SOY PROTEIN AND HUMAN NUTRITION

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NUTRITIONAL QUALITY OF SOYBEAN PROTEIN ISOLATES: STUDIES IN CHILDREN OF PRESCHOOL AGE

Benjamin Torun

The need for new sources of protein and food mixtures with a high protein content for human nutrition has increased and the trend towards their more widespread use seems to continue. The higher availability and lower cost of vegetable proteins compared with animal proteins make the former more suitable as new sources or to increase the protein content or nutritional value of existing foods. This can be achieved through mixtures of vegetables with complementary amino acid patterns (Behar, 1963), through genetically improved vegetable proteins, such as opaque-2 corn (Mertz et al, 1964; Concon, 1966) or through vegetable proteins with a good native amino acid score, such as soybean protein. Digestibility must also be taken into account when vegetable proteins are considered for human consumption (FAO/WHO, 1975.).

One of the nutritional applications of these protein sources is in the supplementary feeding of children. This and the growing interest in soybeans for human nutrition in Central America prompted the testing of their protein quality in children of preschool age. Towards this end two soybean protein isolates with low trypsin inhibiting activity were used[1]. Table 1 shows their chemical composition and amino acid pattern and Table 2 shows their content of essential amino acids and chemical score relative to the FAO/WHO reference (1973). *Supro 620* has high viscosity and gel-forming properties and *Supro 710* has lower viscosity, non-gelling properties and is more easily dispersible in water.

[1]Ralston Purina Company, St. Louis, Missouri.

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Table 1. Physical, chemical and nutritional characteristics of soybean protein isolate[1]

Physical Properties		*Amino Acid Content (%)*[2]	
Color	Cream	Alanine	3.46
Flavor	Bland	Arginine	6.39
Odor	None	Aspartic acid	9.32
pH (water slurry)	7.1	Cystine	1.37
		Glutamic acid	16.03
Chemical Composition		Glycine	3.46
		Histidine	2.21
Protein (N x 6.25)	85.2%	Isoleucine[3]	3.97
Fat (acid hydrolysis)	5.4%	Leucine[3]	6.63
Fat (ether extract)	1.1%	Lysine[3]	5.40
Fiber (crude)	0.1%	Methionine[3]	1.14
Ash	3.7%	Phenylalanine[3]	4.43
Moisture	5.3%	Proline	4.49
Calcium	0.2%	Serine	4.38
Phosphorus	0.8%	Threonine[3]	3.21
Sodium	1.3%	Tryptophan[3]	1.17
Potassium	0.05%	Tyrosine	3.11
		Valine[3]	3.98
Other Characteristics		Ammonia	1.73
Urease, pH increase	0.01		
Residual trypsin inhibitor, units/mg	7.5		
PER	2.10		
Casein corrected PER	1.63		

[1]*Data for* Supro 620. *Characteristics of* Supro 710 *were similar.*
[2]*g/100 g of product.*
[3]*Essential amino acids.*

EXPERIMENTAL DESIGN AND PROCEDURES

Nitrogen (N) balance techniques were used to evaluate the protein quality of the isolates (Allison, 1955). Using regression analysis, the regression coefficients between N intake or absorption and N retention of the test protein were compared with those of milk as a reference protein. The regression coefficient of intake on retention provides a measure

Table 2. Essential amino acid content and score of the two soybean protein isolates

Amino Acid	Supro 620		Supro 710	
	Content[1]	Score[2]	Content[1]	Score[2]
Isoleucine	4.66	117	4.93	124
Leucine	7.78	111	8.15	117
Lysine	6.34	115	6.48	117
Methionine + Cystine	2.95	84	2.75	78
Phenylalanine + Tyrosine	8.85	148	9.41	157
Threonine	3.77	94	3.78	94
Tryptophan	1.37	137	1.38	138
Valine	4.67	93	4.87	97

[1]*g amino acid/100 g protein.*
[2]*Relative to FAO/WHO reference, 1973.*

similar to net protein utilization, NPU. The regression coefficient of absorption on retention is a measure similar to the biological value, BV. In either case, the better the quality of the protein the greater the regression coefficient (Bressani and Viteri, 1970; Viteri and Bressani, 1972).

