

## Chemical, Physical, and Nutritional Properties of High-Protein Flours and Residual Kernel from the Overmilling of Uncoated Milled Rice. II. Amino Acid Composition and Biological Evaluation of the Protein<sup>1</sup>

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### ABSTRACT

The amino acid composition of the protein in 60 samples from the overmilling of 12 different lots of uncoated milled rice was similar. Varietal differences were apparent, particularly in cystine, methionine, and tryptophan. Average amino acid values for the 60 samples are within 10% of the chemical values as reported by the FAO in 1970, except for arginine and tyrosine, for which the values reported here are 24 and 64% higher, respectively. The average, with standard deviation, of protein efficiency ratios determined on 20 fractions of rice, including 12 high-protein flours and 8 other samples from two lots of rice, was  $2.03 \pm 0.20$  adjusted to 2.5 for casein. Relatively little difference was found in the amino acid composition or in the protein efficiency ratios of the various samples from a given lot of rice. The results show that the flours, in addition to containing more protein, had a balance of amino acids as good as that of the original rice.

Rice flours containing from 12 to 20% protein have been produced by abrasive milling of commercial rice (1,2,3) but only two reports are available on the amino acid composition of the protein. Normand et al. (1) determined the amino acid composition of the protein in 12 high-protein flours milled successively from a sample of Bluebonnet 50 rice, and noted no essential differences in amino acid content as compared with the original kernel. Houston et al. (2) reported the amino acid analysis of high-protein flour and original and residual kernels of Calrose. Compositions of the original and residual kernels were similar, but lysine was higher in the flour than in the original rice. Only one value for protein efficiency ratio, 1.84, has been reported for high-protein rice flour, that by Milner (4) on a sample of California Pearl rice.

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As part of a comprehensive study of the nutritive value of high-protein flours, we determined protein efficiency ratios (PERs) and the amino acid composition of the protein in kernels and flours obtained by abrasive milling from 12 lots of southern and western U.S. rices. The results are reported here.

### MATERIALS AND METHODS

Samples of 12 lots of commercially milled rice included the original rice, first-, second-, and third-pass flours, and residual kernel after the third milling pass. The rice samples and the milling process have been described previously (3).

Amino acids were determined on acid hydrolysates, on an automated amino acid analyzer, according to the principle of ion-exchange column chromatography. Cystine, as cysteic acid, and tryptophan were analyzed separately. Each of the 60 samples were analyzed at least in duplicate, i.e., single aminograms on duplicate hydrolysates. Results are expressed in grams of amino acid per 16 g. nitrogen.

An appropriate weight of material, 15 to 60 mg., was refluxed with 250 ml. 6N HCl, reagent grade, not glass-distilled, for 24 hr. at atmospheric pressure. The hydrolysate was cooled to 10°C., filtered through Whatman No. 42 filter paper, and evaporated in two stages in a rotary evaporator. Evaporation was assisted by the use of a water bath at 40° to 45 °C. The residue was transferred to a graduated cylinder and made to an appropriate volume, usually 10 ml., with a buffer solution at pH 2.2 containing *nor*-leucine as a tracer.

Aliquots of the acid hydrolysates were chromatographed on a Jeolco amino acid analyzer Model JLC-5AH, with special resins.<sup>2</sup> Reproducibility was 2 to 5%. Amino acid nitrogen as percent of the Kjeldahl nitrogen ranged from 81 to 87, with only 7 of the 60 samples having values less than 84. Results were not corrected for loss of amino acids during hydrolysis nor for incomplete hydrolysis, because correction factors have not been worked out for our conditions of hydrolysis. Reported methionine is the sum of the values from the methionine and the methionine sulfoxidine peaks.

