(14) Jack, E. L., Henderson, J. L., Hinshaw, E. B., *J. Biol. Chem.* **162,** 119 (1946).
(15) Jack, E. L., Smith, L. M., *J. Dairy Sci.* **39,** 1 (1956).
(16) Kartha, A. R. S., *J. Am. Oil Chemists' Soc.* **31,** 85 (1954).
(17) Longenecker, H. E., *Chem. Revs.* **29,** 201 (1941).
(18) Patton, Stuart, Evans, Laura, McCarthy, R. P., *J. Dairy Sci.* **43,** 95 (1960).
(19) Savary, P., Flanzy, J., Desnuelle, P., *Biochim. et Biophys. Acta* **24,** 414 (1957).
(20) Scott, W. E., Herb, S. F., Magidman, Paul, Riemenschneider, R. W., J. Agr. Food Chem. **7,** 125 (1959).
(21) Shorland, F. B., Hansen, R. P., *Dairy Sci. Abstr.* **19,** 168 (1957).
(22) Smith, L. M., Freeman, N. K., Jack, E. L., *J. Dairy Sci.* **37,** 399 (1954).

Received for review April 18, 1960. Accepted June 14, 1960. Division of Agricultural and Food Chemistry, 137th Meeting, ACS, Cleveland, Ohio, April 1960. Address given at ACS Award in Chemistry of Milk, sponsored by The Borden Foundation, Inc.

RICE PROTEIN SUPPLEMENTATION

Further Studies on the Nutritional Improvement of Rice

M. C. KIK

Department of Agricultural Chemistry, University of Arkansas, Fayetteville, Ark.

The proteins of whole and milled rice can be effectively supplemented by those of whole milk solids, lactalbumin, and defatted whole egg. Data are presented on the content of amino acids (essential and nonessential), vitamins, and other constituents of milk solids, and defatted whole egg. Lactalbumin was tested for total amino acid content. The results of these experiments show the extent to which rice rations can benefit from addition of small amounts of milk solids, lactalbumin, and whole egg. This is of importance in those areas of the world where much rice is consumed and where these high protein foods are economically available.

In an attempt to improve rice diets further, studies were made with whole milk powder, nonfat dried milk (skimmilk), lactalbumin, and nonfat whole egg powder as supplements to whole rice and to white milled rice. An investigation was started on the effect of replacing whole rice proteins with those of milk solids and powdered whole egg; 1, 3, and 5% of these solids replaced equivalent amounts of proteins in milled and whole rice rations which were fed to albino rats for 70 days. Another series of experiments was made on the effect of adding small amounts of these solids to rations containing rice as the only source of protein. This paper also reports studies on the content of all members of the vitamin B complex, amino acids (including nonessentials), calcium, phosphorus, and iron, and on the growth value of the proteins of samples of commercial dry milk solids, lactalbumin, and whole egg powder.

Experimental Procedure and Results

Commercial samples of dry milk solids (spray-dried whole milk, nonfat dry milk solids, or skimmilk), lactalbumin, and fat-extracted whole egg powder were used for the determination of vitamins, minerals, amino acids, and supplementary and growth values of the proteins.

Supplementary values were determined in studies using albino rats as experimental animals fed milled white rice rations containing 5.5% protein and whole rice rations containing 6.38% protein. Milled and whole rice furnished the only source of protein in these rations and at levels to incorporate the necessary protein. The per cent of protein content of the white milled rice was 6.45, of whole brown rice 7.25, of dried whole milk 25.0, of fat-extracted whole egg 65.8, of lactalbumin 80.7, and of skimmilk 36.2. The composition of the rest of the rations was 4% of salt mixture No. 1 (*6*), 4% of hydrogenated vegetable shortening, 2% of cellu flour, 2% of cod liver oil, and 1% of wheat germ oil as sources of vitamins A, D, and E, and the rest as glucose (cerelose).

In the protein replacement experiments, the other solids were added at three levels—1, 3, and 5%; the proteins of these solids were added at the expense of the rice proteins, leaving the total protein in the ration the same. In the protein addition experiments, the solids were added also to the basal ration at these levels of 1, 3, and 5% at the expense of cerelose, and the protein content was slightly increased.

The following crystalline components of the vitamin B complex were administered daily to each animal separately from the rations with double doses on Saturday: 25 γ each of thiamine, riboflavin, pyridoxin, and niacin; 150 γ of calcium pantothenate; 3 mg. of *p*-aminobenzoic acid; 6 mg. of choline chloride; and 1 mg. of inositol. The animals, 28 to 32 days old when started on the experiment and weighing about 50 grams each, were divided in two groups, each having equal numbers of both sexes; they were fed for 70 days. Each animal was weighed weekly and accurate weekly records for food consumption were kept. From these data the protein efficiency ratios were calculated and expressed as gains in body weight per gram of protein intake.

