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APPENDIX V-w

PROTEIN QUALITY OF CONVENTIONAL AND HIGH PROTEIN RICE VARIETIES AND DIGESTIBILITY OF GLUTINOUS AND NON-GLUTINOUS RICE BY PRESCHOOL CHILDREN

TO:

Dr. Samuel G. Kahn
Science Research Officer
Office of Nutrition
Agency for International Development
Washington, D.C. 20523

FROM:

Instituto de Investigacion Nutricional
Apartado 55
Miraflores (Lima), Peru

and

George G. Graham, M.D. &
William C. MacLean, Jr., M.D.
615 North Wolfe Street
Baltimore, Maryland 21205

SUBJECT:

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Milled rice is the staple grain for much of the world's population. Populations that traditionally depend on rice for the bulk of their energy and protein tend to be of short stature. This fact is best exemplified in the countries of tropical Asia. Most varieties of rice provide approximately 7% of energy as protein of reasonably good quality; the amino acid score, based on lysine content is about 60 (1). The digestibility of this protein, however, is less than that of other staples, in particular, wheat (2). Perhaps the most critical factor limiting growth with rice-based diets is the low energy density of cooked rice. The bulk of such diets makes it difficult to meet energy requirements with rice. These problems are of greatest importance for growing infants and children, whose requirements are greater and capacity less.

Recently, high protein varieties of rice have been developed (3). Since rice consumption is usually determined by the weight or volume of cooked rice, these varieties offer the promise of increased protein intakes for rice eating populations. Addition of high density energy (e.g., vegetable oil) while still keeping the percentage of energy supplied by protein at an acceptable level also becomes possible. Experience with other staples suggests that increases in protein quantity may be offset by decreases in protein quality or altered digestibility (3). An inverse relationship of protein content and lysine content has been noted for rice (1). The present studies were undertaken to compare isonitrogenously the protein quality of a sample of high protein rice (IR 480-5-9) with that of a sample of a

common variety of lower protein content (IR 32) and with casein. Additional studies were also carried out to assess digestibility, especially of the carbohydrate component, of these two non-glutinous rices and of a glutinous variety of rice, IR 29.

MATERIALS AND METHODS

Subjects: Eight Peruvian Mestizo male children, ages 11 to 18 months, participated in the studies[1]. All had been admitted for the acute treatment of and long-term recuperation from third degree (Gomez) malnutrition. All were well into convalescence at the time of study and fulfilled the following criteria: free of infection and parasites, no evidence of malabsorption, adequate weight gain while consuming 100 Kcal/kg body weight/day, and serum albumin concentration >3.6 mg/dl. All children participated in both the studies of protein quality and of digestibility.

Diets: Three varieties of rice (*Oryza sativa*) were provided by the International Rice Research Institute (IRRI), Los Baños, Laguna, The Philippines. IR 32 is a conventional variety of rice that provides 3.59 Kcal/gm dry weight, 7.1% protein (N x 6.25). IR 480-5-9 is a recently developed high protein variety of rice providing 3.64 Kcal/gm, 11.4% protein. Both these varieties are non-glutinous. IR 29 is a glutinous variety of rice and provides 3.66 Kcal/gm, 7.9% protein.

All three varieties were cooked as suggested by the IRRI to assure maximal hydrolysis of the starch component. In the case of IR 32 this involved addition of water equal to twice the dry weight

of rice with a boiling time of 20 minutes. IR 480-5-9 required 1.9 times its weight and IR 29 1.3 times its weight in water with boiling times of 18 and 20 minutes, respectively. All diets were then prepared in blenderized liquid form to provide an energy density of 0.67 Kcal/ml. Sodium and potassium intakes were adjusted to provide 2 mEq Na and 3 mEq K per kg body weight per day. Other minerals and vitamins were supplemented to meet U.S. Recommended Dietary Allowances. Diets were analyzed for N, Na and K regularly throughout the studies to verify that actual intakes were as calculated.

Studies of Protein Quality: The composition of the two rice and casein control diets for these studies is shown in Table I. All diets provided 240 mg N and 100 Kcal per kg body weight per day. Approximately 75 Kcal from IR 32 were required to provide the predetermined amount of N. The remaining energy in this diet was supplied by a blend (20:80) of cottonseed and soy oils. Less IR 480-5-9 was required to supply the N content of this diet. Sucrose was added so that rice plus sucrose provided 76% of calories, the remainder of energy was provided from the oil blend. The control diet provided 1.5 gm protein (N x 6.25) as casein. A mixture (47:53) of cornstarch and sucrose was used to match the carbohydrate content of the two rice diets. Cottonseed-soy oil provided 26% of the calories.