Cystine was determined separately. Seventy to 150 mg. of sample was taken for analysis. Oxidation of the cystine moiety in the intact protein to cysteic acid, with performic acid, was carried out as described by Jamalian and Pellett (5). Hydrolysis of the protein was accomplished as described above. The hydrolysate was chromatographed on a Biorad Ag 50W-X8 ion-exchange column by the method of Sanda (6). The eluted cysteic acid was reacted with ninhydrin, and the color was measured in a photoelectric colorimeter at 570 nm. Tryptophan was determined by enzymatic hydrolysis with papain, followed by reaction of the liberated tryptophan with Ehrlich's reagent (*p-N*-dimethylaminobenzaldehyde) according to the method of Lombard and de Lange (7).

PERs were determined by the rat-growth method of Osborne et al. (8), with modifications, on 20 rice samples.

For the 1966 rices, male weaning rats of the Long-Evans strain, 20 days old and weighing 40 to 48 g., were housed in separate cages and fed a stock diet for 3 days. They were then divided into groups of 10 animals with average weights of 51 to 53 g. for 11 of the groups and 61 to 62 g. for 10 groups. Each group was fed either a

<sup>2</sup>Developed by ANC Research.

test or a casein diet for 14 days. Rats were weighed twice a week, and food jars were weighed and replenished three times weekly. Each animal was fed a vitamin mix three times weekly, and received per day: thiamine hydrochloride, 50  $\gamma$ ; riboflavin, 100  $\gamma$ ; niacinamide, 600  $\gamma$ ; calcium pantothenate, 320  $\gamma$ ; pyridoxine hydrochloride, 96  $\gamma$ ; folic acid, 10  $\gamma$ ; biotin, 10  $\gamma$ ; vitamin B<sub>12</sub>, 0.2 g.; vitamin K (menadione), 4  $\gamma$ ; vitamin A acetate, 82 I.U.; irradiated ergosterol, 8.2 I.U.; DL- $\alpha$ -tocopherol, 1.8 I.U.

Fourteen rice samples were tested as follows: 1) First-pass flours through 40-mesh screen of all six varieties of the 1966 rices; and 2) whole-kernel, second and third-pass flours through 40-mesh screen, and residual kernel of one long-grain variety, Bluebonnet 50, and one short-grain, Colusa. The whole and residual kernels were ground to pass through 60- to 80-mesh screens.

The composition of the rice diets was: 90% rice product; 3.5% salt mix, UCB-1Rb (7); and 5% cottonseed oil (or oil plus corn starch to give 5% fat when the rice product contained fat). Test diets containing 90% rice were fed without being adjusted to the customary 10% protein, because some of the rices contained less protein. Because the rice diets contained different amounts of protein, seven casein diets at levels of protein near those of the test diets were fed as controls. The composition of the casein diets was similar to that of the test diets, except that casein and corn starch replaced the rice product. The high-nitrogen casein<sup>3</sup> was of a food and pharmaceutical grade as specified by the Animal Nutrition Research Council.

A similar procedure was used for the 1968 rices; only first-pass flours were tested for these six lots. Three casein diets at levels of protein near those of the test diets were fed as controls. After the experimental period of 14 days, sufficient diet was available to continue the test for an additional 14 days. Results for both the 14-day and the 28-day periods are reported.

## RESULTS AND DISCUSSION

In general, the amino acid composition of the protein in the various fractions of any particular lot of rice was remarkably uniform (Tables I and II). The similarity in values can be noted by the small standard deviation of the averages of the five fractions as typified by the data for Colusa (Table II) and for all 12 lots (Table I). Differences between the highest and lowest values of a given amino acid in the five fractions of a single lot of rice averaged about 8% for the 12 lots, with only an occasional difference over 15%, mostly in methionine and proline. Amino acid analyses, including acid hydrolysis of the sample, are subject to experimental error which may range from 1 to 10%.

