# Evaluation of the Protein Quality and Milled Rices Differing in Protein Content

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Rat feeding experiments done at dietary protein levels of not more than 5% demonstrated a decrease in quality of protein with an increase in protein content of four milled rice samples. Protein-quality indexes based on weight gain, such as PER, net protein ratio, and nitrogen growth index gave values overlapping with those of casein, because rats fed milled rice generally had lower nitrogen and higher fat content than those fed casein. More realistic indexes of protein quality were obtained from the nitrogen-balance index at the same dietary protein level and from nitrogen-growth index for diets high in rice content. All these indexes showed that protein quality tended to decrease with an increase in protein content of milled rice, but that the decrease was less than proportional to the increase in protein content. Differences in protein quality were related to levels of lysine, sulfur amino acids, threonine, and tryptophan, and to the ratio of solubility fractions of rice protein.

Among the cereal proteins, rice protein has been found to be of relatively good quality (Deshpande *et al.*, 1955; Harper *et al.*, 1955; Howe *et al.*, 1965; Pecora and Hundley, 1951; Rosenberg *et al.*, 1959). The main nutritional disadvantage of the rice grain is its low protein content (usually 6 to 8%).

The protein content of any variety may vary by as much as 7 percentage points due to environment (Cagampang *et al.*, 1966; Juliano *et al.*, 1964a). Several studies have shown, through amino acid analysis of samples of milled and of brown rice, that the protein content of the grain is negatively correlated with the lysine content of the protein (Cagampang *et al.*, 1966; Juliano *et al.*, 1964b). However, the drop in lysine content was less than 25% of the corresponding increase in protein content. For these reasons, researchers attempting to improve the nutritional value of rice have concentrated on increasing its protein content rather than the level of lysine, its first limiting amino acid (Juliano *et al.*, 1968).

When breeders develop high-protein, commercial varieties, it will be difficult to compare the protein quality of samples that differ in protein content. Existing varieties, including the high-yielding varieties developed by the International Rice Research Institute, normally have less than 10% protein. They cannot be evaluated by common techniques of biological assay for protein, such as PER (Protein Efficiency Ratio) or NPU (Net Protein Utilization), which require 9 to 10% protein in the diet.

Recently, Bressani and Elias (1971) reported on an assay method applicable to plant proteins with less than 10% protein. The method is a modification of the nitrogen-growth and nitrogen-balance indexes of Allison (1964). It involves measuring the growth response and increase in carcass nitrogen of weanling rats fed diets containing different levels of the experimental protein. The objective of our study was to determine the relation of the weight gain of weanling rats to protein content of milled rice, protein level in the diet, amino acid composition of rice protein, and carcass nitrogen and fat content.

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## MATERIALS AND METHODS

**Rice Samples.** We used samples of three commercial varieties of rice (*Oryza sativa* L.). Samples of IR8 and BPI-76-1 came from crops grown at the IRRI farm, but the IR8 was grown in the 1969 dry season and the BPI-76-1 was grown in the 1969 wet season. A sample of Intan was obtained from the 1969 dry season crop of a farmer in Calamba, Laguna. The samples were dehulled and milled in a Satake, type SB-2B, One-Pass Rice Husker-Pearler and air-shipped to the Institute of Nutrition for Central America and Panama (INCAP) for assay of protein quality.

**Methods.** Protein content was determined by measuring macro-Kjeldahl nitrogen (American Association of Cereal Chemists, 1962) and multiplying by the factor 5.95. Milled-rice hydrolysates were analyzed in a Beckman amino acid analyzer, model 120C, using the 2-hr run with PA-28 and PA-35 resins (Beckman Instruments, 1966). To improve the tyrosine values, we used double evacuation of the hydrolysis tube according to the procedure of Kohler and Palter (1967). Purified nitrogen was introduced into the evacuated tube before rethawing and before sealing the tube, however. The correction factors of Kohler and Palter were used to adjust cystine, isoleucine, methionine, serine, threonine, and valine values obtained by the standard hydrolysis procedure without performic acid oxidation. Tryptophan was determined colorimetrically by the method of Opienska-Blauth *et al.* (1963) on rice flour ground to 100-mesh fineness and digested for 24 hr with 0.4% pronase at 35° to 40° C (Hernandez and Bates, 1969).

