Utilization of Soy Protein Isolate Mixed with Rice Protein in Japanese Women

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(Received July 20, 1984)

Summary Utilization and requirement of soy protein isolate (SPI) and SPI-rice combination were examined in twenty-five female students. After 1 day on protein-free diet, each subject received a low-protein diet for 10 days. The protein sources were SPI for ten subjects and SPI-rice combination, in which the ratio of two proteins was 6:4, for fifteen subjects. The nitrogen intakes were about 45, 65 and 85 mg/kg in both the two series of experiments. Energy intake was at an approximate maintenance level of 36.1 ± 3.0 kcal/kg.

Apparent nitrogen balance improved with increase in nitrogen intake. The regression equations between nitrogen intake (X, mg/kg) and balance (Y, mg/kg) are shown in the following:

SPI: \[ Y = 0.411X - 40.8 \quad (n = 10, \quad r = 0.812) \]

SPI and rice protein: \[ Y = 0.392X - 32.7 \quad (n = 15, \quad r = 0.739) \]

From the above equations, the maintenance intakes of SPI and SPI-rice combination for an apparent nitrogen equilibrium were calculated to be 99 and 83 mg N/kg, respectively. Digestibilities were 98.2 ± 5.0% for SPI and 93.1 ± 6.1% for SPI-rice combination. The NPUs of SPI at intake levels of 40, 60 and 80 mg N/kg were 47 ± 24 (n=4), 49 (n=2) and 44 ± 3 (n=4), respectively. The NPUs of SPI and rice mixed protein at intake levels of 45, 70 and 90 mg N/kg were 67 ± 13 (n=5), 51 ± 7 (n=5) and 54 ± 12 (n=5), respectively. It was concluded from the present study that both SPI and the SPI-rice combination had a high nutritive efficiency comparable with that of egg protein.

Key Words soy protein isolate, rice protein, soy-rice mixed protein, nitrogen requirement, NPU, slope ratio method

Rice and soy bean are both important traditional food items in Japan. In 1981, average protein intakes from soy bean (containing its products, tofu, miso etc.) and rice were found to be 7.1 and 13.4 g/person/day, respectively (I). In recent years, new soy bean products have been introduced, such as soy bean flours, concentrates

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and isolates by advanced food technology. As the usage of these products has been increasing in a variety of food industries, the actual intake of soy bean protein is thought to be higher than formerly. The position of soy protein in the protein nutrition of not only Japanese but also the people in other advanced countries will become increasingly important in the future.

The nutritional value of soy protein isolate (SPI) and its minimum requirement for apparent nitrogen equilibrium has been reported by many investigators (2-6). According to them, SPI has a high nutritive value, about 80% of that of egg protein. The slightly lower quality of soy protein relative to egg was supposed to be due to its low content of sulfur-containing amino acids. The improvement of the protein quality by adding methionine to soy protein (2, 4, 5) or combinations of soy and beef (2, 4) and soy and fish (6) was investigated. As rice protein contains methionine in significant quantities, it is thought that rice protein can effectively supplement soy protein.

In the present study, we examined nitrogen utilization of SPI and SPI-rice combination, and compared it with that of egg protein reported previously (7).

EXPERIMENTAL

Twenty-five female students aged 18 to 22 years participated in two series of experiments using soy protein isolate (SPI) and SPI-rice combination as protein source. Table 1 summarizes the characteristics of the subjects. During the experiments they lived in a metabolic unit and continued their usual activities.

After 1 day on protein-free diet, each subject received a low-protein diet for 10 days. The protein sources were soy protein isolate (Fujipro R; Fuji Oil Co., Osaka, Japan) for ten subjects and soy protein isolate and rice for fifteen subjects. The ratio of the two proteins was 6:4 and the amino acid score of the mixed protein was calculated as 91. The powdery product of the soy protein isolate was prepared as cookies with corn starch, sugar and shortening. Polished rice was cooked with water. In the SPI-rice combination experiment, one third of the daily intake of SPI or rice was given in each of the three meals. An example of the dietary composition is shown in Table 2. Energy was supplied mainly as corn starch, sucrose and shortening. Dietary fat supplied about 25% of the total energy intake. When polished rice was included in the experimental diet, equal amounts of fat and energy were substituted from partly shortening, corn starch and sugar. The nitrogen intakes were about 45, 65 and 85 mg/kg in both experiments. Approximate maintenance energy intake was determined individually by detailed inquiry about the food consumed before the experiment. When the body weight of a subject decreased or increased distinctly during the early stage of the experimental period, a certain amount of sucrose was added to or substituted for in the diet.