When an occasional anomalous value was obtained, it could usually be related to a somewhat lower percentage of amino acid nitrogen recovered in the acid hydrolysate. The low recovery was probably due to oxidation of some of the more sensitive amino acids, such as cystine, tyrosine, and methionine. In the hydrolysis procedure used, air was excluded to a considerable degree by the addition of a large quantity of acid in relation to the sample size, a ratio of more than 4,000:1 by weight. This also diluted the concentration of material, and prevented interaction

<sup>3</sup>"Sheffene," obtained from Sheffield Chemical, Division of National Dairy Products Corp., Norwich, N.Y.

TABLE I. AMINO ACIDS IN SAMPLES FROM THE OVERMILLING OF MILLED RICE,  
AVERAGE AND STANDARD DEVIATION OF 12 LOTS

Amino Acid	Amino Acids, g. per 16 g. N					Residual kernel after third pass	Average of All Determinations <i>n</i> = 60	Standard Deviation	
	Whole kernel	Flour through 40-mesh screen			Individual determinations <i>n</i> = 60			Average of five fractions <i>n</i> = 5	
		First pass	Second pass	Third pass					
Lysine	4.00 ± 0.09	4.04 ± 0.16	3.85 ± 0.17	3.80 ± 0.14	3.95 ± 0.13	3.93	0.17	0.10	
Histidine	2.60 ± 0.07	2.79 ± 0.08	2.72 ± 0.09	2.69 ± 0.10	2.60 ± 0.10	2.68	0.11	0.08	
Arginine	9.39 ± 0.11	9.18 ± 0.19	9.42 ± 0.22	9.52 ± 0.21	9.44 ± 0.22	9.39	0.22	0.13	
Aspartic acid	9.21 ± 0.31 <sup>a</sup>	9.16 ± 0.31 <sup>d</sup>	9.13 ± 0.45	9.38 ± 0.65	9.28 ± 0.36	9.23	0.44	0.10	
Threonine	3.49 ± 0.19	3.70 ± 0.08	3.51 ± 0.22	3.52 ± 0.19	3.50 ± 0.08	3.54	0.18	0.09	
Serine	4.95 ± 0.23	5.10 ± 0.16	5.08 ± 0.29	5.24 ± 0.13	5.01 ± 0.18	5.08	0.22	0.11	
Glutamic acid	17.57 ± 0.52	17.92 ± 0.82	18.26 ± 0.57	18.71 ± 0.72	17.54 ± 0.70	18.00	0.79	0.49	
Proline	4.58 ± 0.38	4.77 ± 0.46	4.76 ± 0.45	4.70 ± 0.34	4.61 ± 0.40	4.68	0.40	0.09	
Glycine	4.58 ± 0.19	4.92 ± 0.23	4.73 ± 0.46	4.50 ± 0.22	4.52 ± 0.15	4.65	0.30	0.18	
Alanine	5.54 ± 0.23	5.75 ± 0.21	5.55 ± 0.25	5.44 ± 0.24	5.48 ± 0.21	5.55	0.25	0.12	
Cystine	1.69 ± 0.17	1.70 ± 0.15	1.70 ± 0.16	1.70 ± 0.14	1.70 ± 0.15	1.70	0.15	0.04	
Valine	5.77 ± 0.37	5.86 ± 0.30	5.79 ± 0.21	5.81 ± 0.23	5.91 ± 0.21	5.83	0.27	0.06	
Methionine	2.25 ± 0.27	2.19 ± 0.37	2.27 ± 0.19 <sup>e</sup>	2.16 ± 0.28	2.28 ± 0.29	2.24	0.28	0.05	
Isoleucine	4.10 ± 0.09 <sup>bc</sup>	4.03 ± 0.20	4.04 ± 0.14	4.04 ± 0.14	4.15 ± 0.13	4.07	0.15	0.05	
Leucine	8.15 ± 0.29	8.07 ± 0.39	8.20 ± 0.23	8.31 ± 0.19	8.20 ± 0.29	8.19	0.29	0.29	
Tyrosine	5.19 ± 0.28 <sup>b</sup>	5.03 ± 0.39	5.30 ± 0.13 <sup>e</sup>	5.36 ± 0.20	5.31 ± 0.20 <sup>e</sup>	5.24	0.28	0.13	
Phenylalanine	5.12 ± 0.19 <sup>b</sup>	5.07 ± 0.27	5.16 ± 0.18	5.20 ± 0.23	5.14 ± 0.16	5.14	0.20	0.05	
Tryptophan	1.74 ± 0.30	1.73 ± 0.29	1.73 ± 0.29	1.74 ± 0.30	1.72 ± 0.29	1.73	0.29	0.01	