In the study (*3*) of the supplementary value of the proteins of whole milk solids for those of white milled rice, an equivalent amount of the proteins of rice was replaced by the proteins of 1, 3, and 5% whole milk powder, leaving the protein level at 5.55. In experiments reported here, 1, 3, and 5% of whole milk were added, which increased the protein level to 5.86, 6.24, and 6.62%, respectively (Table I, *A*, ration 9 listed).

The protein content of the various foods was determined from nitrogen

Table I. Supplementary Relationship between Various Solids

(12 animals in each group. Average results per animal for a 10-week period)

	Ration	Protein in Ration, %	Gains in Body Weight, Grams	Gains in Body Weight, %	Protein Intake, Grams	Protein Efficiency Ratio[a], Grams	Protein Efficiency Ratio[a], %
	A. Proteins in Whole Milk Solids and Rice[b]						
1	Whole rice	6.38	82.3 ± 3.7	...	43.8	1.87 ± 0.03[c]	...
2	Whole rice + 1% whole milk	6.38	111.7 ± 5.5	35.7[d]	50.4	2.21 ± 0.06	18.1[a]
3	Whole milk solids	6.38	75.7 ± 4.5	...	44.6	1.69 ± 0.05	...
4	Whole rice + 1% whole milk	6.56	127.1 ± 3.4	54.3[d]	54.9	2.31 ± 0.13	23.8[d]
5	Whole milk solids	9.0	148.3 ± 4.0	...	72.5	2.04 ± 0.10	...
6	Whole rice + whole milk	9.0	175.8 ± 9.9	18.5[d]	86.8	2.02 ± 0.08	−1.0
7	Polished rice + whole milk	9.0	182.8 ± 13.0	23.2[d]	84.8	2.17 ± 0.10	6.3
8	Polished rice	5.55	63.6 ± 4.6	...	42.2	1.50 ± 0.04[c]	...
9	Polished rice + 1% whole milk	5.86	78.4 ± 5.0	23.1[d]	44.0	1.78 ± 0.07	18.6[d]
	B. Proteins in Dried Nonfat Milk Solids and Rice[b]						
10	Polished rice	5.55	63.6 ± 4.6	...	42.2	1.50 ± 0.04[c]	...
11	Polished rice + 1% N.F.M.	5.55	99 ± 3.3	55.6[d]	50.7	1.94 ± 0.04	29.3[d]
12	N.F.M.	6.38	124.4 ± 4.0	...	59.2	2.11 ± 0.06	40.6[d]
13	Polished rice + 1% N.F.M.	5.73	97.4 ± 6.0	53.1[d]	46.7	2.08 ± 0.08	38.6[d]
14	N.F.M.	5.68	97.7 ± 2.8	..	46.3	2.11 ± 0.05	40.6[d]
15	Whole rice	6.31	72.0 ± 4.4	...	41.5	1.73 ± 0.06	15.3[d]
16	Whole rice + 1% N.F.M.	6.31	87.0 ± 6.5	20.8[d]	43.6	1.98 ± 0.11	32.0[d]
17	N.F.M.	9.0	187.0 ± 11.8	160.0[d]	89.3	2.08 ± 0.13	32.0[d]
	C. Lactalbumin and Protein in Rice[b]						
18	Polished rice	5.42	71 ± 4.7	...	35.4	2.01 ± 0.07[c]	...
19	Polished rice + 1% lactalbumin	5.42	96 ± 5.2	35.2	39.8	2.41 ± 0.08	14.9[d]
20	Lactalbumin	5.42	20 ± 3.4	...	23.8	2.09 ± 0.19	...
21	Lactalbumin	9.0	179 ± 6.6	...	72.5	1.79 ± 0.08	...
22	Whole rice	6.31	72 ± 4.4	...	41.6	1.93 ± 0.06	...
23	Whole rice + 1% lactalbumin	6.31	93 ± 6.4	29.1[d]	41.3	2.23 ± 0.03	15.5[d]
	D. Proteins in Fat-Extracted Whole Eggs and Rice[b]						
24	Polished rice	5.55	73 ± 3.4	...	41.9	1.74 ± 0.03[c]	...
25	Polished rice + 1% whole egg	6.05	113.8 ± 8.8	55.9[d]	51.5	2.24 ± 0.04	28.7[d]
26	Whole egg	6.38	113.9 ± 8.8	...	51.5	2.21 ± 0.03	27.0[d]
27	Whole rice	6.38	82.3 ± 3.7	...	43.9	1.87 ± 0.03	...
28	Whole rice + 1% whole egg	6.38	143.3 ± 7.0	74.1[d]	58.2	2.46 ± 0.04	31.5[d]
29	Whole egg	9.00	142.2 ± 8.5	...	78.9	1.78 ± 0.08	...