Throughout the experiment subjects were weighed daily before breakfast after voiding urine. Urine was collected every 24 h and feces in the low-protein diet period were pooled. The total nitrogen contents of SPI, rice, urine and feces were analyzed.
Table 1. Characteristics of subjects.

| Subj. code | Age (yr) | Height (cm) | Weight<sup>a</sup> (kg) | BMR<sup>b</sup> (kcal/kg) | Fat<sup>c</sup> (%) | LBM<sup>d</sup> (kg) |
|------------|----------|-------------|--------------------------|---------------------------|-------------------|----------------|
| Soy protein isolate |
| 01 | 19 | 164 | 52.2 | 25.7 | 21.5 | 40.6 |
| 02 | 20 | 147 | 51.7 | 23.2 | 28.2 | 38.4 |
| 03 | 19 | 155 | 50.8 | 20.8 | 29.0 | 36.2 |
| 04 | 20 | 160 | 55.3 | 24.9 | 30.3 | 40.4 |
| 05 | 20 | 154 | 59.4 | 19.7 | 31.7 | 39.8 |
| 06 | 20 | 152 | 55.7 | 20.6 | 25.6 | 40.5 |
| 07 | 20 | 152 | 48.4 | 24.4 | 29.0 | 35.0 |
| 08 | 19 | 157 | 49.6 | 21.5 | 24.9 | 37.4 |
| 09 | 19 | 155 | 60.2 | 22.0 | 32.6 | 40.8 |
| 10 | 19 | 157 | 58.5 | 16.6 | 34.5 | 38.6 |
| Mean | 19.5 | 155.5 | 54.2 | 21.9 | 28.7 | 38.4 |
| SD | 0.5 | 4.5 | 4.2 | 2.7 | 3.9 | 2.0 |

| Soy protein isolate and rice |
|-----------------------------|
| 11 | 19 | 169 | 57.9 | 19.3 | 22.2 | 43.5 |
| 12 | 19 | 160 | 56.8 | 21.6 | 26.7 | 40.6 |
| 13 | 20 | 156 | 47.2 | 19.3 | 22.7 | 35.8 |
| 14 | 22 | 165 | 55.0 | 23.5 | 22.5 | 44.0 |
| 15 | 21 | 160 | 58.0 | 17.5 | 32.7 | 39.4 |
| 16 | 19 | 154 | 48.7 | 21.6 | 24.2 | 36.1 |
| 17 | 19 | 160 | 52.9 | 20.2 | 23.9 | 39.7 |
| 18 | 19 | 163 | 51.7 | 22.4 | 25.5 | 37.7 |
| 19 | 19 | 169 | 57.2 | 19.6 | 22.2 | 43.5 |
| 20 | 18 | 157 | 51.7 | 22.9 | 28.9 | 36.0 |
| 21 | 20 | 149 | 59.7 | 18.4 | 19.6 | 46.8 |
| 22 | 20 | 157 | 56.4 | 24.1 | 25.1 | 40.5 |
| 23 | 21 | 160 | 50.2 | 24.8 | 17.8 | 40.1 |
| 24 | 18 | 160 | 60.6 | 22.9 | 26.9 | 42.9 |
| 25 | 21 | 160 | 58.0 | 17.5 | 32.7 | 39.4 |
| Mean | 19.7 | 159.9 | 54.8 | 21.0 | 24.9 | 40.4 |
| SD | 1.2 | 5.3 | 4.1 | 2.4 | 4.2 | 3.3 |

<sup>a</sup>Weight on the first day of the experimental period. <sup>b</sup>Measured by indirect calorimetry on one day during the experimental period. <sup>c</sup>Calculated from the body density measured hydrometrically using the equation suggested by Brožek et al. (1963). <sup>d</sup>Difference between body weight and body fat weight.