<sup>a</sup>California Calrose excluded.

<sup>b</sup>Belle Patna excluded.

<sup>c</sup>Saturn excluded.

<sup>d</sup>Bluebonnet 50 excluded.

<sup>e</sup>California Pearl, parboiled, excluded.

TABLE II. AMINO ACID CONTENT OF SAMPLES FROM THE  
 OVERMILLING OF MILLED RICE, COLUSA VARIETY

Amino Acid	Amino Acids, g. per 16 g. N				Residual Kernel after third pass	All Fractions Avg. & Std. Deviation
	Whole kernel	Flour through 40-mesh screen				
		First pass	Second pass	Third pass		
Lysine	3.98	4.10	3.83	3.96	3.76	3.93 ± 0.13
Histidine	2.48	2.58	2.70	2.60	2.54	2.58 ± 0.08
Arginine	9.53	9.31	9.48	9.45	9.70	9.49 ± 0.14
Aspartic acid	9.37	9.21	8.85	9.14	9.70	9.25 ± 0.31
Threonine	3.56	3.74	3.35	3.50	3.58	3.55 ± 0.14
Serine	5.01	5.17	5.16	5.46	5.24	5.21 ± 0.16
Glutamic acid	17.80	18.28	18.25	18.29	18.24	18.17 ± 0.21
Proline	4.58	5.32	4.97	5.20	4.32	4.88 ± 0.42
Glycine	4.75	4.82	4.51	4.56	4.72	4.67 ± 0.13
Alanine	5.74	5.76	5.78	5.38	5.78	5.69 ± 0.17
Cystine <sup>a</sup>	1.48	1.58	1.53	1.56	1.58	1.55 ± 0.04
Cystine <sup>b</sup>	1.46	1.65	1.40	1.36	0.81	1.34 ± 0.31
Valine	6.21	5.68	5.94	5.74	5.94	5.90 ± 0.21
Methionine	2.36	2.16	2.50	2.34	1.94	2.26 ± 0.22
Isoleucine	4.15	4.22	4.16	4.16	4.10	4.16 ± 0.04
Leucine	8.16	7.72	8.46	8.12	8.15	8.12 ± 0.26
Tyrosine	5.50	4.93	5.29	5.34	5.04	5.22 ± 0.23
Phenylalanine	5.34	5.32	5.38	5.40	5.28	5.34 ± 0.05
Tryptophan	2.04	2.01	2.02	2.03	2.02	2.02 ± 0.01
Amino acid N, %	87.2	87.2	87.1	87.0	86.6	87.0 ± 0.2
N in sample, % dry basis	1.09	1.83	1.72	1.66	0.99	...

<sup>a</sup>Determined by conversion to cysteic acid.

<sup>b</sup>Determined from acid hydrolysate.

between the amino acids and other constituents of the rice, such as hydrolytic products of starch. Although not an infallible system, it was satisfactory in a high percentage of samples. A comparison of the data for cystine in Colusa, obtained with and without conversion to cysteic acid, shows relatively good values for cystine without conversion, but much more uniform values with conversion.

This uniformity of amino acid composition in the various milling fractions is in agreement with the work of Juliano<sup>4</sup> who stated that similar aminograms for the protein of milling fractions of milled rice (results presented in this paper) reflect the same composition of rice protein bodies with the rice endosperm.

