Efficiency of Utilization of Soy Protein Isolate in Japanese Young Men

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Summary The nutritional quality of soy protein isolate (SPI) was evaluated in young men by the nitrogen balance method using fish protein as a standard. Twenty-one male university students were given SPI (Supro 620, Ralston Purina Co., St. Louis, Mo., U.S.A.), fish protein (cod fish) or a 50:50 mixture of the two proteins as the sole source of protein. SPI and fish were prepared as paste products (kamaboko). Four experimental periods were used in which each subject received 0.35, 0.45, 0.55 and 0.65 g protein/kg/day, respectively, given in random order. Each period consisted of one day on protein-free diet and ten days on the experimental diet, with an interval of three days on a free-choice (*ad libitum*) diet between periods. Energy intake was constant for each individual to maintain their body weight (44.6 ± 2.4 kcal/kg/day). The linear regression equations obtained between nitrogen intake (X : mg N/kg/day) and the apparent nitrogen balance (Y : mg N/kg/day) were as follows: SPI, $Y = 0.298X - 35.2$; fish protein, $Y = 0.365X - 31.8$; mixed protein, $Y = 0.423X - 38.3$. The nitrogen requirement for maintenance of nitrogen equilibrium determined from the regression equation was 118.1 ± 15.4 mg N/kg/day for SPI, 87.1 ± 17.2 mg N/kg/day for fish protein and 90.5 ± 17.1 mg N/kg/day for mixed protein. The NPU's calculated at the respective maintenance nitrogen intakes were 38.9, 52.8 and 50.8 for SPI, fish protein and mixed protein, respectively. There was no significant difference between the nutritive values of mixed protein and fish protein. The nutritive value of SPI relative to fish protein was estimated as 82%, 74% and 74% by the slope-ratio method, and values for the nitrogen requirement and NPU, respectively.

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In Japan, soybean is an important traditional food item together with rice and fish, and serves as a good source of protein. Soybean is eaten in various forms such as miso (fermented soybean paste), soy sauce, tofu (soybean curd) and natto (fermented soybean product) as well as simply as cooked soybeans. In 1977, protein intake from soybean was estimated to be 7.6 g/person/day, corresponding to be about 10% of the total daily protein intake of Japanese. In contrast, the primary use of soybeans in the United States is for oil and the residual meal after extraction of oil is used as animal feed or fertilizer.

With advances in food technology, new soybean products have been introduced, such as soybean flours, concentrates and isolates. In recent years, the consumption of soybean products in a variety of food industries has increased rapidly for economic reasons and because of the multiple functional properties of these products such as for gelation, increase in viscosity, emulsification, dough formation, aeration, and improvement of texture (1). Soy protein isolates are used in processed meat, fish paste products (kamaboko, chikuwa, fish sausages), bakery products and dairy-type products. They are also used as protein supplements in infant formulas and hospital diets.

Besides having wide applications for use in processed foods because of their unique functional properties, soy protein isolates like other vegetable proteins have advantages over animal proteins with respect to lipid metabolism (2). Moreover their nutritional value is important, because protein malnutrition is a world-wide problem and a plentiful supply of cheap high quality protein foods is urgently required to meet the needs of the rapidly growing world population. Although soy protein has a low content of sulfur-containing amino acids, it is considered to be a good quality vegetable protein.

Thus in view of the growing use of soybeans in human foods and the importance of soy protein in protein nutrition, we studied the nutritional value of soy protein isolate and its minimum requirement for apparent nitrogen equilibrium compared with those of fish protein. We also examined the improvement of the nitrogen balance achieved by mixing an equal amount of fish protein with soy protein isolate. Anthropometric measurements as well as hematological examinations and urinalyses were conducted in an attempt to detect possible changes in body composition and metabolic status of the subjects.

MATERIALS AND METHODS

Three series of experiments using soy protein isolate, fish protein and a mixture of the two proteins as protein source were conducted on 21 male university students. The characteristics of the subjects are shown in Table 1. The subjects were all

Table 1. Characteristics of the subjects.

	Subject	Age (year)	Height (cm)	Weight (kg)	Blood pressure (systolic/diastolic) (mmHg)
SPI	M	21	173	68.2	
	N	21	161	53.9	
	O	28	164	54.6	
	P	22	164	74.0	
	Q	26	174	77.5	
	Mean	24	167	65.6	
	SD	3	6	10.9	
Fish	A	21	171	63.3	125/75
	B	20	164	56.5	135/82
	C	19	171	58.1	145/90
	D	24	171	69.8	120/75
	E	22	179	64.3	115/75
	F	22	172	63.2	130/85
	G	20	168	57.6	120/70
	K	22	166	71.6	125/84
	Mean	21	170	63.1	127/80
	SD	2	5	5.6	10/7
Mix.	A	21	171	63.4	105/70
	B	21	164	59.2	133/92
	C	20	170	59.4	148/93
	D	24	171	68.3	118/75
	E	22	178	62.3	122/73
	H	25	170	60.3	95/58
	I	21	166	52.0	108/72
	J	21	168	61.0	117/83
	Mean	22	170	60.7	118/77
	SD	2	4	4.6	17/12

healthy and lived in a metabolic ward of our laboratory throughout the study. During the feeding period, they continued their daily routine activities and their physical activities were roughly measured with a pedometer. Subjects C and D could not participate in one of four experimental periods with fish protein because of febrile upper respiratory infections.