At INCAP, the samples were ground with a Raymond Pulverizer to 60-mesh fineness. The samples were then analyzed for nitrogen content by the Kjeldahl method (American Association of Cereal Chemists, 1962) and stored at 4° C in plastic bags until the diets were prepared. Vitamin-free casein (Nutritional Biochemicals Corp.) with 13.8% Kjeldahl nitrogen was used as the reference protein in the feeding trials.

The rice samples were assayed in two trials. Intan and two IR8 samples were in the first trial, and the BPI-76-1 sample, which became available at a later date, was in the second. The protein quality of Intan and of the two IR8 samples was assayed in two ways. In the first assay, rice flour or casein was added to a basal diet in amounts to provide 1, 2, 3, 4, and 5% of protein. The basal diet contained 4% mineral mixture (Hegsted *et al.*, 1941), 5% refined cottonseed oil, 1% cod liver oil, 90% refined corn starch, and 5 ml/100 g of diet of a complete vitamin mixture (Manna and Hauge, 1953). The rice or casein replaced an equivalent weight of the corn starch. In the second assay, diets were prepared which contained 90% rice flour with the same amounts of supplements indicated above for the basal diet. As a control, enough casein was added to the basal diet to equalize the percentage of protein in the 90% rice flour of each variety.

In the second trial, the BPI-76-1 rice and casein diets were adjusted to contain 0, 2, 5, 8, 11, and 12% protein.

In all studies, the diets were fed *ad libitum* to groups of ten 21-day-old rats. The rats were of the Wistar strain from the INCAP colony. All groups within each experiment had the same average initial weight. Each group consisted of five males and five females.

The rats were placed in individual all-wire screen cages with raised screen bottoms. Water was available to them at all times. Food intake was measured every 7 days. Spilled food was collected daily and used to correct the amount ingested. The animals were weighed every 7 days for 28 days. At the end of the experimental period, the rats were killed, cut open ventrally from jaw to tail, and air-dried to constant weight in an oven. The dried carcasses were then ground in a micro Wiley mill. Samples were analyzed for nitrogen by the macro Kjeldahl method and for total body fat content by Soxhlet extraction with hexane for 72 hr.

## RESULTS

Most amino acids of rice, when expressed as percentages of the protein, remained more or less constant, except leucine, which increased with increasing concentration of protein, and lysine, sulfur amino acids, threonine, and tryptophan, which decreased (Table I). However, the contents of all amino acids per unit weight of milled rice increased with increasing protein content. Casein contained more lysine, threonine, and tryptophan but less total sulfur amino acids than milled-rice protein.

Table II shows the mean weight gains, total carcass nitrogen and fat, and the protein efficiency ratios. The PER values reveal that the Intan rice had better protein quality at all levels of dietary protein (1, 2, 3, 4, and 5%) than the other two rices in this group.

The net protein ratio or the ratio of the weight gain corrected for maintenance needs (represented by the values obtained from rats fed 0% nitrogen diets) to protein intake is a measure of protein quality (Allison, 1964). These values, calculated from the data in Table II, were: Intan, 3.71;