With some amino acids, differences among varieties were much greater than among fractions of the same lot of rice. The greatest varietal difference was found with tryptophan in CC, where the highest value, 2.15 g. per 16 g. nitrogen, was 73% greater than the 1.24 g. in CP (Table III). The highest value for methionine, 2.70 in TE, was 61% greater than the lowest, 1.68 in CP, while the highest value for cystine, 1.94 in TL, was 31% greater than the lowest, 1.48, in CS and CC.

Lysine, the first limiting essential amino acid in rice protein, showed little variability in the original kernel among the rice varieties analyzed, with average and standard deviation of  $4.00 \pm 0.09$  g. per 16 g. nitrogen (Table I), ranging from 3.84 for CP to 4.14 for S and TP, a difference of 8%. Differences between the highest and lowest values for the other 14 amino acids averaged 12%, with differences for arginine, glutamic acid, and isoleucine only about 5%.

<sup>4</sup>Private communication.

TABLE III. CYSTINE, METHIONINE, AND TRYPTOPHAN  
(g. AMINO ACID/16 g. N) IN 12 LOTS OF RICE  
OF DIFFERENT VARIETIES AND TREATMENTS

Rice Lot and Variety <sup>a</sup>	Cystine		Methionine		Tryptophan	
	Whole kernel	Five fractions	Whole kernel	Five fractions	Whole kernel	Five fractions
		Avg.		Std. dev.		Avg.
1966						
BP	1.85	1.84 ± 0.04	2.27	2.37 ± 0.17	1.75	1.74 ± 0.01
BB	1.70	1.71 ± 0.05	2.27	2.30 ± 0.31	1.90	1.87 ± 0.06
CR	1.56	1.56 ± 0.03	2.41	2.14 ± 0.20	2.04	2.03 ± 0.02
S	1.93	1.89 ± 0.03	2.32	2.35 ± 0.16	1.88	1.89 ± 0.02
CL	1.65	1.66 ± 0.03	2.29	2.25 ± 0.08	1.99	2.01 ± 0.04
CS	1.48	1.55 ± 0.04	2.36	2.26 ± 0.21	2.04	2.02 ± 0.01
1968						
CB	1.86	1.84 ± 0.02	2.50	2.43 ± 0.06	1.60	1.59 ± 0.02
CC	1.48	1.50 ± 0.02	2.05	2.08 ± 0.16	2.15	2.08 ± 0.04
TE	1.72	1.69 ± 0.04	2.70	2.67 ± 0.18	1.57	1.51 ± 0.04
TL	1.94	1.93 ± 0.01	1.88	2.02 ± 0.27	1.35	1.34 ± 0.02
CP	1.64	1.64 ± 0.04	1.68	1.72 <sup>b</sup> ± 0.09	1.24	1.25 ± 0.02
TP	1.51	1.53 ± 0.03	2.22	2.06 ± 0.18	1.42	1.45 ± 0.03
Avg.	1.69	1.70	2.25	2.22	1.74	1.73
Std. dev.	±0.17	±0.15	±0.27	±0.24	±0.30	±0.29

<sup>a</sup>BP, Texas Belle Patna; BB, Arkansas Bluebonnet 50; CR, California Calrose; S, Louisiana Saturn; CL, California Caloro; CS, California Colusa; CB, California Belle Patna; CC, California Calrose; TE, Texas Belle Patna, early seeding; TL, Texas Belle Patna, late seeding; CP, California Pearl, parboiled, medium; TP, Texas Belle Patna, parboiled, light.

<sup>b</sup>Second-pass flour excluded.

The average percentages of amino acids for the original rice are within 10% of the chemical values (58 samples) for each amino acid as reported by the FAO (9), except for arginine and tyrosine. Our values for the latter two are 24 and 64% higher, respectively, but agree within 3% or less for isoleucine, leucine, valine, alanine, aspartic acid, and proline. Except for glutamic acid which was 9% lower, all of our other amino acid values were from 4 to 15% higher. The data reported by the FAO for arginine and tyrosine may be low; we found tyrosine, in particular, to be very sensitive to hydrolysis.