Apparent digestibility was also calculated and compared with that of the reference protein.

Two separate studies were carried out. *Supro 620* was the test protein in the first study. It was tested with descending levels of intake from 320 to 120 mg N/kg/day, equivalent to from 2.0 to 0.75 g soybean protein/kg/day (N x 6.25). Fifty mg of choline chloride were added daily to the diet in addition to the vitamin and mineral supplements shown in Table 3 based on reports which suggest that the need for choline may increase when methionine is the limiting amino acid, as is the case with soy protein (Du Vigneaud et al, 1941; Treadwell, 1948; Mudd and Poole, 1975). That amount of choline chloride is equivalent to the content of free choline in about 500 ml of cow's milk (Macy et al, 1953).

Table 3. Vitamin and mineral supplements administered daily

Vitamin A	2500 I.U.
Vitamin B_1	1 mg
Vitamin B_2	0.5 mg
Niacinamide	5 mg
Vitamin B_6	0.5 mg
Pantothenic acid	5 mg
Folic acid	30 mcg
Vitamin B_{12}	2 mcg
Biotin	50 mcg
Vitamin C	25 mg
Vitamin D	500 I.U.
Vitamin E	1.5 mg
Iron (as ferrous sulfate)	60 mg
Iodine (as KI)	100 mcg
Manganese sulfate	0.9 mg
Zinc sulfate	1 mg

Supro 710 was the test protein in the second study. Based on the results of the first study, it was tested with descending levels of intake ranging from 200 to 80 mg N/kg/day, equivalent to from 1.25 to 0.5 g soybean protein/kg/day (N x 6.25). Tests were carried out with and without the addition of choline to evaluate the effect of the vitamin, usually with the same children.

A liquid diet was used in the two studies. It was prepared with the soybean protein isolate, sugars, corn starch, peanut oil and salts. Table 4 shows a typical dietary formula ıministered daily

2500 I.U.
1 mg
0.5 mg
5 mg
0.5 mg
5 mg
30 mcg
2 mcg
50 mcg
25 mg
500 I.U.
1.5 mg
60 mg
100 mcg
0.9 mg
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Table 4. Composition of daily liquid diet prepared for a 10-kilogram child. During the day it was divided in five equivalent meals.

Component	Amount	Protein	Fat	Energy
	g	g	g	kcal
Supro 620	22.2	20	1.2	91
Sucrose	100.0	-	-	400
Dextrins-Maltose (Mead Johnson, U.S.)	42.3	-	-	165
Corn starch	15.0	-	-	60
Peanut oil	32.1	-	32.1	284
Mineral mix[1]	6.1	-	-	-
Water[2]	582.3	-	-	-
TOTAL	800.0	20	33.3	1,000

[1]*6.1 g of mineral mix provides, as mEq:* K^+ *60;* Na^+ *10;* Ca^{++} *10;* Mg^{++} *4;* Cl^- *60;* $HPO_4^{=}$ *10;* $CO_3^{=}$ *10;* $SO_4^{=}$ *4.*

[2]*Approximately 10% of the water was flavored with cinnamon by boiling during 30 minutes around 40 g of cinnamon sticks in 1,000 ml of water.*

for a 10 kg child with an intake of 320 mg N/kg/day. The energy content of the diet was 100 kcal/kg/day, since that was the energy level used in previous experiments with milk (the reference protein source). The experience at the Institute of Nutrition of Central America and Panama (INCAP) has been that this energy intake allows rates of growth and weight gains at least as fast as those expected for children of the same size as the ones who participated in the present studies. The only exceptions were children ER and GR who received 90 and 110 kcal/kg/day, respectively, based on several weeks' observations of their dietary intakes and growth patterns which deviated from the other children with 100 kcal/kg/day. Some children also ate 150-190 g of fresh apple which provided 3-5 mg N/kg/day. The liquid formula was prepared weighing the ingredients with an accuracy of ± 0.1 g, mixing them with approximately 80% of the water required and boiling for 15 minutes. After the diet cooled, plain or cinnamon-flavored water was added to take it to the prescribed weight. It was homogenized with a blender and the amount for each meal was weighed in tared cups for each of the corresponding child's meals.