Studies of Rice Digestibility (Table II): Diets based on IR 32, IR 480-5-9 and IR 29 were prepared to provide 240 mg N and 100

Kcal/kg body weight/day. These diets differed from those used in assessment of protein quality in that all three rice diets provided approximately 13.2 g rice (48 Kcal). Nitrogen content of the two lower-protein rice diets was completed with casein. Sucrose was added to adjust energy from carbohydrate to approximately 65% of calories. Cottonseed-soy oil was added to provide approximately 29%. A casein-based control diet was prepared using sucrose, cornstarch and cottonseed-soy oil to parallel the three rice diets.

Protocol: Children consumed the diets in one of the four sequences shown in Table III. All sequences were used twice. Periods 1 to 4 were used for studies of protein quality, periods 5 to 7 for rice digestibility. Casein periods were nine days each. Rice periods were shortened to seven days because of the anticipated poorer quality of rice protein. Metabolic balance studies were carried out as previously described (5) during the last six days of each dietary period. For digestibility studies each child consumed only one of the two non-glutinous rice varieties (IR 32 or IR 480-5-9); all children consumed the glutinous rice, IR 29. Blood was obtained for determination of serum total protein (Biuret) and albumin concentration (acetate gel electrophoresis) at the beginning of the study and at the end of the combined rice periods prior to returning to the control diet (i.e., the ends of periods 3 and 6).

Urine and stool samples were analyzed in three-day pooled collections. Nitrogen was determined by micro-Kjeldahl. Fecal fat was estimated by the method of Van de Kamer (6). During digestibility

studies energy intakes were verified by bomb caloriemtry (7). Fecal samples during these periods were also analyzed for total energy content by bomb calorimetry. Fecal carbohydrate was not measured directly but was calculated by the formula:

$$\text{Fecal CHO}_g = \frac{E-(N \times 6.25 \times 5.65 + \text{Fat}_g \times 9.4)}{4.15}$$

where CHO = carbohydrate in grams, E = fecal energy by bomb calorimetr N = fecal nitrogen, Fat = fecal fat and 5.65, 9.4 and 4.15 are the accepted energy conversion factors for protein, fat and carbohydrate, respectively (8).

Since each child served as his own control, no attempt was made to measure insensible losses of nitrogen. Apparent absorptions and retentions of nitrogen were calculated. Statistical analysis of these and other data was carried out where appropriate using a "t" test for paired comparisons and ordinary least squares linear regression analysis (9).

RESULTS

Protein Quality of IR 32 and IR 480-5-9: Nitrogen balance studies are summarized in Table IV. Apparent nitrogen absorption from both varieties of rice was significantly less than that from casein (rice mean 65.8%, casein mean 84.8%). Apparent retention of nitrogen from IR 32 was 28.6±9.4% of intake, that from IR 480-5-9, 23.0±2.5%. Although suggestive, the difference between the two rice varieties was not significant. Nitrogen retention during the final casein period was higher than during the initial casein period in

six of the eight children, most likely indicative of compensatory nitrogen retention following 14 days consumption of rice protein.

Stool wet weights and dry weights (Table V) were highest when IR 32 was consumed, possibly due to the much larger quantity of th variety required to supply 240 mg of nitrogen. Stool weights were not excessive even with this diet, however. Fecal fat excretions were similarly low on all four diets, ranging from 1.4±0.4 (IR 480 5-9) to 1.5±0.2 (IR 32) g/day while consuming rice, with a mean of 2.1±0.7 g/day while consuming casein. Excessive loss of energy in the stool could not explain the differences in apparent nitrogen retention. Rates of weight gain were similar on all diets and ran from 1.9±2.5 g/kg body weight/day (IR 480-5-9) to 2.9±1.6 g/kg/day (casein); the differences were not statistically significant. Sem albumin concentrations changed little during rice consumption; the mean difference was -0.1 g/dl (range -0.6 to +0.4 g/dl).

Digestibility Studies with IR 32, IR 480-5-9 and IR 29: Fecal wet and dry weights, fecal energy and computed fecal carbohydrate from these studies are shown in Table V. All parameters appeared lowest during consumption of IR 29. Only the differences between IR 29 and the casein control diet for stool wet weight and computed stool carbohydrate were statistically significant. Further analysi was carried out using the combined values for both non-glutinous rices for comparison with IR 29; no additional differences of significance were found.