The experimental design is shown in Fig. 1. After a four-day adjustment period during which subjects were given 1.25 g/kg of protein and maintenance energy from conventional foods, each subject received four low levels of protein successively. Subjects were randomly assigned to each low protein level according to the Latin

Fig. 1. Experimental design. Subjects were randomly assigned to the four levels of test protein during the four dietary periods. a. The average daily nitrogen excretion in the last five days at each protein level was used in calculating nitrogen balance. b. Feces in the last eight days at each protein level were pooled. The average daily fecal nitrogen loss in each period was used in calculating the nitrogen balance and digestibility.

Table 2. Analysis of soy protein isolate.^a

	%
Protein (N × 6.25)	86.5
Moisture	6.0
Ash	4.1
Fat	0.8
Fiber (crude)	0.25
Calcium	0.15
Phosphorus	0.8
Sodium	1.3
Potassium	0.05

^a Supro 620, Ralston Purina Co., St. Louis, Mo., U.S.A.

Square Design to avoid an effect of the previous protein intake on the results. Each period consisted of one day on protein-free diet and ten days on low-protein diet, and was followed by a three-day interval on free-choice (*ad libitum*) diet (Fig. 1).

The protein sources were soy protein isolate (Supro 620; Ralston Purina Co., St. Louis, Mo., U.S.A.) (Table 2) for five subjects, fish protein (cod fish as a standard) for eight subjects and a mixture of equal amounts of soy protein and fish protein for eight subjects, of whom five (Subjects A, B, C, D and E) also

Table 3. Compositions of kamaboko products.

	SPI ^a		Cod	
	Parts	%	Parts	%
SPI	20	17.5	—	—
Cod paste	—	—	100	77.2
NaCl	3	2.6	3	2.3
Potato starch	5	4.4	5	3.9
Sugar	1.5	1.3	1.5	1.2
Water	85	74.2	20	15.4
Total	114.5	100.0	129.5	100.0

^a Soy protein isolate.Table 4. Diet composition.^a

Component	g/day
SPI ^b kamaboko ^c	107.5
Cod kamaboko ^c	155
Sugar	323
Corn starch	300
Margarine	32
Corn oil	30
Agar	3
Baking powder	5
NaCl	2.5
Mineral mixture ^d	6
Vitamin mixture ^d	3

^a Intakes are for a 65 kg subject receiving 0.55 g/kg of mixed protein with 45 kcal/kg of energy. ^b Soy protein isolate (Supro 620, Ralston Purina Co., St. Louis, Mo.). ^c Nitrogen contents are as follows: dried cod, 89.6 mg/g; dried SPI, 147.2 mg/g; cod kamaboko, 18.45 mg/g; SPI kamaboko, 26.6 mg/g. ^d For details, see Reference (13).

participated in studies on fish protein and mixed protein. The soy protein isolate and fish protein were prepared as paste products (kamaboko) (Table 3) and diets were given in three equal meals each day. An example of the diet composition is shown in Table 4. The protein levels tested were 0.35, 0.45, 0.55 and 0.65 g protein/kg/day ($N \times 6.25$) for each protein and these levels were given to each subject in a random order. Energy intakes were constant for each individual and were sufficient to maintain his body weight (mean $\pm$ SD = 44.6 ± 2.4 kcal/kg/day ranging from 38.4 to 49.8 kcal/kg/day).

All urine was collected and the average nitrogen excretion in the last five days

at each protein level was used for calculating the nitrogen balance. Feces in the last eight days of each test period were pooled. True digestibility was calculated at each level of protein intake using the value of 12.4 mg N/kg for metabolic fecal nitrogen (3). The nitrogen balance response to nitrogen intake was observed for each individual and the regression equation relating nitrogen balance to nitrogen intake was calculated from pooled data together with the 97.5% confidence intervals. The minimum nitrogen requirement was estimated from the regression line. Net protein utilization (NPU) at nitrogen equilibrium was also calculated. The nutritional value of soy protein isolate was estimated from the slope of the nitrogen balance response curve and compared with that for fish protein.