by the semimicro-Kjeldahl method. Urinary creatinine was determined by the method of Folin (8). Basal metabolic rate was measured by indirect calorimetry on one of the days during the experimental period. Body fat content was calculated from the body density measured hydrometrically by using the equation of Brožek et al. (9).
Table 2. Composition of the experimental diet.a

| Ingredient               | Intake (g/day) | Protein-free diet | SPI diet | SPI and rice diet |
|--------------------------|----------------|-------------------|----------|-------------------|
| SPI (Fujipro R)          | —              | 30                | 19       |                   |
| Rice, polished           | —              | —                 | 176      |                   |
| Corn starch              | 207            | 187               | 80       |                   |
| Sugar                    | 123            | 112               | 57       |                   |
| Shortening               | 48             | 48                | 46       |                   |
| Agar                     | 5              | 5                 | 5        |                   |
| Sodium chloride          | 3              | 3                 | 3        |                   |
| Vitamin mixtureb         | 2 tablets      | 2 tablets         | 2 tablets|                  |
| Mineral mixtureb         | 4              | 4                 | 4        |                   |

aIntake is given for a subject receiving 1,600 kcal of energy and 0.6 g/kg of protein. As well as the ingredients in this table, subjects consumed tea, green tea and lemon juice. bFor details, see reference (10).

Factors for the conversion of nitrogen values to protein (g/g N) used were 5.71 for SPI and 5.91 for rice.

RESULTS

Changes in body weight, urinary creatinine excretion, nitrogen intake, urinary and fecal nitrogen excretions and nitrogen balance of individual subjects are summarized in Table 3. Mean values of energy intake and energy intake per basal expenditure of all subjects were 36.1 ± 3.0 kcal/kg and 1.71 ± 0.20 kcal/kcal basal metabolism, respectively. Urinary creatinine excretion was constant during the experimental period in all subjects. Urinary nitrogen excretion decreased rapidly to a fairly steady level within a week. Thus, the mean of urinary nitrogen excretion in the last 4 days of the experimental period was used to estimate nitrogen balance. Apparent nitrogen balances were calculated from the nitrogen intake and fecal and urinary excretions. Nitrogen balance improved with the increase in nitrogen intake. Significant rectilinear relations were found between nitrogen intake (X, mg/kg) and balance (Y, mg/kg) as shown in Fig. 1. The regression equations are shown below:

SPI: \( Y = 0.411X - 40.8 \quad (n=10, \ r=0.812) \)

SPI and rice protein: \( Y = 0.392X - 32.7 \quad (n=15, \ r=0.739) \)

From the above equations, the maintenance intake of SPI and SPI-rice combination for an apparent nitrogen equilibrium were calculated to be 99 and 83 mg N/kg, respectively.

Net protein utilization (NPU) and digestibility were calculated using the obligatory urinary and fecal N losses determined in Japanese women (10). Mean values of digestibility were 98.2 ± 5.0% for SPI and 93.1 ± 6.1% for SPI and rice.

*J. Nutr. Sci. Vitaminol.*
Table 3. Energy intake, change in body weight, nitrogen balance and urinary creatinine excretion of individual subjects.