**Table I. Aminogram of Casein and Milled Rice Assayed for Protein Quality**

| Amino acid | Amino acid content[a] | | | | | |
|---|---|---|---|---|---|---|
| | | Milled rice | | | | |
| | Casein | Intan | IR8 | IR8 | BPI-76-1 | LSD[b] (0.05) |
| Alanine | 3.32 | 5.99 | 6.04 | 5.66 | 6.24 | |
| Arginine | 3.47 | 7.90 | 8.78 | 8.82 | 8.58 | |
| Aspartic acid | 8.36 | 9.59 | 9.97 | 9.46 | 10.4 | |
| Cystine | 0.26 | 1.81 | 1.81 | 1.45 | 1.42 | 0.27 |
| Glutamic acid | 24.4 | 18.3 | 19.1 | 18.7 | 21.1 | 1.06 |
| Glycine | 2.02 | 4.82 | 4.80 | 4.52 | 4.66 | 0.20 |
| Histidine | 2.67 | 2.45 | 2.45 | 2.48 | 2.48 | |
| Isoleucine | 6.35 | 4.89 | 5.10 | 4.78 | 5.34 | |
| Leucine | 10.1 | 7.84 | 8.32 | 8.20 | 9.64 | 0.92 |
| Lysine | 7.46 | 4.27 | 3.77 | 3.69 | 3.35 | 0.32 |
| Methionine | 2.67 | 3.45 | 3.02 | 2.44 | 1.80 | 0.36 |
| Phenylalanine | 5.52 | 5.55 | 5.74 | 5.66 | 6.25 | 0.41 |
| Proline | 11.2 | 4.73 | 5.04 | 4.79 | 4.66 | |
| Serine | 7.78 | 6.31 | 5.68 | 6.27 | 6.92 | |
| Threonine | 4.78 | 4.10 | 4.10 | 3.78 | 3.75 | 0.20 |
| Tryptophan[c] | 1.65 | 1.35 | 1.26 | 1.05 | 0.97 | 0.08 |
| Tyrosine | 6.04 | 5.02 | 5.34 | 5.34 | 5.85 | |
| Valine | 7.98 | 6.24 | 6.55 | 6.51 | 7.30 | |
| Ammonia | 2.00 | 3.70 | 2.98 | 2.86 | 2.28 | 0.46 |
| Total | 118.03 | 108.31 | 110.29 | 106.46 | 112.98 | |
| Nitrogen recovery, % | 98.7 | 102.7 | 101.3 | 98.5 | 99.8 | |
| Protein content (%)[d] at 12% moisture | 86.2 | 5.68 | 7.32 | 9.73 | 14.3 | |

[a] In g/16.8 g N for casein and in g/16.8 g N for milled rice. Values for cystine, isoleucine, methionine, serine, threonine, and valine were corrected using the factors of Kohler and Palter (1967). [b] Calculated for the four milled-rice samples. [c] Colorimetric assay of pronase digest by the Opienska-Blauth (1963) reagent. [d] N × 6.25 for casein and N × 5.95 for milled rice.

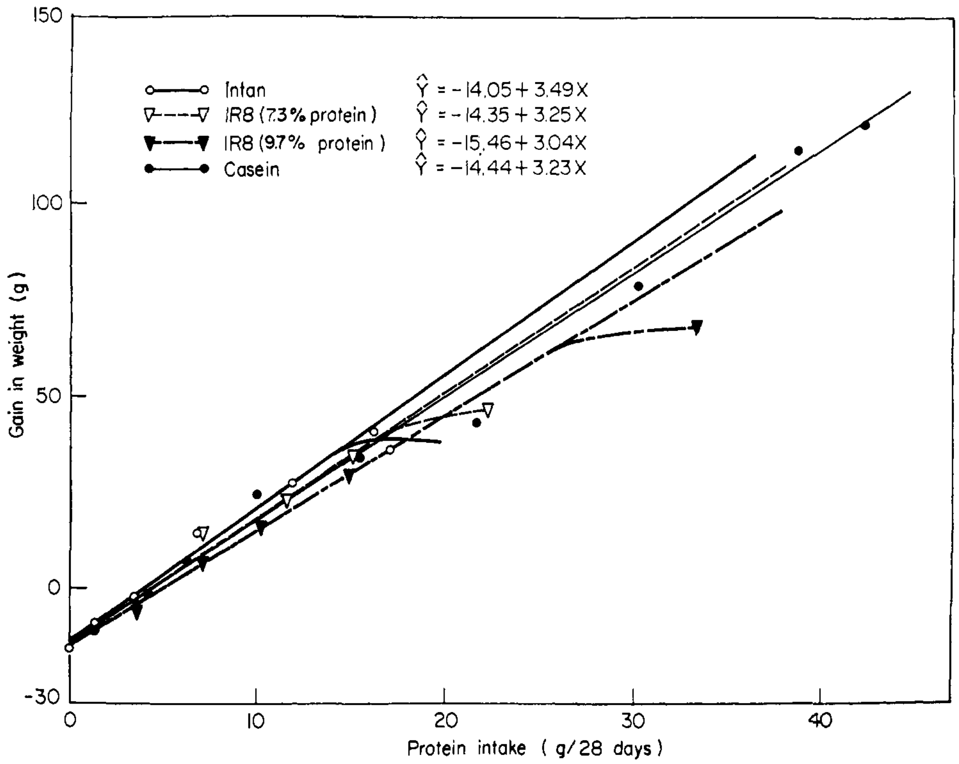


**Figure 1.** Relation between mean protein intake and weight gain of ten weanling rats fed 0, 1, 2, 3, 4, and 5% protein levels of casein and three samples of milled rice differing in protein content for 28 days. Data for 90% rice diets are alo included