Body weight was measured daily at 7:00 a.m. with minimal clothing after voiding urine and before breakfast. Total nitrogen, creatinine (4), urea (5), ammonia (5), uric acid (6), sodium and potassium in urine samples were measured. Blood was drawn from an antecubital vein at 6:30 a.m. after an overnight fast of about 10 hr on the last days of the adjustment period and of each period on low protein diet. Routine hematological analyses (erythrocyte and leucocyte counts, hemoglobin and hematocrit) were carried out, and total protein (7), albumin (8), urea (5), SGOT (9), SGPT (9), glucose (10), triglyceride (11), total cholesterol (12), sodium and potassium in plasma were also measured. Anthropometric measurements to detect possible physical changes, such as measurements of the circumference of the chest, waist and mid-upper arm and skin-fold thicknesses of the subscapular, suprailiac, triceps and biceps areas were conducted at the ends of the adjustment period and each period on low protein diet.

RESULTS

Date on nitrogen balance with the three proteins are summarized in Table 5. Fecal nitrogen did not increase appreciably with increase in soy protein or fish protein intake and therefore the two proteins were almost completely digested. Nitrogen balance for soy protein was significantly lower than that for fish protein ($p < 0.05$) at the same protein intakes of 0.45, 0.55 and 0.65 g protein/kg/day. There were no significant differences between the nitrogen balances of fish protein and mixed protein at any level of nitrogen intake tested.

Individual nitrogen balance data were plotted against nitrogen intake in Figs. 2, 3 and 4. From these results, regression equations relating nitrogen balance to nitrogen intake for individual subjects were calculated and are given in Table 6. Nitrogen balance in subject K given fish protein was inversely related to nitrogen intake and so these data were omitted from further analyses. The slopes of regression lines showed wide individual ranges of 0.210–0.396 for soy protein, 0.158–0.461 for fish protein and 0.171–0.620 for mixed protein. The regression lines obtained from pooled nitrogen balance data are shown in Fig. 5. The slope for soy protein (0.298) was smaller than those for fish protein (0.365) and mixed protein (0.423), but the differences were not statistically significant.

Table 5. Summary of nitrogen balance data for young men given different levels of soy protein isolate, fish protein and a mixture containing 50% soy protein isolate and 50% fish protein.

Protein source	Protein level (g/kg)	Body weight (kg)	Energy intake (kcal/kg/day)	Nitrogen balance				Digestibility ^c
				Intake N	Urinary N ^a	Fecal N ^b	N balance	
SPI	0.35 (5) ^d	65.6 ± 10.9	44.2 ± 2.1	56.0 ± 0.1	63.6 ± 4.0	10.6 ± 2.3	-18.3 ± 3.8	100
	0.45 (5)	65.6 ± 10.9	45.6 ± 1.7	72.0 ± 0.1	75.0 ± 4.2	10.6 ± 3.2	-13.5 ± 6.2 ^e	100
	0.55 (5)	65.6 ± 10.9	45.9 ± 1.9	88.1 ± 0.1	86.0 ± 2.8	12.1 ± 2.8	-10.1 ± 5.4 [*]	100
	0.65 (5)	65.6 ± 10.9	44.5 ± 1.8	104.0 ± 0.1	95.0 ± 3.2	12.6 ± 2.8	-3.5 ± 3.3 [*]	99.8
Fish	0.35 (6)	60.8 ± 3.4	45.1 ± 1.9	55.9 ± 0.5	56.6 ± 6.9	12.2 ± 1.6	-12.9 ± 7.5	100
	0.45 (7)	62.2 ± 5.1	43.8 ± 2.7	71.3 ± 0.9	64.4 ± 4.6	11.7 ± 1.5	-4.8 ± 5.1	100
	0.55 (6)	63.0 ± 4.6	44.4 ± 2.7	87.1 ± 0.5	72.3 ± 4.5	12.9 ± 2.9	+2.0 ± 6.2	99.6
	0.65 (7)	62.3 ± 4.4	43.5 ± 2.2	102.7 ± 0.7	85.4 ± 5.6	12.9 ± 1.5	+4.4 ± 6.3	99.5
SPI + Fish	0.35 (8)	60.9 ± 4.7	44.7 ± 2.5	55.6 ± 0.6	56.3 ± 6.1	12.9 ± 2.3	-13.6 ± 7.2	99.1
	0.45 (8)	60.9 ± 4.6	44.7 ± 2.4	71.5 ± 0.5	67.6 ± 4.9	12.9 ± 2.0	-9.0 ± 5.7	99.3
	0.55 (8)	61.1 ± 4.6	44.1 ± 3.4	87.0 ± 0.5	76.8 ± 8.4	13.7 ± 2.7	-3.5 ± 8.3	98.5
	0.65 (8)	61.2 ± 4.6	44.7 ± 2.4	102.7 ± 0.4	82.4 ± 6.9	13.4 ± 2.2	+6.9 ± 7.5	99.0

^a Values are means for the last five days at each protein level. ^b Mean fecal nitrogen loss in the last eight days at each protein level.^c True digestibility was calculated using a value of 12.4 mg N/kg for fecal metabolic nitrogen. ^d Numbers in parentheses are numbers of subjects. ^e Significantly different from fish protein. * $p < 0.05$.

