Starch, Energy, and Protein Utilization by Rats in Milled Rice of IR36-Based Amylose Extender Mutant

BJØRN O. EGGUM,[1] BIENVENIDO O. JULIANO,[2] CONSUELO M. PEREZ,[2] and GURDEV S. KHUSH[2]

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ABSTRACT

The utilization of starch, energy, and protein in cooked milled rice of amylose extender (*ae*) mutant 2064 and its IR36 parent were examined in growing rats. Despite the higher lysine content of the *ae* mutant (3.83 vs. 3.32 g per 16 g of N), net protein utilization and protein quality of the two rices were comparable because of the lower protein, energy, and starch digestibility of the mutant. Starch, energy, and protein digestibility of the mutant decreased progressively from raw to cooked to cooked-parboiled, with or without the the addition of the antibiotic Nebacitin (used to suppress hindgut fermentation) in the diet. Unlike the same *ae* mutants in corn, the rice *ae* mutant did not have a considerable increase in resistant starch with increased amylose content.

Increasing the amylose content of milled rice starch from 0 to 33% to over 35% affects the processing properties (as in rice noodles and parboiled rice) and nutritional properties of rice. Amylose extender (*ae*) mutants, obtained by crossing japonica *ae* mutants with high-amylose (25–33%) indica rice IR36, have up to 40–45% apparent amylose content in their starch (Juliano et al 1990, Kaushik and Khush 1991). High-amylose corn starch has higher resistant starch than normal corn starch, whether raw (0.5–0.7 vs. 25.8–48.1%) or autoclaved (100–134°C for 45 min in 67% water) and dried (2.9–4.3 vs. 23.4–33.7%) (Berry 1986, Sievert and Pomeranz 1989). Autoclaved high-amylose barley starch has only slightly higher resistant starch (3.0 vs. 0.7–0.9%) than normal barley starch (Björck et al 1990). Resistant starch is defined as any starch, including fecal starch, that escapes digestion in the small intestine and is included in the analysis of dietary fiber (Cummings and Englyst 1991).

The brown rice protein of the *ae* mutant contains 0.5% higher lysine than does IR36 protein (3.32 vs. 3.83%) (Juliano et al 1990). This difference is partially retained in milled rice (IRRI 1991). Similar higher lysine content has been reported for *ae* or high-amylose corn (Glover et al 1975). The *ae* rice starch also showed the highest residual or waxy gene protein content (0.9%) (Villareal and Juliano 1989). This *ae* protein contributes to the higher lysine content of its milled rice protein because of the high lysine content of the waxy gene product of a japonica rice (8.6%) (Wang et al 1990).

Very high amylose rices have potential for use in products such as noodles and are a source of resistant rice starch. This study determined the starch, energy, and protein digestibility of raw, cooked, and cooked-parboiled *ae* mutant milled rice in growing rats. A recent identical study on ordinary milled rice included high-amylose rice (Eggum et al, *unpublished*). Also, the nitrogen balance of cooked mutant rice was compared with IR36 to calculate protein quality in the higher lysine protein mutant.

MATERIALS AND METHODS

Rough rice samples were obtained from the International Rice Research Institute (IRRI) experimental farm in 1990. They were aged at least four months after harvest and then dehulled with a Satake THU 35 sheller (Satake Engineering Co., Tokyo, Japan) and milled with a Satake one-pass rice whitening machine (model MC-250). Bran-polish yield was at least 7% by weight of brown rice. Rough rice was parboiled by soaking for 8 hr at 60°C, steamed in an autoclave for 30 min at 100°C, cooled, air-dried, and processed (Biswas and Juliano 1988).

Milled rice was cooked in a Goldstar model RJ-203 SB auto-

[1]Department of Animal Physiology and Biochemistry, National Institute of Animal Science, Foulum DK-8830, Tjele, Denmark.
[2]Plant Breeding, Genetics, and Biochemistry Division, International Rice Research Institute, 4031 Los Baños, Laguna, Philippines.

matic electric cooker with heater at the lid (Goldstar Philippines Sales, Manila, Philippines); the optimum water-rice ratio was based on apparent amylose content (2.1 for IR36, 2.65 for the mutant) (Juliano et al 1990). The samples were left for an additional 10 min in the cooker after power shutoff; then they were cooled, frozen at −50°C, and freeze-dried. The raw mutant milled rice was presoaked for 30 min in the cooking water; the parboiled rice was presoaked for 1 hr.