Least squares linear regression analyses were carried out to correlate fecal wet weight and fecal dry weight with total fecal energy, fecal fat, and computed fecal carbohydrate (Table VI). Neither fecal wet weight nor dry weight was linearly correlated with fecal carbohydrate. A significant correlation of fecal fat content with both fecal wet weight ($r=0.564$, $P<0.02$) and fecal dry weight ($r=0.526$, $P<0.05$) was found during rice periods. Only the correlation of fecal fat with fecal wet weight ($r=0.657$, $P<0.05$) was significant while consuming the casein-based control diet. Most significant, however, were the correlations between fecal energy and either fecal wet or dry weight ($P<0.01$ in all cases). This was true for both the rice and the casein control diets. Plots of the equations showed only minor differences of the lines for the rice diets, the casein control diet and the combined data.

DISCUSSION

Previous studies have compared nitrogen digestibility and retention from high and low protein rice based diets (1, 10, 11). The comparisons have usually been made with equal quantities of rice being consumed and hence have not been isonitrogenous. We were able to carry out an isonitrogenous comparison only by blenderizing diets. It is unlikely that the nearly 21 grams of IR 32 per kg body weight could otherwise have been consumed daily.

Mean apparent digestibility of nitrogen by our children was approximately 65% from both rice diets. This is lower than the value of 76.9% that has been reported for adults (11). Unlike protein

from other staple grains, virtually 100% of the nitrogen of rice is contained in protein bodies: rice has essentially no matrix protein (12). Most of these protein bodies remain intact during cooking (13) and are apparently resistant to digestion, as they may be recovered intact in the feces of animals consuming rice (14). Furthermore, cooking of rice has been shown to decrease the digestibility of nitrogen by laboratory rats (14). It can be presumed that the lack of soluble matrix protein unique among staple grains to rice and the alterations brought about by cooking act together to decrease digestibility in the human as well. Nitrogen absorption from rice by these children was approximately 78% of that from casein. It is probably this poor nitrogen digestibility as much as anything that limits the retention of nitrogen from rice.

Overall digestibility of rice was excellent. Although consuming nearly 75 Kcal of rice per kg body weight during the initial studies with IR 32, stool weights were no higher than during the second control period, and no higher than we are accustomed to see when infants are fed milk-based formula diets. The studies of digestibility during which equal quantities of rice were consumed confirmed the overall digestibility. In these studies it was assumed that actual (as opposed to apparent) absorption of nitrogen from casein added to rice and in the control diet was virtually complete. Sucrose was also assumed to be fully absorbed; quantities of vegetable oil in the diets were equal. Any differences in total fecal energy should have been a reflection of differences in the rice itself.

Glutinous or waxy rices (both misnomers since rice contains neither gluten nor wax) differ from non-glutinous varieties in that they contain little amylose (±1%). Nearly all starch present is in the form of amylopectin. Non-glutinous rices generally contain 15-35% amylose. Glutinous rices tend to absorb more water on cooking and are of a stickier consistency. They are commonly thought to be more "filling" than non-glutinous varieties. Total fecal energy and estimated carbohydrate content during consumption of IR 29 show that if so this is not due to impaired digestibility.

Fecal energy appeared highest during consumption of IR 480-5-9. (Statistical analysis was not possible due to the small n). Computed fecal carbohydrate was low in these children, as were fecal fat excretions. Apparent nitrogen absorption from IR 480-5-9, however, was 4-6% lower than from the other two rice + casein diets. Because equal quantities of rice were consumed during these periods, a substantial part of the protein in the other two rice diets was supplied by casein. It is likely that the increased fecal energy loss of IR 480-5-9 is again a reflection of poor protein digestibility.

Although n's would have to be increased to attain statistical significance, a number of factors suggest that the increased protein content of IR 480-5-9 has been associated with a decrease in protein quality, when compared with IR 32. Both apparent absorption and retention of nitrogen were lower from IR 480-5-9. Mean fecal energy content was higher and rate of weight gain tended to be lower during the isonitrogenous-isoenergetic comparison. The modest decrease of protein quality, however, should be more than offset by the 60% greater

protein content of the high protein variety. This variety provides 12.5% of energy as protein and even allowing for its poorer digestibility allows the addition of substantial amounts of non-protein energy without decreasing percent-protein energy below an acceptable level, even for growing infants. The addition of small amounts of animal protein or legumes high in lysine should improve protein quality. Because of the protein bodies in rice one cannot be certain that milling or processing will appreciably alter digestibility.