Fig. 2. Relationship between nitrogen intake and nitrogen balance of individual subjects fed soy protein isolate. Subj. M (○), Subj. N (●), Subj. O (×), Subj. P (△), Subj. Q (▲).

Fig. 3. Relationship between nitrogen intake and nitrogen balance of individual subjects fed fish protein. Subj. A (○), Subj. B (●), Subj. C (×), Subj. D (△), Subj. E (▲), Subj. F (□), Subj. G (■).

The mean nitrogen intake for apparent nitrogen equilibrium (intercept of the lines for nitrogen intake with nitrogen equilibrium) determined from individual regression lines was 120.5 mg N/kg (range 101–138) for soy protein, which was significantly higher than 87.7 mg N/kg (range 66–104) for fish protein (Table 6). There was no significant difference between the maintenance nitrogen intakes of fish protein and mixed protein. The coefficient of variation of the maintenance nitrogen intake was 13.3% for soy protein, 16.5% for fish protein and 22.9% for mixed protein. The maintenance nitrogen levels obtained from pooled data, that is, 118.1 mg N/kg for soy protein, 87.1 mg N/kg for fish protein and 90.5 mg N/kg for mixed protein, were similar to those obtained from individual data (Table 6). The protein

Fig. 4. Relationship between nitrogen intake and nitrogen balance of individual subjects fed a mixture of 50% SPI and 50% fish protein. Subj. A (○), Subj. B (●), Subj. C (×), Subj. D (△), Subj. E (▲), Subj. H (⊙), Subj. I (▽), Subj. J (▼).

Fig. 5. Regression lines for soy protein isolate, fish protein, and a mixture of soy protein and fish protein.

- × --- ×, soy protein isolate $Y = 0.298X - 35.2$ ($n = 20$)
Maintenance = 118.1 ± 15.4 mg N/kg/day
- △ ----- △, fish protein $Y = 0.365X - 31.8$ ($n = 26$)
Maintenance = 87.1 ± 17.2 mg N/kg/day
- ——— ○, 50% soy protein isolate + 50% fish protein $Y = 0.423X - 38.3$ ($n = 32$)
Maintenance = 90.5 ± 17.1 mg N/kg/day

allowance, estimated from the intersection of the upper 97.5% confidence band with the zero balance line, was 166 mg N/kg for soy protein, 128 mg N/kg for fish protein and 130 mg N/kg for mixed protein (Figs. 2, 3 and 4).

NPU's calculated at the respective maintenance nitrogen levels from individual

Table 6. Summary of maintenance nitrogen values and regression equations relating nitrogen balance to nitrogen intake.

Soy protein isolate			Fish		50% SPI + 50% Fish		
Subject	Regression equation ^a	Main-tenance ^b	Subject	Regression equation	Main-tenance	Subject	Regression equation
Individual data based on apparent nitrogen balance							
M	$Y=0.396X-40.0$	101.0	A	$Y=0.320X-21.2$	66.3	A	$Y=0.577X-51.3$
N	$Y=0.210X-25.9$	123.3	B	$Y=0.430X-40.4$	94.0	B	$Y=0.249X-30.8$
O	$Y=0.320X-44.1$	137.8	C	$Y=0.322X-29.4$	88.6	C	$Y=0.528X-49.4$
P	$Y=0.345X-37.0$	107.2	D	$Y=0.461X-33.4$	72.5	D	$Y=0.399X-25.2$
Q	$Y=0.218X-29.0$	133.0	E	$Y=0.158X-16.3$	103.2	E	$Y=0.620X-54.7$
			F	$Y=0.445X-46.4$	104.3	H	$Y=0.405X-36.3$
			G	$Y=0.363X-30.8$	84.8	I	$Y=0.435X-36.3$
			K ^c	$Y=-0.274X+0.03$	—	J	$Y=0.171X-22.3$
Mean	0.298 $\bar{X}$	120.5**	Mean	0.357 $\bar{X}$	87.7	Mean	0.423 $\bar{X}$
S.D.	0.081	7.6	S.D.	0.105	14.5	S.D.	0.155
C.V. ^d	27.2	13.3	C.V.	29.4	16.5	C.V.	36.6
Pooled data based on apparent nitrogen balance							
	$Y=0.298X-35.2$			$Y=0.365X-31.8$			$Y=0.423X-38.3$
	($n=20$, $r=+0.774$)			($n=26$, $r=+0.727$)			($n=32$, $r=+0.727$)
	Maintenance: 118.1 ± 15.4*			Maintenance: 87.1 ± 17.2			Maintenance: 90.5 ± 17.1
	NPU = 38.9			NPU = 52.8			NPU = 50.8

^a Y, nitrogen balance (mg/kg/day); X, nitrogen intake (mg/kg/day). ^b Nitrogen intake for maintenance of nitrogen equilibrium (mg/kg/day). ^c K, omitted from group analyses. ^d Coefficient of variation. * Significantly different from maintenance intake of fish protein. * $p < 0.05$.

data were 38.7 ± 5.3 for soy protein, 53.7 ± 9.6 for fish protein and 50.6 ± 11.5 for mixed protein (Table 6). The NPUs obtained from pooled data were similar to those obtained from individual data. Values for the protein quality of soy protein isolate relative to fish protein estimated from pooled data were 82% (0.298 mg N/kg vs 0.365 mg N/kg), 74% (118.1 vs 87.1) and 74% (38.9 vs 52.8) based on the regression coefficients ("slope ratios"), nitrogen requirements ("maintenance intakes") and NPU, respectively.