The data for high-protein rice flours obtained in the present study are in agreement with those of Normand et al. (1), who analyzed the amino acids in 12 flours milled successively from a sample of commercially milled, long-grain rice, predominantly Bluebonnet 50. These workers noted a slight increase in amino acid contents in some fractions as well as a slight decrease in others, but stated that, for practical considerations, their data indicated no essential differences in amino acid content as compared with the original kernel except perhaps in the decrease in tryptophan as the center of the kernel was approached. This decrease in tryptophan was not observed in any of the rices in the present study.

Our values check fairly well with the amino acid composition reported by Houston et al. (2) for the original kernel of the same six lots of 1966 rices used by us in the present study. Amino acid nitrogen recovery was somewhat low (80.7%) for our analysis of Belle Patna, and is associated with low values for some of the amino acids, whereas nitrogen recoveries as reported by Houston et al. were

somewhat low (94.7 and 94.8%) for samples of Caloro and Colusa. Our results for two amino acids are lower: methionine uncorrected in our study, 10 to 27% and cystine by performic acid oxidation in both studies, 33%, while the three basic amino acids—lysine, histidine, and arginine—are about 10% higher.

In the same study Houston et al. also reported the amino acid analysis of high-protein flour and residual kernel of Calrose. The composition of the residual kernel was very close to that of the original rice in both works. In the analysis of the high-protein flour by Houston et al. the nitrogen recovery was only 90.2%, and values for some of the amino acids are low, particularly glutamic acid, leucine, tyrosine, and phenylalanine. In both studies lysine was 0.4% higher in the high-protein flour than in the original rice.

TABLE IV. PROTEIN EFFICIENCY RATIOS OF FIRST-PASS FLOURS THROUGH 40-MESH SCREEN AND OF ALL FRACTIONS OF BLUEBONNET 50 AND COLUSA, 1966 RICES AS DETERMINED BY RAT GROWTH<sup>a</sup>

Protein Source	Protein in Diet %	Food Intake %	Gain in Body Weight		Protein Efficiency Ratio			
			Avg. Std. Dev. g.	Avg. Std. Dev.	Adjusted to casein at 2.5		Avg. Std. Dev.	
					Avg.	Std. Dev.		
Casein	5.3	140	21 ± 8	2.71 ± 0.92				
	8.8	179	44 ± 9	2.78 ± 0.52				
	9.9	184	53 ± 11	2.89 ± 0.39				
	12.4	200	67 ± 11	2.69 ± 0.18				
	13.5	182	70 ± 6	2.85 ± 0.19				
	16.3	193	85 ± 7	2.72 ± 0.19				
				2.77 ± 0.08 <sup>b</sup>				
Flour, first pass								
BP	15.4	188	67 ± 6	2.32 ± 0.15			2.09 ± 0.14	
BB	11.9	175	47 ± 8	2.26 ± 0.26			2.04 ± 0.23	
CR	10.0	211	54 ± 5	2.75 ± 0.26			2.48 ± 0.23	
S	11.0	211	60 ± 6	2.60 ± 0.18			2.35 ± 0.16	
CL	9.1	219	41 ± 9	2.36 ± 0.29			2.13 ± 0.26	
CS	9.0	195	40 ± 7	2.26 ± 0.24			2.04 ± 0.22	
					2.42 ± 0.20 <sup>b</sup>		2.19 ± 0.18 <sup>b</sup>	
Bluebonnet 50								
Whole kernel	6.1	142	21 ± 5	2.24 ± 0.51			2.02 ± 0.46	
Flour, first pass	11.9	175	47 ± 8	2.26 ± 0.26			2.04 ± 0.23	
Flour, second pass	11.4	176	42 ± 8	2.12 ± 0.27			1.91 ± 0.24	
Flour, third pass	10.6	191	43 ± 8	2.12 ± 0.29			1.91 ± 0.26	
Residual kernel	5.7	125	17 ± 6.5	2.37 ± 0.90			2.14 ± 0.81	
					2.22 ± 0.11 <sup>b</sup>		2.00 ± 0.10 <sup>b</sup>	
Colusa								
Whole kernel	4.8	137	15 ± 3	2.31 ± 0.54			2.08 ± 0.49	
Flour, first pass	9.0	195	40 ± 7	2.26 ± 0.24			2.04 ± 0.22	
Flour, second pass	8.2	179	29 ± 5	1.99 ± 0.26			1.80 ± 0.23	
Flour, third pass	7.7	182	28 ± 7	2.00 ± 0.33			1.80 ± 0.30	
Residual kernel	4.4	129	11 ± 6	1.95 ± 0.87			1.76 ± 0.79	
					2.10 ± 0.17 <sup>b</sup>		1.90 ± 0.15 <sup>b</sup>	