FOOTNOTE

These studies were approved by the Joint Committee on Human Investigation, The Johns Hopkins Medical Institutions and the corresponding committee of the Instituto de Investigacion Nutricional, Lima, Peru. Informed consent was obtained from the parents of all children participating in the studies.

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Table I

Composition per 100 Kcal of diets used to assess protein quality of two varieties of milled rice, IR 32 (conventional) and IR 480-5-9 (high protein). All diets provided 240 mg N and 100 Kcal (in 150 ml) per kg body weight per day[1,2]

	Amount	Energy	N	Fat	CHO
	g	Kcal	mg	g	g
IR 32					
Rice	20.95	75.2	240	0.11	17.1
Oil[3]	2.75	24.8	--	2.75	--
IR 480-5-9					
Rice	13.20	48.0	240	0.20	10.06
Oil[3]	2.66	23.9	--	2.66	--
Sucrose	7.01	28.0	--	--	7.01
Casein Control					
Ca Caseinate[4]	1.70	6.3	240	0.03	--
Oil[3]	2.83	25.5	--	2.83	--
Cornstarch	10.06	36.2	--	--	10.06
Sucrose	8.01	32.0	--	--	8.01

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1 Mineral intakes of all diets were constant to provide 2.0 mEq Na, 3 mEq K and 2.3 mEq Cl per kg body weight. This was accomplished by addition of 0.68 g per 100 Kcal of a mineral mixture of the following composition (W/W): NaCl, 17.300%; $MgSO_4$, 12.850%; $CaHPO_4$, 23.450%; $KHCO_3$, 41.000%; KCl, 2.650%; Ferric citrate, 2.000%; $CuSO_4 \cdot 5H_2O$, 0.352%; $ZnSO_4 \cdot 7H_2O$, 0.200%; $MnSO_4 \cdot H_2O$, 0.156%; KI, 0.014%; NaF, 0.028%. The mineral mixture was prepared and assayed by Pfizer Pharmaceuticals, Pfizer Incorporated, Eastern Point Road, Groton, Connecticut 06340.

2 Vitamins were supplemented to all diets to meet U.S. Recommended Dietary Allowances. An appropriate amount of Poly-Vi-Sol (Mead Johnson, Evansville, Indiana) was given each child Q a.m. Poly-Vi-Sol contains: retinyl palmitate, 1,500 I.U.; ergocalciferol 400 I.U.; Vitamin E, 5 I.U.; ascorbic acid, 35 mg; thiamin, 0.5 mg; riboflavin, 0.6 mg; niacin, 8 mg; Vitamin B_6, 0.4 mg; Vitamin B_{12}, 2 μg.

3 Cottonseed (20%)-soy (80%) oil blend.

4 Casec. Mead Johnson, Evansville, Indiana.

Table II

Composition per 100 Kcal of diets used to assess the digestibility of two varieties of milled non-glutinous rice, IR 32 (conventional), IR 480-5-9 (high protein) and a variety of milled glutinous rice, IR 29. All diets provided 240 mg N, 100 Kcal (150 ml) per kg body weight per day[1,2]

	Amount	Energy	N	Fat	CHO
	g	Kcal	mg	g	g
IR 32					
Rice	13.35	48.0	154	0.07	10.9
Ca Caseinate[3]	0.61	2.3	86	0.01	--
Oil[4]	3.25	29.3	--	3.25	--
Sucrose	5.12	20.5	--	--	5.12
IR 480-5-9					
Rice	13.20	48.0	240	0.20	10.06
Oil[4]	3.13	28.2	--	3.13	--
Sucrose	5.96	23.8	--	--	5.96
IR 29					
Rice	13.12	48.0	166	0.17	10.56
Ca Caseinate[3]	0.52	1.9	74	0.01	--
Oil[4]	3.15	28.4	--	3.15	--
Sucrose	5.43	21.7	--	--	5.4
Casein Control					
Ca Caseinate[3]	1.70	6.3	240	0.03	--
Oil[4]	3.30	29.7	--	3.30	--
Cornstarch	10.56	38.0	--	--	10.56
Sucrose	6.50	26.0	--	--	6.50

1,2 See footnotes 1 and 2, Table I.