Analyses

Milled rice was ground for analysis in a Udy cyclone mill (Udy Corp., Fort Collins, CO) with 40- and 60-mesh screens. Moisture content was determined from weight loss after 1 hr at 130°C (AACC 1983). Crude protein was analyzed by micro-Kjeldahl digestion, followed by an AutoAnalyzer (Technicon Corp., Tarrytown, NY) colorimetric ammonia assay in digests diluted to 30 ml (Juliano et al 1968). Apparent amylose content was determined in acetate buffer (pH 4.5–4.8) using AutoAnalyzer iodine colorimetry and the potato amylose-waxy IR29 rice flour standard curve (Juliano et al 1981). Alkali spreading value was determined for six grains per 10 ml of 1.7% KOH after 23 hr at 30°C (Little et al 1958). Gel consistency of 100 mg of rice flour in 2 ml of 0.20*N* KOH was analyzed in 100- × 13-mm test tubes (Cagampang et al 1973).

Tests of amylograph pasting viscosity used 40 g of flour, ground to pass a 40-mesh screen, dispersed in 360 ml of water in a Brabender Viscoamylograph (Brabender OHG, Duisburg, Germany) set at 45 rpm using a 700-cm·g sensitivity cartridge (Juliano et al 1985). The cycle consisted of heating from 30 to 95°C at 1.5°C/min, holding 20 min at 95°C, and cooling to 50°C at 1.5°C/min. Setback is viscosity when cooled to 50°C minus peak viscosity. Consistency is viscosity when cooled to 50°C minus final viscosity at 95°C.

In vitro resistant starch was determined by an industrial U.S. laboratory using a modification of the pancreatic α-amylase-pullulanase method of Berry (1986). The initial heating step was omitted for the raw rice samples.

Nitrogen Balance Studies

Two groups of five male Wistar rats, weighing approximately 70 g, were used for the nitrogen balance experiment (preliminary period of four days and balance period of five days) (Eggum 1973, Eggum et al 1989). The rats were housed individually in Plexiglas cages with stainless steel mesh bottoms (for separate collection of feces and urine) in a controlled environment of 25°C and 50% rh with alternating 12-hr periods of light and darkness. Nitrogen level in the diets was adjusted by adding corn starch (100% digestible), as indicated in Table I (Eggum et al 1989). Each animal received 10 g of dry matter and 183 mg of N for IR36, or 200 mg of N for the mutant, daily throughout the preliminary and balance periods. The N level (dry basis) in the balance study was 1.83% for IR36 and 2.00% for the mutant. Body weight and diet intake were recorded at the end of the period. During the balance period, urine and feces were collected separately. Metabolic N and endogenous N were determined on a diet prepared by adding ether-extracted, freeze-dried egg (100% digestible), equivalent to 4% protein, to the N-free (corn starch) diet (Eggum 1973). True digestibility (TD), biological value (BV), net protein utilization (NPU), and digestible energy were determined from Kjeldahl and calorimetric analyses of diet, feces, and urine (Eggum et al 1989). Protein quality was calculated by multiplying TD by amino acid score, based on the first limiting essential amino acid, 5.8 g of lysine per 16 g of N (WHO 1985), as 100% (FAO/WHO 1990).

TABLE I
Composition of Rice Diets (g/500 g of Diet Dry Matter)

Component[a]	Nitrogen Balance		Resistant Starch[a]
	IR36	Mutant	
Milled rice	408	373	472
Raw corn starch	64	99	0
Minerals[b]	20	20	20
Vitamins[c]	8	8	8

[a] Diets supplemented with antibiotics contained the same weights of ingredients in addition to 3.5 g of Nebacitin (0.77% dry basis).