**Table II. Mean Gain in Weight and Total Carcass Nitrogen and Fat of Weanling Rats and the Protein Efficiency Ratios (PER) of Rice Varieties and Casein Fed at Various Levels of Dietary Protein**

| Protein in diet, % | Mean weight gain, g/28 days | Mean total carcass nitrogen, g | Mean total carcass fat, g | PER |
|---|---|---|---|---|
| | | Nitrogen-free diet | | |
| 0 | −16 ± 1.10[a] | 0.87 ± 0.03[a] | 3.02 ± 0.12[a] | |
| | | Rice Intan (5.7% protein) | | |
| 1 | −9 ± 0.63 | 1.01 ± 0.04 | 3.82 ± 0.42 | |
| 2 | −2 ± 0.79 | 1.21 ± 0.04 | 6.15 ± 0.58 | |
| 3 | 14 ± 1.74 | 1.50 ± 0.05 | 10.40 ± 0.90 | 1.95 ± 0.20[a] |
| 4 | 27 ± 2.33 | 1.80 ± 0.07 | 16.05 ± 1.23 | 2.28 ± 0.17 |
| 5 | 41 ± 2.02 | 2.16 ± 0.07 | 19.56 ± 1.32 | 2.56 ± 0.06 |
| | | Rice IR8 (7.3% protein) | | |
| 1 | −10 ± 0.70 | 1.06 ± 0.04 | 4.06 ± 0.37 | |
| 2 | −4 ± 1.00 | 1.20 ± 0.04 | 5.47 ± 0.55 | |
| 3 | 13 ± 1.29 | 1.56 ± 0.04 | 12.23 ± 0.73 | 1.90 ± 0.12 |
| 4 | 22 ± 1.52 | 1.77 ± 0.06 | 13.09 ± 1.32 | 1.92 ± 0.13 |
| 5 | 34 ± 2.79 | 2.02 ± 0.06 | 17.57 ± 0.98 | 2.20 ± 0.13 |
| | | Rice IR8 (9.7% protein) | | |
| 1 | −10 ± 0.65 | 1.04 ± 0.04 | 4.35 ± 0.25 | |
| 2 | −6 ± 0.49 | 1.27 ± 0.04 | 5.88 ± 0.45 | |
| 3 | 6 ± 1.58 | 1.37 ± 0.04 | 7.78 ± 0.76 | 0.80 ± 0.20 |
| 4 | 16 ± 1.47 | 1.59 ± 0.06 | 12.21 ± 1.23 | 1.54 ± 0.10 |
| 5 | 29 ± 2.88 | 1.66 ± 0.08 | 13.82 ± 1.10 | 1.94 ± 0.12 |
| | | Casein | | |
| 1 | −11 ± 0.90 | 0.98 ± 0.03 | 4.13 ± 0.36 | |
| 2 | −2 ± 0.93 | 1.21 ± 0.04 | 6.10 ± 0.52 | |
| 3 | 7 ± 0.73 | 1.47 ± 0.05 | 8.69 ± 0.46 | 1.14 ± 0.10 |
| 4 | 24 ± 1.90 | 1.85 ± 0.09 | 12.86 ± 0.90 | 2.33 ± 0.13 |
| 5 | 34 ± 3.00 | 2.12 ± 0.09 | 15.51 ± 1.10 | 2.20 ± 0.19 |
| 10 | 121 ± 4.82 | | 27.90 ± 1.67 | 2.86 ± 0.07 |

[a] Standard error.

casein and IR8 (7.3% protein), 3.36; and IR8 (9.7% protein), 3.07.

Weight gain is plotted against nitrogen intake in Figure 1, which also shows the regression equation of the lines. The slope or the nitrogen-growth index (Allison, 1964) was Intan, 3.41; IR8 (7.3% protein), 3.25; casein, 3.23; and IR8 (9.7% protein), 3.04.

The nitrogen and fat content of the carcasses indicated differences in composition between the two dietary protein sources (Table II). Calculated by comparing the 5% protein diets to the 0% protein diets, the ratio of increases in carcass fat to increases in carcass nitrogen was 10.0 for casein, 12.8 for Intan, 12.7 for IR8 (7.3% protein), and 13.4 for IR8 (9.7% protein). Clearly, rats fed milled-rice diets accumulated more body fat than those fed casein.