The studies were carried out with children aged 19 to 44 months who had been admitted to INCAP's Clinical Center for treatment for protein-energy malnutrition. All had recovered nutritionally at least one month prior to beginning the studies based on clinical evaluation, weight expected for height, creatinine-height index and hematological and other biochemical indicators (Viteri and Alvarado, 1970; Torun and Viteri, 1976; Viteri and Torun, 1978); and in the interim they ate a diet which provided about 2 g milk protein and 100 kcal/kg/day. Informed parental consent to participate was obtained, and the experimental protocols were reviewed critically and approved by a Committee on Human Rights and Participation of Humans as Experimental Subjects. Eight children participated in the first study and eight others in the second. Of the latter, seven received the protein isolate with and without additional choline, and one of them (child SB) received the protein isolate with choline on two separate occasions. Table 5 shows their ages, weights and heights. When the same child participated in the study more than once, it was at intervals of at least 2 weeks during which he received a diet with 1.5 g soybean protein, 0.5 g milk protein and 100 kcal/kg/day.

The diet was fed initially at the highest level of N prescribed (320 and 200 mg N/kg/day in experiments 1 and 2, respectively), and every 9 days the amount of soybean protein isolate was reduced by 40 mg N/kg/day. The energy intake was kept constant by adding sugar and/or corn starch to compensate for the decrease in protein isolate. The full cups were weighed immediately before each meal; the diet was given to the children, the cups rinsed with 20-40 ml of water, which the children also drank, and the empty cups were weighed again. The difference in weight after accounting for the rinse water was recorded as the amount of diet ingested. Some children vomited occasionally, usually shortly after finishing a meal. The vomit's weight was calculated by picking it up with pre-weighed towels and by weighing the children's pre-weighed bedding and clothes. It was assumed to be mainly liquid diet, and the same weight of food was given to the child at a later time on the same day to compensate for the vomit. Complete urine and fecal collections were obtained during the

Table 5. Age, height and weight of children who participated in studies with soybean protein isolates

	Supro 620 + Choline	Supro 710 + Choline	Supro 710
Initial			
Age, months	33 ± 7[1]	32 ± 8	31 ± 7
Height, cm	83.8 ± 5.7	84.6 ± 4.9	83.6 ± 4.0
Weight, kg	11.62 ± 1.01	12.58 ± 1.69	12.28 ± 1.41
Weight-for-height, % of expected	97 ± 4	106 ± 11	105 ± 9
Change[2]			
Weight, g/day	10.6 ± 2.8	7.2 ± 6.1	13.9 ± 5.6
Height, mm/month	9.3 ± 2.1	7.5 ± 2.4	9.1 ± 3.3
Weight-for-height, % of expected during experimental period[2]	4 ± 3	1 ± 2	3 ± 2

[1]*Mean ± standard deviation, n = 8.*
[2]*Duration of experimental periods: 54 days for* Supro 620 *and 36 days for* Supro 710.

last 4 of each 9-day period. All losses were accounted for by continuous surveillance of the children and weighing of bedding, clothes and diapers previously weighed or tared. Carmine red and charcoal were used as markers for the periods of fecal collection. Homogeneous aliquots of urine, feces and diet were analyzed for their N content.

In addition to the N balance techniques, the children were weighed nude every morning before breakfast and their height was measured on 2 consecutive days at 2-week intervals. Total plasma proteins were determined refractometrically at the end of every 9-day period from fingertip blood.