Values determined in anthropometric, blood and urine analyses were not significantly affected by differences in the dietary protein source (Appendices 1, 2, 3, 4 and 5).

DISCUSSION

In this study we examined the nutritional quality of well processed soy protein product by giving it to young men at four protein levels within the submaintenance-to-maintenance range and evaluated the nutritive value of soy protein isolate relative to fish protein by various methods based on nitrogen balance data. The digestibilities of soy protein isolate and fish protein were both very high, being about 100%, which is comparable with that of egg protein (13). Since the digestibility of cooked soybean was reported to be 92% (14), isolation of soy protein improved its digestibility. The slope of the regression line relating nitrogen balance to nitrogen intake was 0.298 for pooled data on soy protein as compared with 0.365 for those on fish protein. The slope for soy protein was less than that for egg protein (0.411) estimated previously (13). Using the slope ratio method, the relative nutritive value of soy protein was estimated to be 82% and 73% of the values of fish and egg proteins, respectively. However, the NPU of soy protein at maintenance nitrogen intake, that is 38.9, corresponded to 74% of that for fish protein (NPU = 52.8) and 76% of that for egg protein (NPU = 51.1). Young *et al.* (15) evaluated the protein quality of soy protein as 87% and 77% of that of egg protein from the slope of the regression line based on pooled and individual data, respectively. Thus the quality of the soy protein isolate used in this study roughly corresponded to 80% of that of fish protein and egg protein.

The maintenance nitrogen requirement for apparent nitrogen equilibrium obtained from the regression equation of pooled data was 118 ± 15 mg N/kg/day for soy protein isolate and 87.1 ± 17.2 mg N/kg/day for fish protein. There was no significant difference between the requirement of fish protein determined in this study and that of egg protein (90.1 ± 14.1 mg N/kg/day) reported previously (13), but the soy protein requirement was higher than that of egg protein. From these figures, the relative nitrogen requirement of soy protein isolate was calculated as 74% and 76% of the requirements of fish and egg proteins, respectively. Vemury *et al.* (16) observed a lower nitrogen balance with soy protein concentrate than with egg and beef at nitrogen intakes of 4.0 g N/day of test proteins. Kies and Fox (17) found on the basis of nitrogen balance data that although the nutritional value of

textured soy protein was inferior to that of beef at 4 g nitrogen intake, those of the two proteins were similar at 8 g nitrogen intake per day. Zezulka and Calloway (18) reported that the average nitrogen balances obtained with soy protein isolate were -1.21 , -0.08 , $+0.26$ and $+0.79$ g nitrogen per day at 3.0, 4.5, 6.0 and 7.5 g soy nitrogen intake per day, respectively, with a total nitrogen intake of 9 g/day adjusted by adding non-specific nitrogen in the form of a mixture of glycine and alanine in isonitrogenous amounts. The maintenance nitrogen intake obtained from their nitrogen balance data is considerably lower than that obtained in this study, possibly because of the difference in the total nitrogen intake. Young *et al.* (15) observed a similar value to ours for maintenance nitrogen intake of soy protein isolate (judging by inspection of their figure for nitrogen balance (15); Fig. 5, p. 114). They also calculated the relative nitrogen requirement of soy protein isolate as 80% of the egg protein requirement, which is similar to our value of about 75% described above.

The lower quality of soy protein relative to fish or egg protein may be due to its lower content of sulfur amino acids. Kies and Fox (17) and Zezulka and Calloway (18) observed an improvement of the nitrogen balance by adding methionine to soy protein at low nitrogen intake. Young *et al.* (15) observed a similar effect when 0.6% or 1.1% methionine was added to soy protein at 0.51 g protein/kg/day. But they observed a negative effect when 1.6% methionine was added to 0.51 g soy protein/kg/day, although a positive effect of 1.6% methionine was still observed at 0.8 g soy protein/kg/day. In the present study, we examined nitrogen utilization with soy-fish combination instead of methionine supplementation. Soy protein in combination with an isonitrogenous amount of fish protein had essentially the same nutritive value as fish protein alone judging from data on nitrogen balance, the slope of the regression line, the maintenance nitrogen intake and the NPU. Kies and Fox (17) and Young *et al.* (15) investigated the protein quality of combinations of soy and beef in ratios of 4:0, 3:1, 2:2, 1:3 and 0:4. Kies and Fox (17) obtained a linear increase in nitrogen balance with increase in the ratio of beef to soy at 4.8 g nitrogen per day per person, but Young *et al.* (15) observed no differences in the nitrogen balance with the same combinations of beef and soy protein at 0.6 g protein/kg body weight/day. Thus, the nutritional quality of soy protein may depend on the level of protein intake as well as the individual soy protein product.