Table III presents regression equations between increase in carcass nitrogen and nitrogen intake, and between increase in carcass nitrogen and weight gain. These results rank the three rice samples in the same order as the previous measures, but the values for casein are higher than those of the rice samples. The nitrogen-balance index was casein, 0.53, Intan, 0.49, IR8 (7.3% protein), 0.47, and IR8 (9.7% protein), 0.36.

The generally higher nitrogen and lower fat content of carcasses of rats fed casein may explain the differences in the relative ranking of casein and the three rice samples from nitrogen growth and nitrogen balance indexes. Rats fed casein showed the highest protein increase with weight gain, followed in order by those fed Intan and IR8 (7.3% protein), and IR8 (9.7% protein) diets. The differences in protein contents of the carcass must have been due in part to the higher fat content of rats fed Intan and IR8 (7.3% protein) milled rice protein. Rats fed IR8 (9.7% protein) had lower carcass nitrogen content than those fed casein and the two other rice samples, and had lower fat content than those fed the two other milled-rice samples (Table II).

Based on PER, net protein ratios, nitrogen-growth index, and nitrogen-balance index, the three rice samples showed the same ranking in protein quality, *i.e.*, protein quality tended to decrease with an increase in protein content in milled rice. The decrease in quality was only a fraction of the increase in protein content, however.

Table IV shows the results of the study in which all diets contained 90% rice flour. No difference in PER values (2.02 to 2.04) between varieties was observed. Rats gained more weight on diets of the rice varieties with higher protein levels. Similarly, only the IR8 (9.7% protein) had a lower PER value than its casein control; the other two samples had PER values similar to those obtained from their respective casein controls. The weight gain of rats fed diets with 90% rice was lower than the value expected from the regression lines in Figure 1 obtained at 5% dietary protein or lower, but the corresponding data for casein still fitted the casein regression line.

The results for the BPI-76-1 are shown in Table V together with the casein standard. The PER of BPI-76-1 milled rice at 5% dietary protein was 2.24, and that of the casein standard, 3.28. The growth response in this experiment at 2 and 5% protein levels was higher than in the one involving the other three rice samples in which casein gave a PER value of only 2.20. The PER of BPI-76-1, corrected for the differences in growth response of rats to casein in the two experiments, was 1.50. The net protein ratio for BPI-76-1 rice, using the data for 2 and 5% dietary protein, was 3.08, and for casein was 4.02. The corrected net protein rato for BPI-76-1 was 2.57.

**Table III. Regression Equations between Carcass Nitrogen and Nitrogen Intake and between Carcass Nitrogen and Weight Gain for Three Rice Samples and Casein[a]**

| Protein source | Regression equation between carcass nitrogen and Nitrogen intake (X) | Weight gain (X) |
|---|---|---|
| Rice Intan | $\hat{Y} = 0.91 + 0.49X$ | $\hat{Y} = 1.206 + 0.0241X$ |
| Rice IR8 (7.3% protein) | $\hat{Y} = 0.94 + 0.47X$ | $\hat{Y} = 1.257 + 0.0244X$ |
| Rice IR8 (9.7% protein) | $\hat{Y} = 0.96 + 0.36X$ | $\hat{Y} = 1.252 + 0.0230X$ |
| Casein | $\hat{Y} = 0.89 + 0.53X$ | $\hat{Y} = 1.258 + 0.0270X$ |

[a] Correlation coefficients ranged from 0.91 to 0.96.

**Table IV. Mean Weight Gain of Weanling Rats Fed 90% Rice Diets, and Protein Efficiency Ratio of Milled Rice Samples**

| Protein source | Protein in diet, % | Mean weight gain, g/28 days | PER |
|---|---|---|---|
| Rice Intan | 5.50[a] | 35 ± 2.1[b] | 2.04 ± 0.04[b] |
| Casein | 5.50 | 34 ± 3.0 | 2.20 ± 0.19 |
| Rice IR8 (7.3% protein) | 6.70[a] | 46 ± 5.2 | 2.02 ± 0.12 |
| Casein | 6.80 | 44 ± 5.7 | 1.96 ± 0.15 |
| Rice IR8 (9.7% protein) | 8.80[a] | 68 ± 2.8 | 2.02 ± 0.06 |
| Casein | 8.40 | 79 ± 6.7 | 2.57 ± 0.13 |
| Casein | 10.00 | 114 ± 9.0 | 2.94 ± 0.12 |

[a] Amount of protein provided by 90% rice flour. [b] Standard error.