| Subj. code | Energy intake (kcal/kg) | Nitrogen intake (mg/kg) | Change in BW (kg/11 days) | Urinary creatinine\(a\) (mg/day) | Urinary N\(b\) (mg/kg) | Fecal N (mg/kg) | N balance (mg/kg) |
|------------|-------------------------|-------------------------|---------------------------|----------------------------------|-----------------------|----------------|------------------|
|            | Soy protein isolate     |                         |                           |                                  |                       |                |                  |
| 01         | 38.3                    | 41.5                    | -1.40                     | 1,006±83                        | 60.3                  | 13.4           | -31.3           |
| 02         | 38.7                    | 41.9                    | -2.03                     | 935±71                          | 63.8                  | 12.1           | -33.1           |
| 03         | 39.6                    | 40.0                    | -1.20                     | 831±77                          | 44.5                  | 10.4           | -14.0           |
| 04         | 32.8                    | 39.2                    | +0.05                     | 879±65                          | 52.7                  | 7.5            | -20.1           |
| Mean       | 37.4                    | 40.7                    | -1.15                     | 912                              | 55.3                  | 10.9           | -24.6           |
| SD         | 3.1                     | 1.3                     | 0.87                      | 75                               | 8.6                   | 2.5            | 9.1             |
| 05         | 33.7                    | 57.0                    | -1.10                     | 943±18                           | 67.2                  | 9.1            | -18.6           |
| 06         | 35.9                    | 60.7                    | -2.00                     | 1,010±32                        | 59.6                  | 13.3           | -11.3           |
| Mean       | 34.8                    | 58.9                    | -1.60                     | 977                              | 63.4                  | 11.2           | -15.0           |
| 07         | 36.7                    | 84.0                    | +0.65                     | 908±40                           | 83.8                  | 7.5            | -6.4            |
| 08         | 40.2                    | 81.9                    | -0.15                     | 925±52                           | 78.0                  | 14.9           | -10.1           |
| 09         | 33.5                    | 80.9                    | -0.50                     | 970±35                           | 80.2                  | 7.9            | -6.4            |
| 10         | 34.5                    | 83.4                    | -0.45                     | 981±43                           | 73.7                  | 15.6           | -5.3            |
| Mean       | 36.2                    | 82.6                    | -0.11                     | 946                              | 78.9                  | 11.5           | -7.1            |
| SD         | 3.0                     | 1.4                     | 0.53                      | 35                               | 4.2                   | 4.4            | 2.1             |
|            | Soy protein isolate and rice |                   |                           |                                  |                       |                |                  |
| 11         | 34.5                    | 46.0                    | -0.52                     | 1,009±64                         | 46.0                  | 12.5           | -11.4           |
| 12         | 35.5                    | 45.5                    | -1.42                     | 954±32                           | 41.3                  | 22.5           | -15.6           |
| 13         | 42.4                    | 47.1                    | +0.12                     | 921±30                           | 42.0                  | 15.1           | -6.8            |
| 14         | 36.4                    | 45.9                    | +2.73                     | 1,034±66                         | 56.9                  | 11.8           | -21.9           |
| 15         | 30.2                    | 43.5                    | +0.41                     | 879±40                           | 43.8                  | 10.7           | -10.1           |
| Mean       | 35.8                    | 45.6                    | +0.26                     | 959                              | 46.0                  | 14.5           | -13.2           |
| SD         | 4.4                     | 1.3                     | 1.55                      | 63                               | 6.4                   | 4.7            | 5.8             |
| 16         | 39.1                    | 69.8                    | -0.22                     | 861±20                           | 63.3                  | 15.1           | -7.5            |
| 17         | 36.0                    | 64.3                    | -0.73                     | 892±34                           | 66.2                  | 13.7           | -14.6           |
| 18         | 36.8                    | 65.8                    | -0.07                     | 969±26                           | 62.9                  | 14.4           | -10.5           |
| 19         | 35.7                    | 70.1                    | -0.56                     | 960±89                           | 57.9                  | 12.8           | +0.3            |
| 20         | 38.7                    | 65.7                    | -0.24                     | 817±132                          | 65.1                  | 12.9           | -11.3           |
| Mean       | 37.3                    | 67.1                    | -0.36                     | 900                              | 63.1                  | 13.8           | -8.7            |
| SD         | 1.6                     | 2.6                     | 0.27                      | 65                               | 3.2                   | 1.0            | 5.6             |
| 21         | 34.3                    | 89.1                    | -0.91                     | 959±31                           | 75.5                  | 15.4           | -1.0            |
| 22         | 35.4                    | 81.1                    | -2.93                     | 1,078±53                         | 65.6                  | 17.1           | +0.6            |
| 23         | 39.8                    | 91.1                    | -0.18                     | 1,021±23                         | 64.7                  | 15.1           | +12.3           |
| 24         | 34.7                    | 87.9                    | -2.35                     | 1,105±69                         | 85.8                  | 13.4           | -10.4           |
| 25         | 30.1                    | 91.8                    | +1.09                     | 871±33                           | 67.8                  | 13.7           | +12.2           |
| Mean       | 34.9                    | 88.2                    | -1.06                     | 1,007                            | 71.9                  | 14.9           | +2.7            |
| SD         | 3.5                     | 4.3                     | 1.63                      | 94                               | 8.9                   | 1.5            | 9.6             |

\(a\) Mean±SD of 11 days. \(b\) Average of the last 4 days of the experimental period.
mixed protein. The NPUs of SPI at intake levels of 40, 60 and 80 mg N/kg were obtained as 47 ± 24 (n = 4), 49 (= 2) and 44 ± 3 (n = 4), respectively. The NPUs of SPI and rice mixed protein at intake levels of 45, 70 and 90 mg N/kg were 67 ± 13 (n = 5), 51 ± 7 (n = 5) and 54 ± 12 (n = 5), respectively.