It is concluded from the present study and the above discussion that soy protein isolate has a nutritive value of about 80% of that of fish or egg protein and is a good source of protein for human consumption. However, long-term studies like those of Young *et al.* (15) should be carried out on a wide variety of subjects to detect possible adverse effects of this protein source.

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Appendix 1. Basal metabolism and anthropometric data.

	Protein source	Control	Protein level (g/kg)			
			0.35	0.45	0.55	0.65
Basal metabolism (kcal/kg)	SPI (5)	24.2 ± 0.8	—	—	—	—
	Fish (8)	24.0 ± 2.1	23.4 ± 1.5	23.1 ± 2.0	22.7 ± 2.3	22.9 ± 1.4
	Mix. (8)	24.4 ± 2.0	22.7 ± 0.9	23.9 ± 1.2	24.1 ± 1.2	23.4 ± 1.0
Chest circum. (cm)	SPI (5)	92.3 ± 4.7	91.4 ± 4.2	91.9 ± 3.8	92.1 ± 4.9	92.0 ± 5.2
	Fish (8)	88.6 ± 4.8	88.2 ± 4.5	88.3 ± 4.7	88.8 ± 4.3	88.7 ± 4.7
	Mix. (8)	87.6 ± 4.6	87.2 ± 3.9	87.8 ± 4.2	87.3 ± 4.9	87.0 ± 4.9
Waist circum. (cm)	SPI (5)	75.3 ± 6.0	75.1 ± 4.9	75.3 ± 5.2	75.2 ± 5.1	75.9 ± 5.6
	Fish (8)	72.8 ± 6.2	72.8 ± 5.9	72.7 ± 5.6	72.4 ± 5.3	73.0 ± 5.2
	Mix. (8)	71.7 ± 4.1	72.1 ± 4.9	72.8 ± 4.6	71.6 ± 4.4	71.4 ± 3.9
Mid-upper arm circum. (cm)	SPI	—	—	—	—	—
	Fish (8)	26.0 ± 1.4	25.9 ± 1.5	25.8 ± 1.5	25.6 ± 1.3	25.7 ± 1.4
	Mix. (8)	26.0 ± 1.1	25.5 ± 1.1	25.5 ± 1.1	25.7 ± 1.3	25.6 ± 1.3
Skin fold (mm)						
Subscapular	SPI (5)	9.6 ± 3.2	10.2 ± 3.3	10.4 ± 3.8	10.1 ± 3.2	10.3 ± 3.0
	Fish (8)	11.9 ± 3.8	12.7 ± 4.4	13.0 ± 4.3	12.2 ± 3.0	12.4 ± 4.1
	Mix. (8)	9.9 ± 3.5	9.3 ± 2.9	9.5 ± 3.3	9.6 ± 3.5	9.2 ± 3.0
Suprailiac	SPI	—	—	—	—	—
	Fish (8)	18.9 ± 7.2	19.4 ± 8.0	20.0 ± 8.5	20.5 ± 7.8	19.7 ± 8.4
	Mix. (8)	14.1 ± 4.3	14.3 ± 5.3	14.3 ± 5.6	14.6 ± 5.4	14.0 ± 5.2
Triceps	SPI (5)	7.7 ± 1.2	6.8 ± 1.3	7.5 ± 1.3	7.1 ± 1.6	7.7 ± 1.7
	Fish (8)	9.1 ± 3.7	9.4 ± 4.8	9.2 ± 4.2	9.6 ± 3.4	9.2 ± 2.6
	Mix. (8)	7.7 ± 2.7	7.2 ± 2.3	7.4 ± 2.3	7.4 ± 2.6	7.1 ± 1.6
Biceps	SPI	—	—	—	—	—
	Fish (8)	4.9 ± 1.8	5.3 ± 2.2	5.0 ± 1.9	4.8 ± 2.0	4.9 ± 1.9
	Mix. (8)	4.1 ± 1.7	3.6 ± 1.3	3.6 ± 1.5	4.2 ± 1.7	4.0 ± 1.4

Appendix 2. Blood analysis (1).