RESULTS

The diets were readily accepted and all children were in good health throughout the studies, except for upper respiratory infections, apparently of viral origin, that required symptomatic treatment. Child RR had a generalized rash without fever for two days followed by epidermal desquamation while on *Supro 710* without choline. Five children had periods of moderate diarrhea which disappeared spontaneously without dietary changes or medication.

Growth was adequate in terms of weight and height (Table 5) and there were no consistent changes in the pattern of weight gain when the protein content of the diets decreased.

Digestibility: Table 6 shows the apparent digestibility of the protein isolates tested. It decreased with the lowest N intakes, probably due to the relatively larger contribution of metabolic (obligatory) fecal N losses. There were no differences between the soybean protein isolates, between the use or non-use of choline, nor between the soybean isolates and milk fed at the same levels of nitrogen (Scrimshaw et al, 1958; Bressani et al, 1958, 1963, 1967, 1969, 1972; Viteri and Bressani, 1972).

Nitrogen balance: Table 7 and Figure 1 show the amounts of nitrogen retained at various levels of protein intake. Two different types of relationship between intake and retention were observed in the study with *Supro 620*: there were no significant changes in retention at intakes greater than 200 mg N/kg/day ($r=0.272$, $p>0.1$), whereas retention decreased with intakes below that level. The comparisons of the linear regressions with intakes ≤ 200 mg N/kg/day by analysis of covariance showed that there was no difference between the regression coefficients when either of the protein isolates was used nor when choline was added. There was, however, a shift to the left in the regression line of the second study (*Supro 710*) resulting in an apparent need of less dietary N to attain equilibrium (60 and 58 mg N/kg/day, with and without additional choline, respectively) than in the first study (86 mg/kg/day). The former figures were even lower than the intake of milk protein needed by similar children using the same experimental techniques (68-84 mg N/kg/day) (Viteri and Bressani, 1972; Bressani et al, 1972).

The regression equations of the pooled data for each dietary treatment were similar to the mean of the individual regression lines, as shown in Table 8.

Nitrogen balance index: The nitrogen balance index is derived from the relationship between apparent (i.e., without accounting for obligatory losses) nitrogen absorption and re

Table 6. Apparent digestibility (% of intake) of soybean protein isolates at various levels of nitrogen intake

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Table 6. Apparent digestibility (% of intake) of soybean protein isolates at various levels of nitrogen intake

N Intake[1] *mg/kg/day*	*Supro 620 + Choline*	*Supro 710 + Choline*	*Supro 710*	*Milk*[2]
320	86 ± 5 (87 ± 3)[3]	-	-	84
280	86 ± 7 (88 ± 3)	-	-	83
240	87 ± 3	-	-	82
200	87 ± 3 (88 ± 3)	83 ± 7 (85 + 6)	86 ± 4	83
160	84 ± 4 (85 ± 2)	81 ± 7 (82 ± 7)	85 ± 6	78
120	82 ± 3[4]	75 ± 12 (79 ± 9)	75 ± 10[5] (80 ± 4)	-
80	-	70 ± 14[5] (74 ± 8)	65 ± 12[4] (68 ± 8)	69

[1]Nitrogen intakes ± 3%. Eight children with each treatment.
[2]Calculated from studies at INCAP: Scrimshaw et al, 1958; Bressani et al, 1958, 1963, 1967, 1969, 1972.
[3]% absorbed, mean ± standard deviation. Figures in parentheses: excluding balance of one or two children with diarrhea.
[4,5]Differs from other levels of N intake (Student's paired "t" test): [4] $p < 0.01$, [5] $p < 0.05$.