**Table V. Weight Gain of Rats Fed Rice Variety BPI-76-1 and Casein at Various Levels of Dietary Protein and the Corresponding Protein Efficiency Ratios**

| | Rice | | Casein | |
|---|---|---|---|---|
| Protein in diet, % | Mean weight gain, g/28 days | PER | Mean weight gain, g/28 days | PER |
| 0 | −13 ± 0.75[a] | | −13 ± 0.75[a] | |
| 2 | 2 ± 2.0 | 0.83 ± 0.29[a] | 3 ± 1.7 | 0.75 ± 0.24[a] |
| 5 | 35 ± 3.0 | 2.24 ± 0.15 | 57 ± 3.9 | 3.28 ± 0.13 |
| 8 | 66 ± 2.3 | 2.23 ± 0.15 | 112 ± 4.6 | 3.23 ± 0.06 |
| 11 | 87 ± 7.7 | 1.88 ± 0.07 | 124 ± 9.6 | 2.56 ± 0.10 |
| 12 | 101 ± 7.6 | 1.92 ± 0.07 | 133 ± 7.4 | 2.60 ± 0.07 |

[a] Standard error.

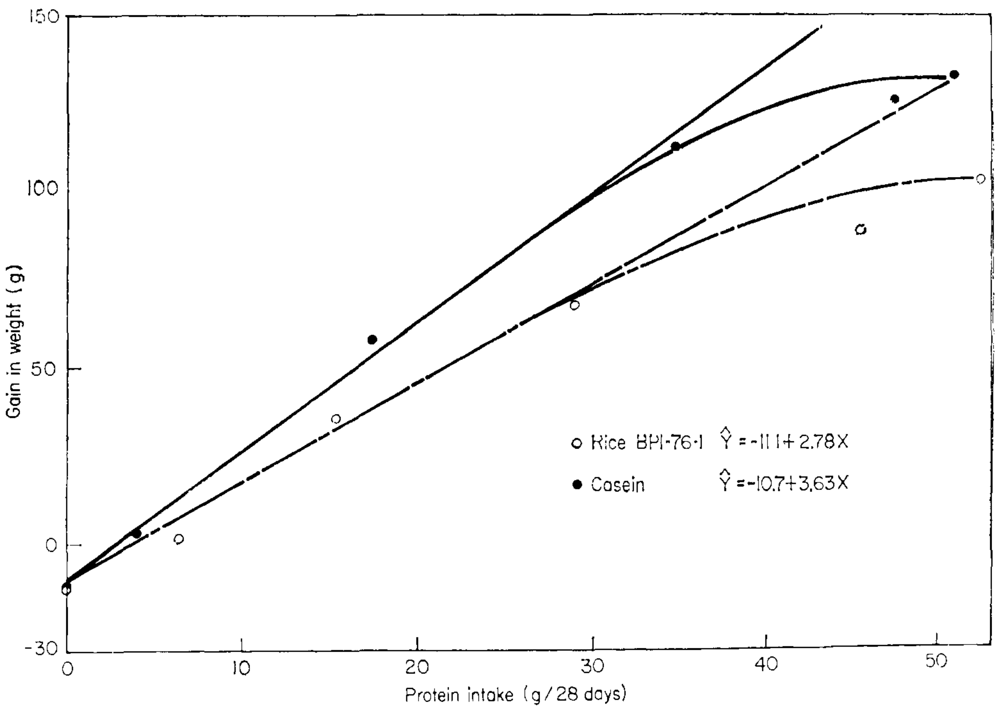


**Figure 2. Relation of mean protein intake to weight gain of ten weanling rats fed 0, 2, 5, 8, 11, and 12% protein levels of casein and BPI-76-1 (14.3% protein) milled rice for 28 days**

The regression equation of the response curve was calculated from all values below an intake of 20 g of protein (<5% dietary protein) since this region showed linearity between the two components for the three other samples. BPI-76-1 gave a nitrogen-growth index of 2.78 and casein gave a nitrogen-growth index of 3.63. Both proteins showed leveling off or nonlinearity of the curve above 10% protein. Based on the slope of the line (Figure 2), BPI-76-1 had only 72.4% of the protein quality of casein. The Intan variety had a value of 110.4%, IR8 (7.3% protein), 100.0%, and IR8 (9.7% protein), 91.4%.