[a] Gain in body weight per gram of protein intake.
[b] Milled white polished rice and whole brown rice.
[c] Standard error.
[d] Significant for $P = 0.05$.

Table II. Determination of Amino Acids

	In Whole Milk Powder, % 25.0% Protein		In Skimmilk Powder, % 36.2% Protein		In Fat-Free Whole Egg, % 65.8% Protein		In Lactalbumin, % 80.7% Protein		In White Milled Rice, % 6.01% Protein (4)	
	In dry matter	In protein	In dry matter	In protein	In dry matter	In protein	In dry matter	In protein	In dry matter	In protein
Alanine	0.80	3.20	1.30	3.58	2.10	3.19	4.24	5.29		
Arginine[a]	1.00	4.00	1.30	3.59	4.31	6.55	3.00	3.72	0.62	10.31
Aspartic acid	2.00	8.00	3.00	8.28	5.23	7.95	8.01	9.93	0.29	4.82
Cystine	0.25	1.00	0.40	1.10	1.54	2.34	3.10	3.84	0.09	1.48
Glutamic acid	5.20	20.8	6.90	19.1	8.93	13.57	12.0	14.80	0.68	11.31
Glycine	1.60	6.40	1.35	3.73	3.06	4.65	3.20	3.97	0.44	7.32
Histidine[a]	0.67	2.68	0.87	2.50	1.78	2.70	2.00	2.48	0.19	3.15
Isoleucine[a]	1.76	7.04	2.15	6.17	4.46	6.78	5.80	7.19	0.31	5.15
Leucine[a]	2.90	11.60	3.40	9.38	6.18	9.39	9.4	11.66	0.57	9.48
Lysine[a]	1.80	7.20	2.58	7.13	4.62	7.02	6.60	8.18	0.22	3.66
Methionine[a]	0.90	3.60	0.96	2.65	2.40	3.65	2.00	2.48	0.23	3.82
Phenylalanine[a]	1.25	5.00	1.76	4.86	3.70	5.62	4.50	5.58	0.29	4.82
Proline	2.20	8.80	2.60	7.46	3.08	4.68	4.00	4.96	0.28	4.65
Serine	1.32	5.20	1.50	4.14	4.00	6.08	3.60	4.46	0.30	4.99
Threonine[a]	1.16	4.64	1.60	4.42	3.70	5.62	4.32	5.36	0.31	5.15
Tryptophan[a]	0.40	1.60	0.50	1.38	0.92	1.40	2.00	2.48	0.05	0.83
Tyrosine	1.25	5.00	1.58	4.53	2.26	3.43	4.10	5.08	0.32	5.32
Valine[a]	1.60	6.40	2.23	6.15	4.87	7.40	5.55	6.85	0.46	7.65

[a] Nutritionally essential.

Table III. Vitamins and Other Constituents in Fat-Free Dry Whole Milk, Skimmilk, and Whole Egg Powder

	Dry Whole Milk	Skimmilk	Whole Egg
	Micrograms Per Gram		
Thiamine	2.8	3.0	4.3
Riboflavin	10.8	13.2	8.4
Nicotinic acid	15.0	14.0	4.0
Pantothenic acid			
Total	34	36.8	46.7
Free	15	23.2	39.0
Biotin	0.20	0.32	0.35
Folic acid			
Total	0.04	0.04	0.06
Free	Traces	Traces	Traces
Pyridoxine	12.0	9.3	7.6
Inositol	2300	1320	1200
Choline	1734	1750	2400
Aminobenzoic acid	0.64	0.85	0.70
	Per Cent		
Calcium	0.98	1.30	0.20
Phosphorus	0.71	0.91	0.83
Iron	0.0053	0.0060	0.0050
Nitrogen	3.97	5.67	10.28
Protein (nitrogen × factor)	25.3	36.2	65.8
Fat (in the original)	26.2	0.05	34.6
Moisture	2.3	5.6	2.5
Ash	5.3	7.2	2.3
Fiber	0	0	1.4

analyses using the factor 6.38 for the nonfat milk solids, 6.49 for lactalbumin, and 6.25 for whole egg. For conversion of total nitrogen to protein, the factor 5.95 was used for rice, as Jones (*2*) reported that the nitrogen content of oryzenin, 16.8%, fairly represents the nitrogen in the rice proteins as a whole.