Table 7. Apparent nitrogen retention (intake-urinary N-fecal N) of soybean protein isolates, mg/kg/day, at various levels of nitrogen intake

N Intake[1] *mg/kg/day*	*Supro 620 + Choline*	*Supro 710 + Choline*	*Supro 710*
320	88 ± 27[2]	–	–
280	81 ± 26	–	–
240	81 ± 17	–	–
200	74 ± 8	80 ± 14	81 ± 21
160	49 ± 14[3]	65 ± 14[3]	62 ± 10
120	22 ± 18	35 ± 10	32 ± 16
80	–	11 ± 11	14 ± 12

[1]*Nitrogen intakes ± 3%. Eight children with each treatment.*
[2]*Nitrogen retention, mean ± standard deviation.*
[3]*Treatments differ (Student's "t" test), $p < 0.05$.*

tention (Allison and Anderson, 1945; Hoffman and McNeil, 1949). Table 8 and Figure 2 show the linear regressions of apparent retention on apparent absorption. The regression coefficients at absorption ≤190 mg/kg/day correspond to the nitrogen balance index. The results were similar to those described for the relationships between intake and retention: two types of relationship were observed with N absorptions above or below 190 mg/kg/day, there were no differences between the regression coefficients of both protein isolates, the addition of choline had no effect and there was a shift to the left in the regression line with *Supro 710* relative to *Supro 620*. The regression equations of the pooled data for each dietary treatment were also similar to the means of the individual regressions.

Plasma proteins: Table 9 shows the concentrations of plasma proteins. The analyses within each dietary treatment (using Student's paired "t" test) showed that the concentration of proteins decreased with N intakes of 120 or 80 mg/kg/day. There were no differences between the two protein iso-

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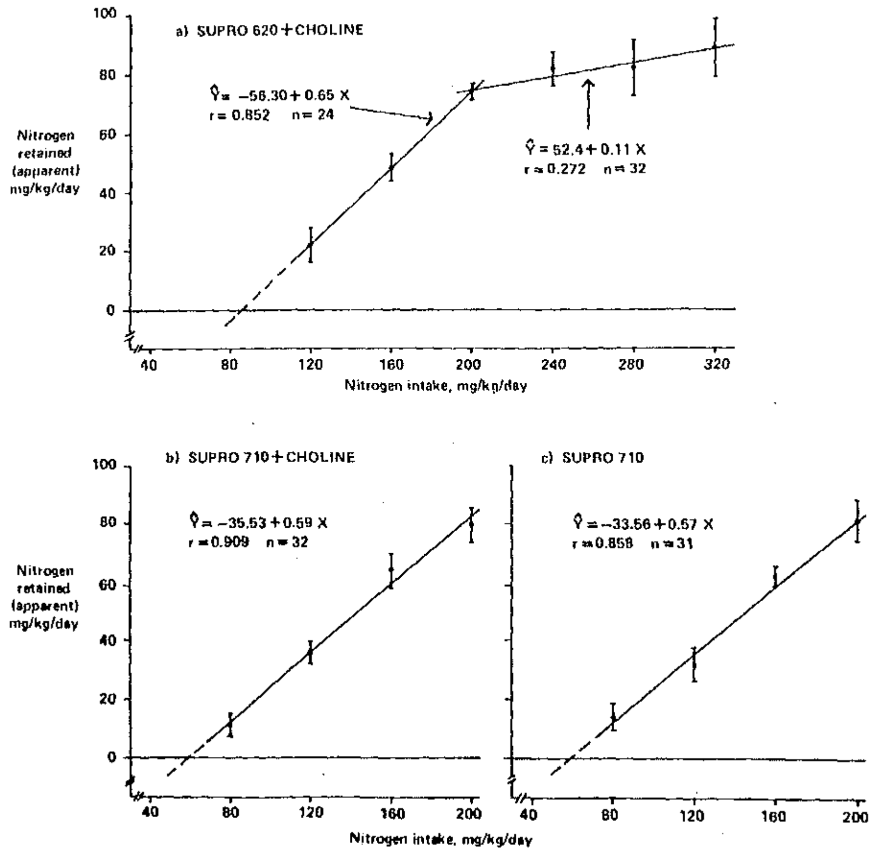

FIGURE 1: Relationship between nitrogen intake of soybean protein isolates and apparent nitrogen retention (intake-urinary N-fecal N), expressed as mg N/kg/day. The regression coefficients at intakes $\leq$ 200 mg N/kg/day do not differ. Points are means ± standard error (n=8, except 7 at intake of 80 mg N/kg/day in c).

lates nor with the addition of choline at any level of protein intake.

