Protein Quality of the High Yielding Varieties of Rice

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Two high yielding varieties of rice, IR-8 and Taichung (Native) 1 (dwarf *indica*), were evaluated for their protein quality by chemical and biological methods and compared with an Indian variety, NP-130 (tall *indica*). Taichung (Native) 1 was found to contain higher quantities of essential amino acids (such as lysine and sulfur-containing amino acids) in its protein than IR-8 and NP-130. Biological tests like protein efficiency ratio (PER) and net protein ratio (NPR) also indicated nutritional superiority of Taichung (Native) 1 over IR-8 and NP-130.

In recent years a number of high yielding varieties of rice with dwarf plant types have been released to the farmers of India. Two of the varieties, IR-8 and TN-1, have become popular because of higher yield.

Rice is the staple food of more than half the Indian population. Rice is also the main source of protein for most of the rice eaters. It has been reported that the bulk of the Indian population derive 72% of dietary protein from cereals (ICMR Spl. Report, 1953).

The high yielding varieties of rice which are gaining popularity are thus likely to become the main source of protein for most of the rice eaters in India. Investigations were therefore carried out to evaluate the protein quality of IR-8 and TN-1 as compared to an Indian variety NP-130 by chemical and biological methods. Though protein quality of the Indian rice varieties has been tested from time to time (Swaminathan, 1937; Acharya *et al.*, 1942; Parthasarathy *et al.*, 1964), no information is available on the protein quality of the high yielding varieties.

MATERIALS AND METHODS

Seeds of IR-8, Taichung (Native) 1 (TN-1), and NP-130 were collected from a field experiment conducted by the Agronomy Division of the Indian Agricultural Research Institute in the IARI farm. In this experiment nitrogen, at the rate of 120 kg/ha, was applied to the high nitrogen responsive dwarf varieties IR-8 and TN-1 and was applied at the rate of 40 kg/ha to NP-130. P_2O_5 and K_2O were applied at the rate of 40 kg/ha to all three varieties. (NP-130 is a fine-grained, low nitrogen responsive, tall *indica* grown extensively in northern India.) The seeds were dehusked in a Japanese dehusking machine and cleaned in a Japanese winnower. No polishing was done. The brown rice samples were ground to pass through a 20-mesh sieve and used immediately for the preparation of animal diets. For chemical analysis, samples of brown rice were ground to pass through a 100-mesh sieve, defatted with cold diethyl ether, air dried, and again passed through a 100-mesh sieve.

The protein contents of the samples were calculated by multiplying Kjeldahl N by 5.95. Samples were dried in a hot air oven at 105° C for determination of moisture. The results of all chemical estimations were calculated in an oven dry basis.

Preparation of Protein Hydrolysates. 50 mg of dry and defatted sample was hydrolyzed with 5 ml of 6 *N* HCl *in vacuo* for 22 hr in a sealed tube at 110° C. The contents of the tube were removed with the help of a few milliliters of distilled water, filtered, and then evaporated to dryness in a flash evaporator at 50° C. The residue was extracted with 2 ml of citrate buffer of pH 2.85.

Quantitative Estimations of Amino Acids. Amino acid estimations were done in a Technicon autoanalyzer (60-cm column of chromobeads Type-C2, maintained at 60° C). Tryptophan was estimated by the method of Spies and Chambers (1949).

Diets. The diets for all the biological experiments were prepared at 8% protein level. The composition of 100 g of diet was as follows: brown rice—calculated weight to give 8% protein; hydrogenated fat—10 g; salt mix—2 g; and sufficient glucose added to make up the total weight to 100 g. Adequate quantities of vitamins were added to the diets.

Protein Efficiency Ratio (PER). PER was determined by the method of Osborne *et al.* (1919). Weanling albino rats, matched as to their litter mate, sex, and weight (*ca.* 40 g) were divided into four groups. Five rats were taken in each group. Besides the three rice varieties a variety of recently evolved dwarf wheat, Sharbati Sonora (Varughese *et al.*, 1966) was included in the experiment for comparison. The wheat diet was prepared at 8% protein level. The animals were fed on the experimental diets for 28 days.