<sup>a</sup>Ten male rats per group; experimental period, 14 days.

<sup>b</sup>Average and standard deviation of the averages.

Average PER with standard deviation for 20 test diets containing rice protein, when adjusted to casein at 2.5, was  $2.03 \pm 0.20$  for a 14-day experimental period (Tables IV and V). Animals fed rice diets had weight gains and PERs of about 80% those of animals fed casein diets of equivalent protein content.

The average PER adjusted to casein for five fractions of Bluebonnet 50 was 2.00 and for Colusa, 1.90; values varied from 1.95 to 2.37, and from 1.76 to 2.14. Because the casein control groups gave such similar PERs, the average value of 2.77 for the six groups fed different levels of casein was used for the adjustment. No statistically significant differences according to the t-test ( $P = 0.01$ ) were found among the five fractions of Bluebonnet 50 nor among the fractions of Colusa. Animals fed diets containing 6% or less of protein (1 to 2 g. protein nitrogen intake per 14 days) were more variable in their response, as can be seen from the higher deviations of the PER (0.51 to 0.92), than were those animals fed higher-protein diets (Table IV). Rats eating 2 to 3.5 g. protein nitrogen in 14 days (diets

TABLE V. PROTEIN EFFICIENCY RATIOS OF FIRST-PASS FLOURS OF 1968 RICES AS DETERMINED BY RAT GROWTH<sup>a</sup>

Protein Source	Protein in Diet %	Food Intake %	Gain in Body Weight		Protein Efficiency Ratio	
			Avg. g.	Std. Dev.	Avg.	Std. Dev.
<b>14 days</b>						
Casein	9.2	163	44 ± 10		2.86 ± 0.33	
	12.6	148	43 ± 2		2.30 ± 0.09	
	14.9	166	68 ± 6		2.75 ± 0.18	
CB	12.4	146	37 ± 7		2.05 ± 0.40	2.23 ± 0.43
CC	9.4	145	34 ± 6		2.48 ± 0.13	2.17 ± 0.11
TE	14.0	154	42 ± 11		1.95 ± 0.46	1.77 ± 0.42
TL	15.3	137	46 ± 8		1.99 ± 0.24	1.81 ± 0.22
CP	12.1	130	30 ± 5		1.92 ± 0.24	2.09 ± 0.26
TP	13.2	122	28 ± 7		1.81 ± 0.22	1.97 ± 0.24
Average <sup>c</sup>					2.03 ± 0.23	2.01 ± 0.19
<b>28 days</b>						
Casein	9.2	376	89 ± 20		2.54 ± 0.25	
	12.6	360	103 ± 13		2.27 ± 0.12	
	14.9	394	137 ± 11		2.34 ± 0.16	
CB	12.4	343	87 ± 16		2.02 ± 0.16	2.22 ± 0.18
CC	9.4	327	64 ± 10		2.07 ± 0.13	2.04 ± 0.13
TE	14.0	376	95 ± 16		1.80 ± 0.20	1.92 ± 0.21
TL	15.3	330	102 ± 13		1.82 ± 0.12	1.94 ± 0.13
CP	12.1	270	56 ± 6		1.71 ± 0.11	1.88 ± 0.12
TP	13.2	286	66 ± 11		1.73 ± 0.14	1.91 ± 0.15
Average <sup>c</sup>					1.86 ± 0.15	1.98 ± 0.13