Table 8. *Regression equations of apparent nitrogen retention (Y) on nitrogen intake (I) or absorption (A) of soybean protein isolates*[1]

	Regression Equations of N Retention on N Intake	N Absorption
Supro 620 + Choline	a^2	d^2
Pooled Data	Y = −56.3 + (0.65 x I)	Y = −47.2 + (0.70 x A)
Mean of Individual Regressions	Y = −56.3 + (0.65 x I)	Y = −45.1 + (0.69 x A)
	(±41.8)[3] (±0.20)[3]	(±36.2) (±0.21)
Supro 710 + Choline	b^2	e^2
Pooled Data	Y = −35.5 + (0.59 x I)	Y = −25.2 + (0.66 x A)
Mean of Individual Regressions	Y = −35.5 + (0.60 x I)	Y = −24.1 + (0.65 x A)
	(±18.4) (±0.13)	(± 9.6) (±0.08)
Supro 710	c^2	f^2
Pooled Data	Y = −33.6 + (0.57 x I)	Y = −19.8 + (0.59 x A)
Mean of Individual Regressions	Y = −33.6 + (0.58 x I)	Y = −19.6 + (0.59 x A)
	(±15.9) (±0.13)	(± 9.4) (±0.12)

[1]*Data from intakes ≤ 200 mg/kg/day and absorptions < 190 mg/kg/day.*

[2]*Analysis of covariance: no difference between regression coefficients in a, b, c nor in d, e, f. The residual variance of the coefficient in e was smaller than in d and f ($p < 0.01$).*

[3]*Standard deviation of the individual intercepts and regression coefficients, respectively.*

Table 9. Concentration of plasma proteins (g/100 ml) at various intakes of soybean protein

N Intake[1] *mg/kg/day*	*Supro 620* + *Choline*	*Supro 710* + *Choline*	*Supro 710*
320	6.9 ± 0.2[2]	-	-
280	7.0 ± 0.3	-	-
240	6.9 ± 0.3	-	-
200	6.8 ± 0.3	6.5 ± 0.3	6.8 ± 0.4
160	6.6 ± 0.4	6.4 ± 0.3	6.3 ± 0.2
120	6.4 ± 0.5[3]	6.3 ± 0.5	6.3 ± 0.3[4]
80	-	6.0 ± 0.4[3]	5.9 ± 0.3[3]

[1]*Nitrogen intakes ± 3%. Eight children with each treatment.*

[2]*Mean ± standard deviation.*

[3]*Less than at other levels of intake (Student's paired "t" test), p ≤ 0.05 or < 0.01.*

[4]*Less than with N intake of 200 mg/kg/day, p < 0.01.*

with intakes of 160 mg/kg/day (Table 7) and the intercept of the regression equations which resulted in a displacement to the left of the regression lines for *Supro 710* relative to *Supro 620*. This, in turn, resulted in different estimates of the dietary protein needed to attain nitrogen equilibrium (Figures 1-3). To this end, intakes of *Supro 710* equivalent to 58-60 mg N/kg/day would be needed in contrast to 86 mg N/kg/day of *Supro 620* and 84 mg N/kg/day of milk protein, as calculated by Viteri and Bressani (1972) from various studies at INCAP using similar techniques with similar children. There is no clear explanation for these apparently low requirements of *Supro 710*. A similar shift of the regression intercepts of N retention on intake or absorption has been observed in dogs using the same protein sources on different occasions and it seemed to be related to the type of diet or nutritional status of the animals prior to beginning the balance studies (Bressani, personal communication). The children who participated in the present studies were equally well-