In the study of the supplementary value of the proteins of whole milk solids for those of whole brown rice, the procedure followed was similar to that described for the white milled rice. In the replacement tests, the protein level was left at 6.38% (Table I, *A*, ration 2 listed), and in the addition tests, the protein level was increased from 6.38 to 6.56, 6.92, and 7.28%, respectively (Table I, *A*, ration 4 listed). The results of these supplementary studies are given in Table I. Only one level of each tested supplement is listed; similar results were obtained on higher levels.

The results, expressed as average gain per animal (Table I, *A*), indicate that the proteins of whole brown rice can also be improved by supplementation with whole milk solids like those of milled white rice, as previously demonstrated in ad libitum and paired feeding experiments (*3*).

The values are higher in the combination whole rice–whole milk than in each food separately and this constitutes a demonstration of a true supplementary relationship between two proteins (Table I, *A*, rations 1, 2, and 3). The results obtained from rations 5, 6, and 7 provide further proof of a supplementary relationship between proteins of milled and whole rice and of whole milk solids.

Table I, *B*, shows that the proteins of dried nonfat milk solids (skimmilk) have a supplementary effect for those of milled white rice and whole brown rice; Table I, *C*, shows that a supplementary relationship exists between lactalbumin and the proteins of milled white rice and of whole brown rice.

As previously shown (*3*) in ad libitum and paired feeding experiments, the proteins of whole egg supplemented those of white milled polished rice in rations where the protein level of 5.55 was kept constant and in whole rice rations fed rats at a constant level of 6.38% protein. Addition of small amounts (1, 3, and 5%) of whole egg to a white polished rice ration leads to large increases in growth and protein efficiency ratio (P.E.R.), although the protein levels of the rations are increased to a certain extent (ration 25, only one level listed, Table I, *D*). The proteins of whole brown rice are supplemented by those of whole egg as shown by the results obtained with ration 28. In the whole rice–whole egg ration 28, a higher value in gain and P.E.R. was obtained than found in either the whole rice ration alone or in the whole egg ration alone and this points to a true, real supplementation. Animals fed extracted whole egg at a 9% protein level of intake showed an average gain of 142 grams and a P.E.R. of 1.78 grams which is lower than the values found for whole milk, nonfat dry milk solids, and lactalbumin.

The results of these experiments indicate that the proteins of white milled polished rice and of whole brown rice can be further improved by supplementation with those of whole milk solids, nonfat dry milk solids, lactalbumin, and extracted whole egg. This is of importance in those areas where rice is consumed to a large extent and where milk solids, lactalbumin, and whole eggs are readily available at a reasonable cost.

A complete description of the experimental procedure for the determination of amino acids, vitamins, and the minerals calcium, phosphorus, and iron is presented in a previous publication (*5*).

Amino Acid Content. The results of the amino acid determinations in fat-free samples of whole milk solids, nonfat dry milk solids, lactalbumin, and whole egg, expressed as percentages, are given in Table II. These values are calculated on the air-dried samples and are also expressed as the percentage in the crude protein. The content of the amino acids (essential) agrees well with that reported elsewhere (*1*). Values for amino acids in rice have been reported previously (*4*) and are reproduced in Table II.

This table shows that the proteins of milk solids, whole egg, and lactalbumin have much higher amino acid content than rice proteins. Tryptophan, lysine, threonine, and the combination of cystine and methionine are much lower in rice proteins and it might be assumed that these amino acids are mainly responsible for the high supplementary effect observed in the growth experiments, especially as lysine is considered to be the main limiting amino acid in rice proteins.

A number of vitamins were determined by methods referred to elsewhere (*4*). The results of these tests are given in Table III, which also contains data on calcium, phosphorus, iron, and other constituents.

Acknowledgment

The author wishes to acknowledge the technical assistance given by Leslie Easterling and Joy Dowell.

Literature Cited

(1) Block, R. J., Weiss, K. W., "Amino Acid Handbook," Charles C Thomas, Publisher, Springfield, Ill., 1956.
(2) Jones, D. B., U. S. Dept. Agr. Circ. **183** (1931).
(3) Kik, M. C., Arkansas Agr. Expt. Sta. Bull. **508** (1951).
(4) *Ibid.*, Bull. **589** (1957).
(5) Kik, M. C., J. Agr. Food Chem. **3,** 600 (1955).
(6) Sure, B., *J. Nutrition* **22,** 499 (1941).

Received for review December 23, 1959. Accepted May 19, 1960. Published with the approval of the director of the Arkansas Agricultural Experiment Station.