[b] Containing (in grams): $CuSO_4 \cdot 5H_2O$ (1.00), K_2HPO_4 (218.8), NaF (0.507), $CaCO_3$ (68.6), calcium citrate $Ca_3C_{12}H_{10}O_{14} \cdot 4H_2O$ (308.3), $CaHPO_4 \cdot 2H_2O$ (112.8), KI (0.041), $MgSO_4$ (38.3), $MgCO_3$ (35.2), ammonium Fe(III) citrate $Fe(NH_4)_3(C_6H_5O_7)_2$ (7.65), $MnSO_4 \cdot H_2O$ (0.201), NaCl (77.1), KCl (124.7), $AlNH_4(SO_4)_2 \cdot 12H_2O$ (0.090), and $ZnSO_4 \cdot 7H_2O$ (11.0).

[c] Containing (in grams): vitamin A (0.25; 250,000 IU), vitamin D_3 (0.002; 65,000 IU), thiamine (0.25), riboflavin (0.2), nicotinamide (1.25), pantothenic acid (0.5), vitamin E (2.0; 2,000 IU), pyridoxine HCl (0.4), choline chloride (70.0), menadion sodium bisulfite (0.41), folic acid (0.07), cyanocobalamin (0.003), biotin (0.025), sucrose (876.0), and soy oil (48.0).

Digestibility Studies

Male Wistar rats (75 g) were placed in individual metabolic cages. The animals, five on each diet, were allowed free access to water, but feed intake was restricted to 10 g of dry matter (DM) per day. All diets had variable protein and starch contents in 94.4 g of milled rice DM, 4.0 g of minerals, and 1.6 g of vitamins per 100-g diet (Björck et al 1986) (Table I). The N level (dry basis) was 2.02% for raw rice, 2.00% for cooked rice, and 1.99% for cooked-parboiled rice. Each diet was tested with and without the addition of Nebacitin (Apodan, Copenhagen, Denmark), an antibiotic drug containing 2:1 (w/w) bacitracin and neomycin sulfate at 0.7 g per 100 g of DM. After the four-day adaptation period, feed residues, feces, and urine were collected during the five-day experimental period. The feces were collected, freeze-dried, ground in a mortar, and kept at −18°C until analyzed. TD, BV, NPU, and digestible energy were estimated as described by Eggum (1973). Fecal samples and diets were analyzed for starch content at IRRI after dispersion with dimethylsulfoxide, digestion with *Chalara paradoxa* amylase, and then digestion with glucoamylase (Eggum et al, *unpublished*). Total resistant starch, and indigestible energy and protein were calculated from the rice diet with 0.7% Nebacitin (100 minus percent of digestible starch, energy, or protein). Unfermented resistant starch, and indigestible energy and protein were similarly calculated from the rice diet without Nebacitin. Fermentable resistant starch, and indigestible energy and protein were calculated as the difference between total and unfermented fractions.

Diets and fecal samples were observed under a polarizing microscope after dispersion in 0.1% Congo red with a mortar and pestle. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) determined the presence of the 60-kDa waxy gene product in the fecal samples (Villareal and Juliano 1989). Fecal samples from the Nebacitin-treated diets were subjected to differential scanning calorimetry (DCS) in 80% moisture at 10°C/min in a Perkin-Elmer 7 series thermal analyzer (performed at the New Products Division, A. E. Staley Mfg., Decatur, IL).

Raw data were subjected to analysis of variance, followed either by calculation of standard deviation (SD) or Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

Physicochemical Properties

The mutant milled rice was a mixture of opaque and translucent grains (2:1, wt/wt) with higher amylose content than IR36 (Juliano et al 1990). The mutant starch showed the B-type X-ray diffraction pattern (not shown) similar to that of the original *ae* mutant (Yano et al 1985), in contrast to the A pattern of rice starch. At 60% rh, the equilibrium moisture content of the *ae* mutant was 1.2 percentage points higher than that of the IR36 parent (Table II). The higher moisture content of the B pattern probably contributed to the higher moisture content of the mutant grain. Protein content of the rices were similar. The higher alkali digestibility of the mutant was deceptive. The gelatinization temperature (GT) of the mutant was intermediate-high (73–80°C),

which was somewhat higher than the intermediate GT of the IR36. The original *ae* mutant also had higher GT than the parent starch (Yano et al 1985). Gel consistency of the mutant was similar to that of the IR36 parent and softened on parboiling. Amylograph viscosity of the mutant was lower than that of the parent, and no distinct peak and viscosity breakdown during cooking at 95°C were seen. Parboiling further decreased amylograph viscosity. Similar changes were observed in high-amylose rice such as IR36 (Biswas and Juliano 1988), but amylograph viscosity was higher than that of the *ae* mutant.