Table 10. Comparative results of nitrogen balance studies with soybean protein isolates and milk, at intake levels 200 mg N/kg/day

	Regression Coefficient Between N Retention[1] and N Intake[1]	Regression Coefficient Between N Retention[1] and N Absorbed[1]	N Equilibrium Attained with N Intake[1] of	Compound (Mean) Score Relative to Milk
Whole Milk[2]	.64	.73	84	
Supro 620	.65 (101)[3]	.70 (96)	86 (98)	98
Supro 710	.59 (92)	.63 (86)	59 (142)	107

[1]*N intake, absorption and retention expressed as mg/kg/day.*
[2]*Source: Viteri and Bressani, 1972.*
[3]*Figures in parentheses: % relative to milk.*

nourished and ate similar diets before the experiments began. Those with *Supro 620*, however, had a slower and more gradual decrement in protein intakes since the experimental diets began with 320 mg N/kg/day, while *Supro 710* started with 200 mg N/kg/day. Most of the children studied with milk also received one or more dietary levels of intake higher than 200 mg N/kg/day. Another difference was that all children studied at INCAP with milk and with *Supro 620* were relatively sedentary while the children who ate *Supro 710* were stimulated to be physically more active. It has been shown that children who were recovering from protein-energy malnutrition with diets which provided 2.5 g protein and 120 kcal/kg/day grew better when they were physically more active, although there was no difference in N retention at that level of intake (Torun et al, 1975; Torun and Viteri, submitted for publication).

Based on those considerations it can be speculated that the children who participated in the second study had a decrease in obligatory fecal N losses or that they used "more efficiently" the protein fed at levels of 1.25 g/kg/day and lower, resulting in the displacement to the left of the regression lines of N retention on intake or absorption. The experimental evidence indicates that it is important to standardize the conditions used in studies based on N balance techniques, not only during the experiment itself, but prior

