67.4%, calculated at the 292-mg nitrogen intake level.

Children. Table 9 shows the nitrogen balance results in the children fed whole milk and the soybean protein textured food at an intake of 2 g of protein/kg/day. Nitrogen absorption and retention were essentially the same for both protein foods at this level of protein intake. The differences in nitrogen intake and retention between the 2 groups were not statistically significant, although they were so with respect to nitrogen absorbed when expressed on an absolute basis. Table 10 shows nitrogen balance results in the children fed decreasing levels of protein intake from the soybean protein textured food. Nitrogen retention and absorption decreased as nitrogen intake decreased. Regression lines between nitrogen intake (NI) and nitrogen retention (NR) and between nitrogen absorption (NA) and

nitrogen retention (NR) were calculated from nitrogen intakes below 160 mg. The first equation was $NR = -59.8 + 0.433\ NI$ ($r = 0.64$) and the second, $NR = -44.7 + 0.534\ NA$ ($r = 0.61$). Nitrogen equilibrium was obtained when the children received approximately 138 mg of nitrogen from the soybean protein textured food, as compared with 97 mg from milk.¹¹

DISCUSSION

From the initial studies with rats, the results indicate that the protein value of the soybean protein textured food is equivalent to about 92% of the nutritive value of casein and of dehydrated beef,

¹¹ Bressani, R., F. Viteri, D. Wilson, J. Alvarado and M. Béhar 1966 'The protein value of several animal and vegetable proteins in children. Federation Proc., 25 (2, part 1): 299 (abstract).

TABLE 8
Nitrogen balance results in dogs

Protein	Nitrogen					Wt
	Intake	Fecal	Urine	Absorbed	Retained	
	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>kg</i>
SPTF ¹	630 ± 20 ²	65 ± 16	228 ± 35	565 ± 25	337 ± 45	7.416
DB ³	619 ± 28	111 ± 18	247 ± 27	508 ± 27	261 ± 26	8.084
SPTF	516 ± 42	63 ± 15	188 ± 22	453 ± 44	265 ± 51	8.408
DB	—	—	—	—	—	—
SPTF	419 ± 23	63 ± 11	158 ± 38	356 ± 26	198 ± 45	8.741
DB	469 ± 24	98 ± 27	211 ± 24	371 ± 30	160 ± 32	8.443
SPTF	261 ± 36	55 ± 13	138 ± 24	206 ± 32	68 ± 40	8.947
DB	292 ± 33	69 ± 21	149 ± 22	223 ± 24	74 ± 33	9.884
SPTF ⁴	190	45	172	145	—27	9.462
DB	156 ± 16	66 ± 9	111 ± 29	90 ± 21	—21 ± 19	9.959
SPTF	99 ± 17	40 ± 7	102 ± 16	59 ± 15	—43 ± 25	9.074
DB	93 ± 5	43 ± 8	101 ± 25	50 ± 9	—51 ± 21	10.008
SPTF	48 ± 9	31 ± 7	70 ± 10	17 ± 7	—53 ± 14	9.065
DB	57 ± 15	38 ± 14	81 ± 16	19 ± 16	—62 ± 21	9.844
SPTF	0	23 ± 6	65 ± 14	—	—	8.596
DB	0	31 ± 11	66 ± 12	—	—	9.473

¹ SPTF = soybean protein textured food.
² SD.
³ DB = dehydrated beef.
⁴ Average of one dog for 2 four-day balance periods.

TABLE 9
Nitrogen balance of children fed milk and soybean protein textured food (SPTF) at 2 g protein/kg/day

Protein food	Nitrogen						
	Intake	Fecal	Urine	Absorbed	Retained	Absorption	Retention
	<i>mg/kg/day</i>	<i>mg/kg/day</i>		<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>% of intake</i>	
Milk	342	52	210	290	80	84.8	23.4
SPTF	312	46	183	266	82	85.2	26.6
t value (df 31)	1.43(ns) ¹			2.99(s) ²			0.10(ns)

¹ Not significant.
² Significant.