Aminograms of IR36 and its mutant verified earlier data showing higher lysine content of the *ae* mutant (IRRI 1991) (Table III). The corresponding amino acid scores (5.8 g of lysine per 16 g of N as 100%) were 57.2% for IR36 and 66.0% for the *ae* mutant.

Protein Quality

Digestible energy of cooked milled rice was lower in the mutant than it was in IR36 (Table III). TD of the mutant was also lower. BV was higher for the mutant, resulting in comparable NPU (BV × TD). Protein quality based on amino acid score × TD, however, was also comparable for the two cooked rices. Thus, the higher lysine content in the *ae* mutant did not result in higher NPU because of poorer TD relative to IR36 protein. Statistical analysis confirmed significant varietal differences for all the properties measured in growing rats (Table IV).

Starch Digestibility Studies

Unfermented resistant starch was 2.4% in the mutant cooked rice and 0.1% in cooked IR36 (Table III). In vitro resistant starch based on α-amylase-pullulanase digestion was slightly higher in raw than in cooked and cooked-parboiled mutant rice (Table V). Corresponding values for high-amylose rice are 0.64–0.76% (raw), 1.54–1.64% (cooked), and 1.57% (cooked-parboiled) (Eggum et al, *unpublished*).

Although they had no effect on protein digestibility, processing and Nebacitin treatment had significant effects on all other properties measured in rats (Table IV). Total in vivo resistant starch in growing rats was higher in cooked and cooked-parboiled rice than in raw rice, mainly because of an increase in the unfermented fraction (Table V). Total, fermentable, and unfermented resistant starch levels of raw mutant rice were higher than those of high-amylose rice (0.1–0.2, 0.1–0.2, and 0%, respectively) (Eggum et al, *unpublished*). A large fraction of the resistant starch was unfermented in the hindgut and was expelled in the feces, even in the presence of Nebacitin. Light microscopic examination of the fecal sample from rats fed the Nebacitin-treated raw mutant diet showed some raw starch granules. DCS of the fecal samples (22–23% starch, gelatinization endotherm not shown) confirmed that ungelatinized starch appeared only in the feces of rats on the Nebacitin-treated raw rice diet. Only feces of rats fed Nebacitin-treated cooked and cooked-parboiled rice diets showed the amylose-lipid complex melting endotherm at 105–140°C of gelatinized starch (not shown). Thus, cooking in boiling water essentially gelatinized the mutant starch.

TABLE II
Properties of Raw and Parboiled Mutant Milled Rices Used in the Resistant Starch Study in Rats as Compared to IR36 Raw Milled Rice[a]

Property	IR36 Raw	IR36 Mutant	
		Raw	Parboiled
Moisture, % wet basis	12.2 ± 0.3	13.4 ± 0.4	11.8 ± 0.3
Crude protein, % wet basis	10.5 ± 0	10.5 ± 0.1	10.4 ± 0.1
Apparent amylose, % dry basis	26.1 ± 0	36.2 ± 1.4	35.5 ± 1.4
Alkali spreading value	5.9 ± 0.3	6.8 ± 0.6	5.2 ± 0.5
Gel consistency, mm	40 ± 3	38 ± 4	100 ± 0
Amylograph viscosity, BU			
Peak	745	355	5
Setback	285	375	35
Consistency	565	370	15

[a] Mean ± SD ($n = 2$, except unreplicated amylograms).

Corresponding values for resistant starch in high-amylose rices are 0.5–3.6% (total), 0.1–0.2% (unfermented), and 0.4–3.4% (fermentable) in cooked rice; and 2.0–4.3% (total), 0.3–0.6% (unfermented), and 1.7–3.7% (fermentable) in cooked-parboiled rice (Eggum et al, *unpublished*). Despite similarities of total resistant starch in cooked and cooked-parboiled mutant and high-amylose rice, mutant rice has more unfermented fraction, particularly if cooked-parboiled (Table V).