	Protein source	Control	Protein level (g/kg)			
			0.35	0.45	0.55	0.65
RBC counts ($\times 10^4/\text{mm}^3$)	SPI (5)	505 $\pm$ 18	492 $\pm$ 24	488 $\pm$ 25	483 $\pm$ 10	491 $\pm$ 42
	Fish (7)	438 $\pm$ 18	438 $\pm$ 15	435 $\pm$ 16	428 $\pm$ 17	437 $\pm$ 17
	Mix. (8)	441 $\pm$ 23	429 $\pm$ 23	423 $\pm$ 21	426 $\pm$ 17	424 $\pm$ 23
WBC counts ($\times 10^3/\text{mm}^3$)	SPI (5)	6.90 $\pm$ 2.10	6.20 $\pm$ 2.80	5.70 $\pm$ 1.30	5.90 $\pm$ 2.00	5.80 $\pm$ 1.80
	Fish (7)	5.65 $\pm$ 1.30	5.16 $\pm$ 1.13	4.82 $\pm$ 1.13	4.83 $\pm$ 0.83	4.77 $\pm$ 1.24
	Mix. (8)	5.18 $\pm$ 1.03	4.95 $\pm$ 0.85	5.09 $\pm$ 0.98	5.47 $\pm$ 0.99	5.45 $\pm$ 0.94
Hemoglobin (g/100 ml)	SPI (5)	15.4 $\pm$ 0.8	15.2 $\pm$ 0.7	14.8 $\pm$ 0.8	14.7 $\pm$ 0.7	14.9 $\pm$ 1.1
	Fish (7)	16.1 $\pm$ 0.5	16.0 $\pm$ 0.5	15.8 $\pm$ 0.5	15.5 $\pm$ 0.8	15.7 $\pm$ 0.3
	Mix. (8)	16.3 $\pm$ 1.3	15.7 $\pm$ 0.7	15.4 $\pm$ 0.6	15.7 $\pm$ 0.8	15.6 $\pm$ 0.5
Hematocrit	SPI (5)	44.8 $\pm$ 1.5	43.5 $\pm$ 2.4	42.5 $\pm$ 2.6	42.4 $\pm$ 2.3	43.0 $\pm$ 3.4
	Fish (7)	45.8 $\pm$ 1.7	45.7 $\pm$ 1.6	45.0 $\pm$ 1.3	44.6 $\pm$ 2.3	45.0 $\pm$ 1.2
	Mix. (8)	45.7 $\pm$ 3.0	44.5 $\pm$ 1.5	43.7 $\pm$ 1.4	44.3 $\pm$ 2.1	43.9 $\pm$ 1.4

Appendix 3. Blood analysis (2).

	Protein source	Control	Protein level (g/kg)			
			0.35	0.45	0.55	0.65
Total protein (g/100 ml)	SPI (5)	7.70 $\pm$ 0.30	7.80 $\pm$ 0.30	7.70 $\pm$ 0.40	7.80 $\pm$ 0.30	7.80 $\pm$ 0.50
	Fish (7)	7.10 $\pm$ 0.56	6.90 $\pm$ 0.45	6.79 $\pm$ 0.36	6.54 $\pm$ 0.23	6.76 $\pm$ 0.47
	Mix. (8)	7.05 $\pm$ 0.42	6.55 $\pm$ 0.31	6.48 $\pm$ 0.38	6.49 $\pm$ 0.32	6.50 $\pm$ 0.38
Albumin (g/100 ml)	SPI (5)	4.80 $\pm$ 0.20	4.80 $\pm$ 0.20	4.70 $\pm$ 0.20	4.80 $\pm$ 0.20	4.80 $\pm$ 0.30
	Fish (7)	4.28 $\pm$ 0.28	4.34 $\pm$ 0.25	4.21 $\pm$ 0.28	4.16 $\pm$ 0.42	4.27 $\pm$ 0.36
	Mix. (8)	4.47 $\pm$ 0.39	4.27 $\pm$ 0.32	4.25 $\pm$ 0.22	4.21 $\pm$ 0.23	4.26 $\pm$ 0.17
Urea-N (mg/100 ml)	SPI (5)	17.5 $\pm$ 5.5	12.6 $\pm$ 3.8	12.8 $\pm$ 5.3	12.8 $\pm$ 4.0	14.3 $\pm$ 4.2
	Fish (7)	10.4 $\pm$ 2.0	4.6 $\pm$ 1.6	4.8 $\pm$ 2.5	4.7 $\pm$ 2.0	7.0 $\pm$ 3.4
	Mix. (8)	10.2 $\pm$ 2.2	3.7 $\pm$ 1.8	4.3 $\pm$ 1.4	4.9 $\pm$ 2.2	5.3 $\pm$ 1.3
GOT (Karmen U.)	SPI (5)	15 $\pm$ 4	14 $\pm$ 3	14 $\pm$ 3	13 $\pm$ 3	14 $\pm$ 4
	Fish (7)	16 $\pm$ 5	11 $\pm$ 5	13 $\pm$ 9	12 $\pm$ 2	13 $\pm$ 5
	Mix. (8)	15 $\pm$ 4	13 $\pm$ 9	14 $\pm$ 8	12 $\pm$ 6	12 $\pm$ 6
GPT (Karmen U.)	SPI (5)	11 $\pm$ 4	10 $\pm$ 3	12 $\pm$ 2	10 $\pm$ 3	11 $\pm$ 4
	Fish (7)	8 $\pm$ 6	8 $\pm$ 7	9 $\pm$ 9	6 $\pm$ 4	8 $\pm$ 6
	Mix. (8)	9 $\pm$ 4	7 $\pm$ 9	7 $\pm$ 6	8 $\pm$ 9	8 $\pm$ 10

Appendix 4. Blood analysis (3).