TABLE 10

Nitrogen balance of children fed decreasing levels of protein from soybean protein textured food

Protein intake	Nitrogen						
	Intake	Fecal	Urine	Absorbed	Retained	Absorption	Retention
<i>g/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	<i>mg/kg/day</i>	% of intake	
1.5	231 ± 14 ¹	42 ± 11	140 ± 11	190 ± 11	49 ± 10	81.8	21.2
1.0	156 ± 7	36 ± 14	111 ± 13	120 ± 15	9 ± 16	76.9	5.8
0.75 ²	114 ± 10	32 ± 3	92 ± 13	82 ± 12	-10 ± 9	71.9	-8.8
0.50	78 ± 3	30 ± 7	75 ± 12	48 ± 7	-27 ± 16	61.5	-34.6
—	—	24 ± 6	61 ± 13	—	—	—	—

¹ SD.² Average of 5 children only.

based on the PER values obtained at the 11.6, 11.7 and 12.2% protein diet, respectively. The soybean protein textured food gave essentially the same weight gain as casein and dehydrated beef at comparable protein levels. The palatability of the food was extremely good as judged from the amount of food consumed by the rats, varying between 347 to 441 g/rat in 28 days. The blood chemistry of the animals fed the soybean protein textured food was similar to the blood picture obtained with animals fed casein. Fresh liver weight correlated with body weight for both casein and the soybean protein textured food. High fat content of the liver in rats fed the low protein level is commonly found, and it concurs with results of investigators working with various protein sources at low levels of protein intake. It is known that low protein diets, in spite of good amino acid balance, cause accumulation of fat in the liver (7-9).

The NPU method of protein evaluation applied to the soybean protein textured food also indicated that it has a slightly lower nutritive value than casein and, on a relative basis, 94.4% of the casein value concurring with calculations based on PER. The low PER value for the protein isolate can be attributed to the presence, in the protein, of residual soybean growth inhibitors, or to other substances derived from the process of extraction and precipitation of the protein. From the results it appears that either the inhibitor or another substance is eliminated in the process of changing the isolate into fiber. The significant increase in both PER and NPU from the fiber to the final product is probably the result of egg albumin being added, eliminating in this manner at least part

of the methionine deficiency inherent in soybean protein (10, 11). In general, PER correlated well with NPU for all products, except for the isolate. However, the discrepancy can be explained on the basis of the duration of the test for each assay. NPU assays run for 10 days (4) while PER assays take 28 days, permitting any physiologically adverse substance to accumulate and act against the performance of the animal. The animals consuming the isolate gained weight during the first 2 weeks of the PER assay, but lost weight during the last two, which resulted in lower growth and PER values.

The results obtained when the materials were cooked indicate that this process eliminated the growth-inhibiting substances present in the isolate. Very little effect was obtained when the fiber or the final product were heated, although there was a definite tendency to consume more food. It is well known that soybean flour must be properly heat-treated to obtain maximal nutritional value, although excess heat can decrease its protein quality (10, 12, 13). Improvement in the protein quality resulting from controlled heat treatment is due to the destruction of soybean trypsin inhibitors (14, 15), and to probable modifications of the protein, permitting a more complete digestibility and utilization of the sulfur amino acids, which are limiting in soybean protein (13, 15, 16). Recently, Longenecker et al. (17) reported that soybean protein isolate may contain good quality protein, but often requires mild heat treatment to bring out the maximal protein value.

From the results of amino acid supplementation, the soybean protein textured food appears to be mildly deficient in both

lysine and methionine, since the addition of both acids together increased PER values particularly when the food was further heat-treated. The effect was not as marked when the material was unheated, suggesting that the treatment applied, although mild, probably caused some decrease in the availability of both amino acids. It is also probable that even the final product still contained some inhibitor which did not allow for the supplements to express their beneficial effects on increasing protein quality. The response obtained with young growing dogs fed the soybean protein textured food was similar to that obtained with dehydrated beef, indicating again its excellent protein quality. No adverse physiological effects were noted during the entire experimental period. Assuming that the final food still contained physiologically adverse substances, these did not show their effect on the dogs, probably because the food was heated before consumption, thus eliminating the possible factors. The nitrogen balance results obtained with dogs indicate again the high protein quality of the soybean protein textured food studied.

With respect to the studies with children, the soybean protein textured food was readily accepted by all and there were no adverse effects at any time during the experiment.

Using the endogenous fecal and urinary nitrogen excretion values, and the nitrogen balance values at the 1 g of protein level of intake, the true protein digestibility and the biological value of the protein in the soybean protein textured food was calculated according to the formula of Mitchell (4, 18). These values are 92.3 and 65.3%, respectively. At an intake of 0.75 g of protein per kg body weight, true protein digestibility was found to be 93.0%, and the biological value, 70.8%.

Similar calculation from data obtained on children of ages similar to the ages of the subjects used in this study, and fed milk protein, indicated that the true protein digestibility and biological value are 92.0 and 80.6%.¹²

It can be concluded then that the protein quality of the soybean protein textured food is high (about 80% of the protein quality of milk), with adequate digesti-

bility; it is readily acceptable and free of adverse physiological effects.

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¹² See footnote 11.

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