**DISCUSSION**

We examined the nutritional qualities of SPI and SPI-rice combinations at three protein levels within the submaintenance-to-maintenance range.

The digestibilities of SPI and SPI-rice mixed protein were 98 and 93%, respectively. Compared with the digestibility of cooked soy bean, 92% (11), the digestibility of SPI was very high, comparable with that of egg protein (7, 12). Other investigators (6, 13) have also reported high digestibility of SPI. However, the digestibility of SPI-rice mixed protein was slightly lower than that of SPI or egg, and was comparable with that of polished rice (91%) (11).

Protein quality of SPI evaluated in the present experiment was sufficiently high. The slope of the regression line relating nitrogen balance to nitrogen intake was 0.411 for SPI. It was higher than that for egg protein (0.325) estimated previously in female subjects (7), but the difference was not statistically significant. The maintenance nitrogen requirement for apparent nitrogen equilibrium obtained from the regression equation was 99 mg N/kg for SPI. It was a little higher than that of egg protein (91 mg N/kg) (7). The NPU of SPI at maintenance nitrogen intake was 43,
whereas that of egg protein was 47 (7). From these figures, the nutritional quality of SPI was calculated as at least 90% of that of egg protein. Wang et al. (6) reported the nutritive value of SPI was about 80% of that of egg protein from the slope ratio method, the NPU at maintenance nitrogen intake or from the maintenance requirement for apparent nitrogen equilibrium. Young et al. (2) evaluated the protein quality of SPI as 87 and 77% of that of egg protein from the slope of regression lines. Thus it is concluded from the present study and the above discussion that SPI has a nutritive value of at least 80 to 90% of that of egg protein in adult subjects.

Furthermore, SPI-rice combination achieved a high nutritive value comparable with that of egg protein. In Fig. 1, the two regression lines for egg and SPI-rice combination overlap. The nutritive values of SPI-rice mixed protein calculated from the slope of the regression line (0.392), the maintenance nitrogen requirement for apparent nitrogen equilibrium (83 mg N/kg) and the NPU at maintenance nitrogen intake (51) showed higher efficiency than egg protein. However, the deviations, the slopes or Y intercepts of the regression lines relating nitrogen balance to nitrogen intake were not significantly different from each other between SPI, SPI-rice combination and egg protein. In experiments on human subjects, unlike on laboratory animal (inbred defined strains), a wide difference in individuals is often observed. Therefore, interpretation of the experimental data should be considered carefully. Further precise investigations should be done to determine whether protein qualities of SPI, SPI-rice combination and egg protein are equivalent or not. For example, the same subject could be fed on a diet containing egg, SPI or SPI-rice combination as the protein source in turn at several intake levels, and nitrogen balances in each dietary period should be compared.

Wang et al. (6) observed that SPI-fish combinations had essentially the same nutritive values as fish protein alone judging from data on nitrogen balance, the slope of the regression line, the maintenance nitrogen intake and NPU. Kies and Fox (4) obtained an improvement in nitrogen balance with the combination of soy and beef. Improvements of the protein quality demonstrated in these studies are supposed to result from the complementary effect of methionine on soy protein. Actually, Kies and Fox (4) and Zezulka and Calloway (5) observed an improvement of the nitrogen balance by adding methionine to soy protein at low nitrogen intake. Young et al. (2) observed a similar effect when 0.6 or 1.1% methionine was added to soy protein but a negative effect when 1.6% methionine was added to 0.51 g soy protein/kg/day. Recently, Young et al. (13) reported that no beneficial effects of methionine supplementations were observed when the test nitrogen intake level was 128 mg N/kg/day. Young et al. (2) also observed no differences in the nitrogen balance with the combination of soy and beef at 0.6 g protein/kg/day. Thus, improvement of the quality of soy protein by adding methionine or by combination with other proteins is not yet proved. However, certain questions on the nutritive value of SPI in human adults remain to be elucidated.
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