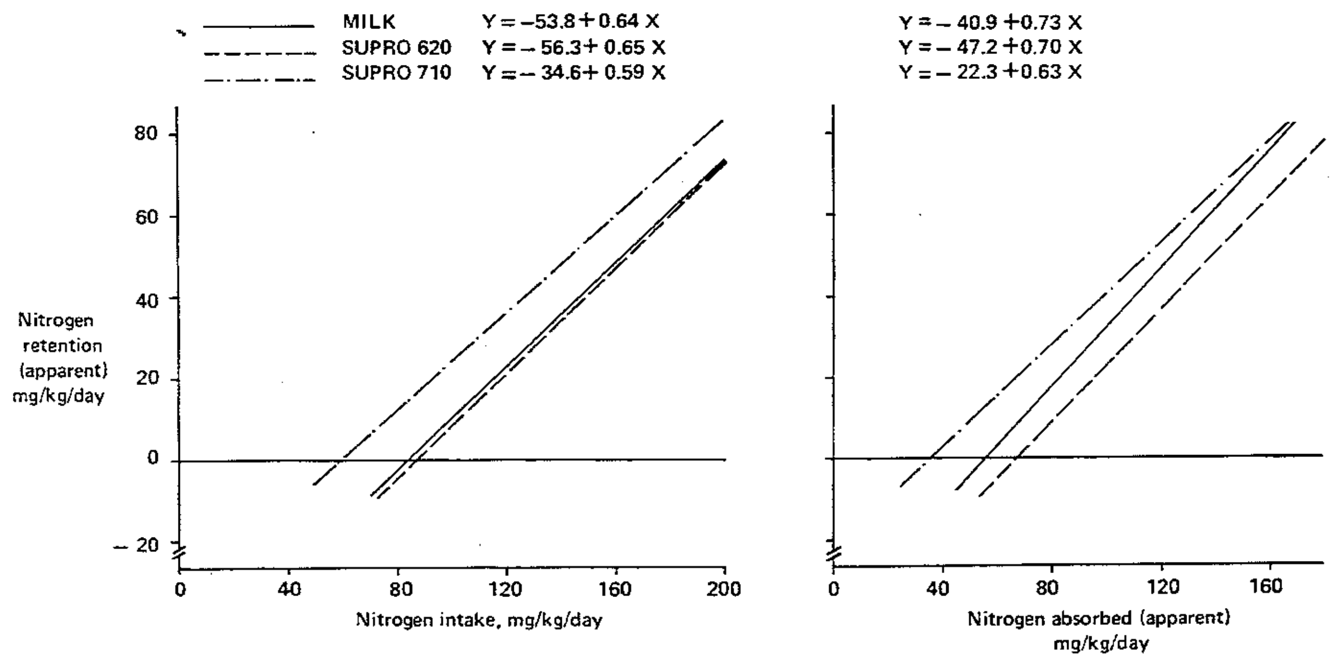

FIGURE 3. Relationship between nitrogen intake or apparent absorption (without correcting for fecal obligatory N losses) and apparent nitrogen retention (intake-urinary N-fecal N) of soybean protein isolates and whole milk.

to its beginning. It is possible that if depletion had taken place at the same rate in the two studies and if there had been no difference in physical activity, the results would have been similar with both protein isolates. It is interesting to note that the effect was greater on the intercept than on the regression coefficient. Therefore, the lack of uniformity in the experimental conditions seems to affect the apparent requirement for a protein more than its biological value relative to other proteins.

The addition of choline did not have any effects, at least under the present experimental conditions and at the levels of protein fed. In view of that and since seven of the eight children studied ate *Supro 710* both with and without additional choline, the results of the two experimental treatments with that protein isolate were combined. The new regression equations of N retention on intake (I) and absorption (A), with 63 data points, were $Y= -34.6 + 0.59\ I$ ($r=0.884$) and $Y= -22.3 + 0.63\ A$ ($r=0.939$).

The two soy protein isolates studied were well tolerated by the children and their digestibilities were comparable to those of milk even when the balance periods with diarrhea were included in the analysis (Table 6). Nitrogen retentions as function of intake or absorption were compared with milk protein in Table 10 and Figure 3. Based on the nitrogen balance index, the biological values of *Supro 620* and *Supro 710* were 96% and 86% that of milk, respectively. Looking at the regression coefficient of retention on intake as equivalent to NPU, the soybean protein isolates had values of 101 and 92% relative to milk. Finally, using the compound score suggested by Viteri and Bressani (1972), which is the mean of the comparisons with a reference protein source of the regression coefficients of apparent N retention on intake and on apparent absorption and of the N intake needed to attain N equilibrium, the two soybean protein isolates scored 98 and 107% relative to milk protein.

In conclusion, the soybean protein isolates tested compared quite adequately with milk based on nitrogen balance techniques, ranging from 86% to 107% depending on the method of comparison selected. Furthermore, all children retained adequate amounts of nitrogen to allow for insensible losses and growth requirements with intakes of 160 mg N (equivalent to 1 g protein) per kilogram per day (FAO/WHO, 1973). This level of intake coincided with that at which the concentrations of plasma proteins were maintained and, again, compares satisfactorily with the recommended intakes of milk protein for children of this age (FAO/WHO, 1973). Nevertheless, it must be borne in mind that it is not practical to think of soybeans as the sole protein source for preschool-age chil-

FIGURE 3. Relationship between nitrogen intake or apparent absorption (without correcting for fecal obligatory N losses) and apparent nitrogen retention (intake-urinary N-fecal N) of soybean protein isolates and whole milk.

dren. But their high nutritional value makes them attractive to complement other proteins of lower quality or to substitute partially for others of equally high quality. However, the nutritional value of such protein combinations must be assessed in each specific case, either theoretically (e.g., based on amino acid scores and digestibility (FAO/WHO, 1975) or experimentally. The latter is, of course, more accurate since theoretical estimates do not take into consideration individual variability nor the specific conditions of the experimental evaluation, such as those illustrated in the present studies.

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