<sup>a</sup>Ten male rats per group.

<sup>b</sup>Adjusted to casein at 2.5.

<sup>c</sup>Average and standard deviation of test groups.



containing 8 to 11% protein) gave PERs with standard deviations of about 0.30; those eating 4 to 5 g. nitrogen (diets over 11% protein), about 0.18.

Average PER for the six first-pass flours of the 1966 rices was 2.19 when adjusted to casein. PERs for CR and S, 2.48 and 2.35 (Table IV), were significantly higher than for the other varieties, except that of S was not significantly higher than that of CL. There were no significant differences between PERs for CR and S nor among the other flours.

Average PER for the first-pass flours of the six lots of 1968 rices was 2.01 for the 14-day feeding period and 1.98 for the 28-day period when adjusted to casein. Casein and rice diets containing 9 and 14 to 15% protein were run at the same time, while those containing about 12% protein were begun 2 weeks later. PER for the casein control at the latter protein level was lower than for the other casein groups. Except for CC, PERs among these flours were not significantly different. The PER for CC was significantly higher ( $P = 0.01$ ) than only those for TE and TL.

Sufficient first-pass flour from the 1968 rices was available to extend the experimental period to 28 days, a period which has been shown to give more consistent results although usually with slightly lower values because of slowing of the growth rate. With the longer time and when adjusted to casein, PER for CB was significantly higher than for the other five flours, where  $P = 0.01$ , except that for CC,  $P = 0.02$ . There were no significant differences among the other five flours.

The average PER with standard deviation for all 12 first-pass flours, 14-day period, was  $2.10 \pm 0.20$ , adjusted to casein. Excluding the flours with the two highest and the two lowest values, there were no significant differences among them. Three flours with the highest PER—CR, S, and CB, with 2.48, 2.35, and 2.23, respectively—were not significantly different from each other. The PER for Calrose (CR) was significantly higher than for the remaining nine flours including a second lot of Calrose (CC), and that for S was higher than all except for CS and CP. PER for CB was not significantly different from any of the flours. Of the two flours with the lowest PERs, that for TL, at 1.81, was significantly lower than for five others—the two lots of Calrose, S, CS, and BP—while PER for TE, at 1.77 and with a larger variance, was significantly lower than those for only three flours—the two lots of Calrose and S.

PER and net protein values of 1.84 (casein 2.57) and 61, respectively, were reported by Milner (4) for a rice flour containing 14.2% protein, which represented 8.1% weight fraction of a California Pearl rice analyzing 7.8% protein. This PER falls within the range of values obtained in the present study.

Differences in amino acid composition of the rice protein do not appear to be related to differences in PERs. Of those rice fractions tested biologically, the lowest values, whether for PER, lysine, threonine, or cystine plus methionine, were from 20 to 30% lower than the highest values. The highest and lowest values for threonine and for cystine plus methionine were scattered throughout the range of values for PER. The higher values for lysine tended to be associated with high PER, as with the first-pass flours of CR and S. But some of the higher PERs occurred in rice fractions with somewhat lower percentages of lysine.

Variance in the response of animals to test diets, particularly in short-term experiments, and in conditions for evaluating PER, as well as experimental error in amino acid analyses, make it difficult to ascertain clear-cut relations between PERs and amino acid content when differences, if they actually exist, are less than 30%.

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