Net Protein Ratio (NPR). NPR was determined by the method of Bender and Doell (1957), as reported by Eggum (1968). One-month-old albino rats balanced with respect to litter mate, sex, and combined body weight for each group were divided into four groups. Five rats were taken in each group. Rice diet was prepared at 8% protein level. A nonprotein diet was prepared by replacing grain by glucose in the diet. Three groups of the rats were given the three rice diets and the fourth group was given the nonprotein diet. The experiment was conducted for 9 days. Weights of the animals were recorded on alternate days. NPR was calculated as follows.

$$\text{NPR} = \frac{\text{wt gain of TPG} - \text{wt loss of NPG}}{\text{wt of protein consumed}}$$

Where TPG = test protein group (rice diets) and NPG = nonprotein group.

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Table I. Amino Acid Composition[a,b] of Rice Varieties (g/16.8 g N)

Essential amino acids	FAO[c]	IR-8	TN-1	NP-130
Lysine	4.2	3.26[f]	4.53	3.05
Histidine	2.4[d]	3.22	3.14	2.27
Threonine	2.8	4.03	4.25	3.26
Methionine	2.2	2.13	2.00	2.25
Cystine		1.29	2.23	1.75
Methionine + cystine	4.2	3.42	4.23	4.00
Isoleucine	4.2	4.00	3.83	4.32
Tryptophan	1.4	1.68	1.48	1.35
Valine	4.2	5.75	7.55	6.58
Leucine	4.8	8.53	7.18	8.50
Phenylalanine	2.8	4.40	4.85	3.89
Tyrosine		1.89	2.58	2.34
Arginine	2.0[e]	9.11	7.97	5.53
Nonessential amino acids				
Aspartic acid		10.07	9.49	12.16
Serine		5.01	5.01	7.49
Glutamic acid		22.27	24.05	22.39
Proline		4.98	5.03	5.53
Glycine		4.97	4.56	3.63
Alanine		4.35	4.90	4.47
Ammonia		2.57	2.29	3.68
Protein (g/100 g of sample)		10.06	13.21	10.95

[a] Recalculated to 95% nitrogen recovery for all rice samples. [b] Average of duplicate samples. [c] FAO pattern of amino acid requirements (Food and Agr. Org., 1957). [d] Requirement of infant or albino rat (Food and Nutrition Board, 1963). [e] Amino acid requirements suggested by Rose (1937). [f] The underlined values are limiting amino acids using FAO pattern as optimal.

Table II. Essential Amino Acid Index (EAAI), Biological Value (BV), and Chemical Score of Rice Proteins

Rice variety	EAAI	BV	Chemical score
IR-8	73.28	68.18	34.50
TN-1	78.50	73.87	47.90
NP-130	69.85	64.44	32.30

$$\mathrm{EAAI} = \sqrt[n]{\frac{\mathrm{Lys}_P}{\mathrm{Lys}_s} \times \frac{\mathrm{Try}_P}{\mathrm{Try}_s} \times \ldots\ldots \times \frac{\mathrm{Hist}_P}{\mathrm{Hist}_s}}$$

Where P refers to food protein (rice protein), s is the standard protein (egg protein), and n is the number of amino acids (counting pairs such as methionine and cystine as one). All the essential amino acids listed in Table I are taken for the calculation. BV = (1.09) EAAI − 11.7. Chemical score is the percentage of most limiting amino acid (lysine) in the protein as compared to the requirement pattern given by Rama Rao *et al.* (1964).

RESULTS AND DISCUSSION

The amino acid composition of the three rice varieties is presented in Table I as percentage of crude protein. The Food and Agriculture Organization (FAO, 1957) reference pattern of amino acid requirements is given for comparison. The requirement of arginine given in Table I is according to the pattern proposed by Rose (1937) and that of histidine is the one given by the Food and Nutrition Board of the National Academy of Science (1963) for infants or rats.

It is observed from Table I that TN-1 is deficient in least number of amino acids as compared to the FAO pattern. Lysine and threonine are considered to be the most limiting amino acids in rice proteins (Watanabe, 1959; Ingaki *et al.*, 1963; Chen *et al.*, 1967). Supplementation of rice diets with sulfur-containing amino acids (Parthasarathy *et al.*, 1964) and tryptophan (Nakamura *et al.*, 1966) have also given improved growth rates of experimental animals. Thus tryptophan and sulfur-containing amino acids might also be considered to be limiting to a certain extent in rice proteins along with lysine and threonine. It is observed from Table I that TN-1 is not deficient in any of the above limiting amino acids as compared to FAO pattern, whereas IR-8 is deficient in lysine and S-containing amino acids, and NP-130 is deficient in lysine, S-containing amino acids, and tryptophan.