	Protein source	Control	Protein level (g/kg)			
			0.35	0.45	0.55	0.65
Glucose (mg/100 ml)	SPI (5)	—	—	—	—	—
	Fish (7)	84.4 ± 5.9	94.4 ± 9.4	88.5 ± 6.4	87.8 ± 7.8	88.1 ± 10.5
	Mix. (8)	77.8 ± 5.9	87.1 ± 5.4	86.9 ± 8.3	85.5 ± 6.1	85.0 ± 5.1
Triglyceride (mg/100 ml)	SPI (5)	222 ± 134	166 ± 88	132 ± 76	134 ± 54	140 ± 77
	Fish (7)	95 ± 21	90 ± 23	93 ± 22	96 ± 17	90 ± 18
	Mix. (8)	93 ± 12	90 ± 21	97 ± 15	97 ± 23	85 ± 13
Total cholesterol (mg/100 ml)	SPI (5)	178 ± 20	151 ± 21	152 ± 29	150 ± 23	146 ± 13
	Fish (7)	144 ± 14	147 ± 13	141 ± 15	136 ± 8	142 ± 21
	Mix. (8)	145 ± 23	127 ± 11	130 ± 16	136 ± 16	131 ± 6
Na (mEq/liter)	SPI (5)	143 ± 1	142 ± 1	142 ± 2	142 ± 2	142 ± 2
	Fish (7)	142 ± 5	138 ± 3	135 ± 4	136 ± 4	137 ± 5
	Mix. (8)	140 ± 2	137 ± 3	135 ± 4	136 ± 6	138 ± 4
K (mEq/liter)	SPI (5)	4.2 ± 0.5	4.1 ± 0.3	4.1 ± 0.3	4.2 ± 0.2	4.0 ± 0.3
	Fish (7)	4.2 ± 0.5	3.9 ± 0.3	3.9 ± 0.3	3.9 ± 0.5	3.8 ± 0.2
	Mix. (8)	4.5 ± 0.8	4.0 ± 0.4	4.1 ± 0.8	4.0 ± 0.3	4.0 ± 0.3

Appendix 5. Urinalysis.

	Protein source		Protein level (g/kg)			
			0.35	0.45	0.55	0.65
Creatinine (g/day)	SPI (5)		1.57 ± 0.25	1.59 ± 0.23	1.59 ± 0.22	1.62 ± 0.25
	Fish (7)		1.56 ± 0.12	1.58 ± 0.09	1.61 ± 0.12	1.59 ± 0.13
	Mix. (8)		1.56 ± 0.08	1.57 ± 0.09	1.58 ± 0.08	1.59 ± 0.09
Urea-N (g/day)	SPI (5)		3.06 ± 0.61	3.72 ± 0.65	4.32 ± 0.76	4.79 ± 0.85
	Fish (7)		2.10 ± 0.47	2.61 ± 0.44	3.05 ± 0.54	3.82 ± 0.36
	Mix. (8)		2.09 ± 0.40	2.72 ± 0.37	3.26 ± 0.51	3.58 ± 0.49
Ammonia-N (mg/day)	SPI (5)		270 ± 60	300 ± 70	340 ± 80	350 ± 70
	Fish (7)		290 ± 83	317 ± 55	329 ± 62	337 ± 44
	Mix. (8)		284 ± 76	302 ± 57	308 ± 77	312 ± 76
Uric acid (mg/day)	SPI (5)		500 ± 60	550 ± 90	570 ± 90	620 ± 110
	Fish (7)		483 ± 50	494 ± 61	500 ± 32	514 ± 27
	Mix. (8)		439 ± 67	483 ± 69	526 ± 95	527 ± 45
Na (g/day)	SPI (5)		—	—	—	—
	Fish (7)		3.07 ± 0.53	3.35 ± 0.52	3.64 ± 0.42	3.91 ± 0.39
	Mix. (8)		2.79 ± 0.47	3.27 ± 0.62	3.39 ± 0.47	3.60 ± 0.71
K (g/day)	SPI (5)		—	—	—	—
	Fish (7)		1.04 ± 0.27	1.13 ± 0.37	1.15 ± 0.39	1.23 ± 0.42
	Mix. (8)		1.01 ± 0.31	1.02 ± 0.33	1.04 ± 0.29	1.04 ± 0.35