The PER values for casein at dietary protein levels over 5% were similar in the two experiments. Since the second experiment showed a PER of 3.07 for the casein (10% protein) standard (Figure 2), as compared with 2.94 in the earlier experiment, the PER of BPI-76-1 (90% rice diets) of 1.92 may be corrected to 1.84.

## DISCUSSION

The use of diets with high contents (90%) of rice did not produce differences in PER values of the rice samples, except for BPI-76-1. The PER of Intan was 2.04, of IR8 (7.3% protein), 2.02, of IR8 (9.7% protein), 2.02, and of BPI-76-1, 1.92 (1.84, corrected). Compared with the casein standard at the same dietary protein level, Intan had 92.7% of the protain quality of casein, IR8 (7.3% protein) had 103.1%, IR8 (9.7% protein) had 78.6%, and BPI-76-1 had 73.8%. These results agree with the findings of Blackwell *et al.* (1966). Hischke *et al.* (1968) also found similar PER values for seven samples of oat groats differing in lysine content and concluded that PER is a less sensitive index of protein quality than the amino acid analyzer method.

Varietal differences in protein quality of milled rice were noted in the experiments with low protein levels in the diet between 0 and 5%. Protein quality tended to decrease as

**Table VI. Summary of Protein Quality Indexes for Four Milled Rice Samples and Casein**

| | | I[b] | | | | | II[c] | | |
|---|---|---|---|---|---|---|---|---|---|
| Protein source | Protein content, % | PER (5% protein) | Net protein ratio | Nitrogen-balance index | Nitrogen-growth index | Relative quality[d] | PER (90% rice) | Nitrogen-growth Index | Relative quality[d] |
| Intan | 5.68 | 2.56 | 3.71 | 0.49 | 3.49 | 80.0 | 2.04 | 2.37 | 47.0 |
| IR8 | 7.32 | 2.20 | 3.36 | 0.47 | 3.25 | 75.0 | 2.02 | 2.30 | 45.6 |
| IR8 | 9.73 | 1.94 | 3.07 | 0.36 | 3.04 | 70.5 | 2.02 | 2.17 | 43.1 |
| BPI-76-1[a] | 14.3 | 1.53 | 2.50 | | 2.47 | 57.4 | 1.84 | 2.12 | 42.1 |
| Casein | | 2.20 | 3.36 | 0.53 | 3.23 | 75.0 | | 3.78 | 75.0 |

[a] Corrected for differences in casein values from the two experiments. [b] Protein intake below 20 g (0 to 5% dietary protein). [c] Protein intake resulting in a net increase in rat weight. [d] Based on a value of 75.0 for casein.

protein contents of the milled rice increased. The mean decrease, however, in protein quality was only 23% of the mean increase in protein content. Hence, an increase in protein content of the rice grain results in a net increase in its nutritional value.

The inverse relationship between protein quality and protein content of milled rice was reflected in the nitrogen-growth and nitrogen-balance indexes. Differences in the relative protein quality of casein and milled rice measured by these two indexes may be due to differences in the carcass nitrogen and fat contents of rats fed these two protein sources. The carcasses of rats fed casein generally had the higher nitrogen content and the lower fat content. Apparently total body fat is correlated with weight gain, particularly in rats fed diets with higher protein levels.

The nitrogen-growth index of Intan was 3.49, of IR8 (7.3% protein), 3.25, of IR8 (9.7% protein), 3.04, and BPI-76-1, 2.78. However, measured by net protein ratio or PER, the nutritive value of BPI-76-1 was intermediate between that of the two IR8 samples. This may in part be because the casein standard had higher values in the second trial with BPI-76-1 than it did in the first trial with the other samples. When adjusted for the differences in growth rates between the two trials, all the protein quality indexes showed BPI-76-1 to be inferior to the three lower-protein rice samples.