3 Casec, Mead Johnson, Evansville, Indiana.

4 Cottonseed (20%)-soy (80%) oil blend.

Table III

Sequences of dietary periods in studies designed to assess the protein quality (periods 1 to 4) and digestibility (periods 5 to 7) of different varieties of milled rice[1]

1	2	3	4	5	6	7
Casein	IR 32	IR 480-5-9	Casein	IR 32 + C[2]	IR 29 + C	Casein
Casein	IR 480-5-9	IR 32	Casein	IR 480-5-9	IR 29 + C	Casein
Casein	IR 32	IR 480-5-9	Casein	IR 29 + C	IR 32 + C	Casein
Casein	IR 480-5-9	IR 32	Casein	IR 29 + C	IR 480-5-9	Casein

Diets used in periods 1 to 4 are shown in Table I, those in periods 5 to 7 in Table II.

[2] C = Casein

Table IV

.dual and mean values (± standard deviation) of nitrogen balance studies
ıparing IR 480-5-9, IR 32 and a casein control diet (Periods 1 to 4)

Subjec No.	EN INTAKE g/day			APPARENT N ABSORPTION % of Intake				APPARENT N RETENTION % of Intake			
	–	IR 32	Casein	Casein	IR 480-5-9	IR 32	Casein	Casein	IR 480-5-9	IR 32	Casei
497*	4	2424	2496	87.0	66.5	78.0	79.0	35.5	18.5	38.5	50.5
507	6	2112	2136	81.0	62.5	52.0	88.0	36.0	24.5	22.0	38.0
518*	0	2340	2370	89.5	60.0	75.0	88.5	43.0	23.0	39.0	44.5
528	8	2064	2136	90.0	66.0	69.0	77.0	38.0	22.5	26.0	28.0
533	8	2304	2352	85.6	60.5	68.0	85.5	39.2	27.5	32.0	50.0
538	0	1950	2070	89.0	65.5	57.0	83.5	23.0	23.5	15.0	32.0
542*	0	2250	2310	86.5	63.5	69.0	87.5	34.0	21.5	37.5	47.0
545*	0	1740	1800	80.0	74.5	64.5	80.0	32.5	23.0	19.0	29.5
Mean	3	2148	2209	86.1	64.9[c]	66.6[c]	83.6	35.2	23.0[b]	28.6[a]	39.9
S.D.	3	±227	±219	±3.8	±4.6	±8.6	±4.5	±5.9	±2.5	±9.4	±9.3

* Ind ıild consumed IR 480-5-9 prior to IR 32. In the remainder the order was reversed.

a Dif ıding first casein period, P<0.05.

b Dif ıding first casein period, P<0.01.

c Dif ıding first casein period, P<0.001.

Table V

lean (± standard deviation) fecal wet and dry weights from all seven dietary periods. Total fecal energy and calculated fecal carbohydrate for studies in which equal quantities of rice were consumed are also shown.

	:asein$_1$	IR 480-5-9	IR 32	Casein$_2$	IR 480-5-9*	IR 32 + C*	IR 29 + C	Casein$_3$
Fecal g/	59.0 ±20.0	49.0[a] ±19.0	87.0[a,b] ±26.0	87.0[b] ±29.0	67.0 ±32.0	66.0 ±35.0	52.0[c] ±16.0	77.0 ±20.0
Fecal g/	8.8 ± 1.8	10.4[a] ± 2.1	15.6[a] ± 2.3	10.8 ± 2.3	11.6 ± 3.5	12.7 ± 3.0	9.5 ± 1.7	11.23 ± 1.2
Total Kcal.	--	--	--	--	58.0 ±23.9	49.2 ±12.7	46.5 ± 9.5	47.6 ± 7.2
Fecal g/	--	--	--	--	1.6 ± 1.1	3.5 ± 2.6	1.2[d] ± 1.2	4.3 ± 2.4

* n :

a Ri ;ignificantly, P<0.01.

b Di. ısein period, P<0.02.

c Di: ısein period, P<0.01.

d Di: ısein period, P<0.05.

Table VI

Summary of linear regression analysis of fecal wet and dry weight and total fecal energy content

	Regression Equation	r	P
Fecal Wet Weight - Fecal Energy			
Rice Diets - n = 16	y = 0.45 x + 22.93	0.776	<0.01
Casein Diet - n = 8	y = 0.30 x + 24.15	0.845	<0.01
Combined - n = 24	y = 0.35 x + 26.20	0.689	<0.01
Fecal Dry Weight - Fecal Energy			
Rice Diets - n = 16	y = 0.23 x - 0.80	0.828	<0.01
Casein Diet - n = 8	y = 0.17 x + 3.07	0.951	<0.01
Combined - n = 24	y = 0.23 x - 0.37	0.822	<0.01