Table III. Mean Values of Gain in Weight, Food Intake, Protein Intake, and PER of Rice and Wheat Diets

Diets	Gain in weight	Food intake	Protein intake	PER
IR-8	32.20	180.66	14.18	2.268
TN-1	38.60	188.88	14.97	2.578
NP-130	29.90	173.65	13.46	2.224
Sharbati Sonora (wheat)	15.80	149.44	11.93	1.328
F test				
SE_m	1.265			0.044
C.D. (5%)	3.795			0.132
PER value of casein as determined from time to time with the stock of albino rats maintained in this laboratory				2.75 ± 0.15

Table IV. Net Protein Ratio of Rice Diets

Diet	Protein consumed in 9 days, g	Average wt gain in rice diets, g	Average wt loss in non-protein diet, g	NPR, %
IR-8	4.90	12.33	8.50	78.16
TN-1	4.88	12.92	8.50	90.57
NP-130	4.66	10.83	8.50	50.00

The observed values of protein and lysine in case of TN-1 were rather high and seemingly go against the general finding that the lysine and protein content in rice are negatively correlated (Juliano *et al.*, 1964; Cagampang *et al.*, 1966; Houston *et al.*, 1969). However, the results of biological experiments conducted with TN-1 (Table II) support these high values.

Essential amino acid index (EAAI) (Oser, 1959), biological value (Oser, 1959), and chemical score (Rama Rao *et al.*, 1964) were calculated from the essential amino acid composition of the three rice varieties. The results are presented in Table II. It is observed from Table II that all the calculated values are highest for TN-1, followed by IR-8, and then NP-130. Chemical score which is calculated on the basis of the most limiting amino acid in a protein gives the same relative order for the three rice varieties as EAAI or BV. Thus protein quality can be evaluated by the estimation of a single amino acid, *i.e.*, the most limiting amino acid in a protein.

Protein Efficiency Ratio. Mean values of gain in weight, food intake, protein intake, and PER of the three rice varieties, along with the high yielding wheat variety (Sharbati Sonora), are given in Table III. It is observed from Table III that gain in weight and PER values for TN-1 are significantly higher than IR-8 and NP-130. IR-8 has all the values higher than NP-130, but the differences are not statistically significant. The relatively higher PER value observed for TN-1 may not be entirely due to its superior protein quality. A stimulating effect of rice diets on the growth of rats has been reported by Muelenaere *et al.* (1967). They observed that rice used as a source of lysine stimulated the growth of rats more than an equivalent amount of free lysine or isolated rice protein. According to them also, rice carbohydrates might have some role as growth stimulants.

Net Protein Ratio. The NPR values of the three rice varieties are given in Table IV. The values listed in Table IV are highest for TN-1, followed by IR-8, and then NP-130. The NPR values appear to magnify the differences in protein quality of the three rice varieties to a greater extent than other biological determinations. However, the relative order remains the same as in other determinations. Since determination of NPR is less time consuming (9–10 days) as compared to PER (28 days) and involves less laboratory work as compared to the determination of BV, it is a useful biological test for evaluation of protein quality. According to Bender and Doell (1957), NPR is far more accurate as a measure of protein value than PER and gives highly significant correlation with NPU.

NP-130, though grown in northern India, is a popular variety among the rice eaters of India. All the chemical and biological tests consistently indicated the nutritional superiority of the two high yielding varieties (IR-8 and TN-1) over this Indian variety. The above results, along with our other studies on high yielding varieties of rice (Mitra, 1969), indicate that the wide adoption of the high yielding varieties by the Indian farmers is not going to present any new nutritional problem.

Since the discovery of high lysine Opaque-2 corn (Mertz *et al.*, 1964) a large number of workers are engaged in screening different varieties of food crops for their nutritive value. Adoption of rapid but reliable chemical and biological methods is essential for success in this type of work. It is suggested that chemical score of Rama Rao *et al.* (1964) and NPR of Bender and Doell (1957) may prove useful.

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