The regression lines were linear only below 5% protein in the rice diets, as shown for the BPI-76-1 trial (Figure 2), and the 90% rice trial for the other rice samples (Figure 1). When the slopes for the higher protein diets which produced a net increase in rat weight in Figures 1 and 2 were considered, the resulting nitrogen-growth indexes were also found to decrease as the protein content of milled rice increased (Table VI). When the value for casein was adjusted to 75.0, the index of protein quality at low dietary protein levels was similar to the biological values of 70 and 75% reported for protein of milled rice (Juliano, 1966). When the data showing net weight increase were processed similarly, the resulting values were similar to the Relative Nutritional Values of 44 and 50% (of lactalbumin) for milled rice protein (Hegsted *et al.*, 1968; Hegsted and Worcester, 1967). In fact, Hegsted (1971) found a Relative Nutritional Value of 40% for our BPI-76-1 rice, which is close to our value of 42%. Although both methods ranked the four samples of milled rice to the same order of protein quality, the method based on growth at low dietary protein levels again gave relatively higher values, as high as that of casein.

The levels of essential amino acids, lysine, threonine, sulfur-containing amino acids, and tryptophan also decreased in rice protein as protein content of milled rice increased (Table I). Rice protein is deficient in lysine and threonine (Deshpande *et al.*, 1955; Harper and de Muelenaere, 1963; Harper *et al.*, 1955; Howe *et al.*, 1965; Pecora and Hundley, 1951; Rosenberg *et al.*, 1959). The decreases in essential amino acids corresponded to the observed reductions in protein quality as the protein content of milled rice increased. Whether or not lysine is still the first limiting amino acid in high-protein rices that also have lower levels of threonine, sulfur amino acids, and tryptophan should be studied.

When rats ate equal levels of milled rice, the differences in protein quality were reduced. One possible reason is that high-protein milled rice may have higher levels of all essential amino acids since the decreases in the levels of lysine, threonine, sulfur-containing amino acids, and tryptophan in rice protein were only a fraction of the corresponding increases in the protein content of milled rice. In fact, Clark *et al.* (1971) found that adult human subjects retained significantly more nitrogen when they were fed a BPI-76-1 milled-rice diet than when they were fed a lower-protein rice of another variety even after its nitrogen level was adjusted to the same level as that of BPI-76-1 by the addition of nonessential amino acids. They stated that the high-protein BPI-76-1 milled rice had better nutritional value because it has higher levels of all essential amino acids.

The low (5%) level of prolamin in milled-rice protein probably caused the minimal decrease in protein quality and in the levels of some essential amino acids in the protein of milled rice as protein content increased. Rice protein is different from other cereal proteins: it has a low content of prolamin (alcohol-soluble protein) and a high (80%) content of glutelin (alkali-soluble protein) (Cagampang *et al.*, 1966; Dimler, 1966; Juliano, 1965; Mossé, 1966). Cagampang *et al.* (1966) and Michael *et al.* (1961) showed that increases in protein content occurred only in the glutelin and prolamin fractions. The drop in lysine content of the protein as grain protein increases is mainly due to the increase in the prolamin content of the grain, since prolamin has the lowest lysine content among the proteins of the rice grain (Palmiano *et al.*, 1968; Tecson *et al.*, 1971). Glutelin has the same aminogram as milled rice (Tecson *et al.*, 1971). Larger differences in protein quality have been reported for cereals containing a greater proportion of prolamin and other proteins of lower lysine content, such as corn, barley, sorghum, and wheat (Mossé, 1966). Oat protein (Hischke *et al.*, 1968) and the endosperm protein of high-lysine corn (Mossé, 1966) are similar to rice protein in that they have a high lysine content and a low proportion of prolamin.

The ratio of albumin/globulin/prolamin/glutelin (as a percentage of total protein) obtained by a modified Osborne serial extraction (Cagampang *et al.*, 1966) was 4/9/2/85 for Intan, 4/9/1/86 for IR8 (7.3% protein), 4/11/2/83 for IR8 (9.7% protein), and 3/9/6/82 for BPI-76-1. The higher the protein content of the rice samples, except for Intan, the higher the prolamin content. Intan protein, with higher lysine content than IR8 protein, had a higher prolamin level than IR8 protein. This suggests that varietal differences in the aminogram of the protein fractions may be another source of differences in aminogram of milled rices, in addition to differences in the ratio of protein fractions. Varietal differences in aminogram of prolamin and glutelin from IR8 and BPI-76 have been reported (Palmiano *et al.*, 1968; Tecson *et al.*, 1971).

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