Indigestible energy (100 minus percent of digestible energy) was lowest for raw and highest for cooked-parboiled mutant milled rice (Table V). Adding 0.7% Nebacitin significantly increased indigestible energy, particularly in cooked rices. The increase corresponded mainly to the fermentable fraction. Cooking had more effect on the fermentable fraction of indigestible energy, whereas cooking-parboiling had more effect on the unfermented fraction. Corresponding values for high-amylose raw rice are 3.6–4.1 (total), 2.7–2.9 (unfermented), and 0.9–1.2% (fermentable) in raw rice; 6.3–7.1 (total), 4.4–4.5 (unfermented), and 1.8–2.7% (fermentable) in cooked rice; and 7.4–8.6 (total), 5.2–5.6 (unfermented), and 2.2–3.0% (fermentable) in cooked-parboiled rice (Eggum et al, *unpublished*). Mutant rice had more indigestible energy than high-amylose rice. The unfermented resistant starch content of raw and cooked mutant milled rice was close to the value of 6.9–7.5% reported for total dietary fiber in the mutant rice (B. D. Webb, *personal communication*). However, a greater fraction was unfermented rather than fermentable.

Indigestible protein (100 minus percent of TD) was lowest for raw and highest for cooked-parboiled milled rices (Table V), confirming the data of Eggum et al (1977). Nebacitin treatment did not significantly increase indigestible protein. Thus, the indigestible protein was mainly unfermented in all three mutant

TABLE III
Amino Acid Composition, Digestible Energy, and N Balance of Cooked IR36 and IR36-Based Amylose Extender Mutant in Growing Rats[a]

Property	IR36	*ae* Mutant
Amino acids[b]		
Alanine	4.88 ± 0.01	5.96 ± 0.14
Arginine	7.99 ± 0.06	7.65 ± 0.57
Aspartic acid	8.94 ± 0.16	9.75 ± 0.40
Cysteine	2.15 ± 0	1.94 ± 0.02
Glutamic acid	16.3 ± 0	16.2 ± 0.1
Glycine	4.68 ± 0.30	4.65 ± 0.17
Histidine	2.54 ± 0.05	2.40 ± 0.14
Isoleucine	4.00 ± 0.01	4.76 ± 0.12
Leucine	8.33 ± 0.16	8.49 ± 0.14
Lysine	3.32 ± 0.04	3.83 ± 0.21
Methionine	2.28 ± 0	2.08 ± 0.04
Phenylalanine	6.16 ± 0.03	5.95 ± 0.06
Proline	5.05 ± 0.38	4.95 ± 0.17
Serine	4.85 ± 0.13	5.09 ± 0.21
Threonine	3.61 ± 0.10	3.73 ± 0.06
Tryptophan	1.25 ± 0	1.24 ± 0.01
Tyrosine	3.79 ± 0.03	3.07 ± 0.11
Valine	5.47 ± 0.06	6.33 ± 0.17
Ammonia	1.51 ± 0.06	2.70 ± 0.22
Amino acid score, %	57.2 ± 0.7	66.0 ± 3.6
Protein, N × 6.25	10.5 ± 0	10.9 ± 0
Balance in five growing rats[c]		
Resistant starch, % of digestible material	0.1 ± 0 a	2.4 ± 0.6 b
Digestible energy, % of total	94.8 ± 0.4 a	90.6 ± 0.6 b
True digestibility, % of N intake	89.4 ± 0.7 a	83.3 ± 1.2 b
Biological value, % of absorbed N	76.1 ± 0.9 b	79.3 ± 0.9 a
Net protein utilization, % of N intake	68.0 ± 0.8 a	66.1 ± 1.6 b
Protein quality, %	51.1 ± 1.0 a	55.0 ± 3.8 a

[a] Means ± SD ($n = 2$ for chemical analysis, $n = 5$ for rat data).
[b] (g/16 g of N).
[c] Means in the same line followed by the same letter are not significantly different at $P = 0.05$ by Duncan's (1955) multiple range test.

TABLE IV
Analysis of Variance *F*-Values for Sources of Variation for Resistant Starch, Digestible Energy, and N Balance in Rats

Source of Variation	Degrees of Freedom	*F*-Value[a] In Vivo Resistant Starch	Digestible Energy	True Digestibility	Biological Value	Net Protein Utilization
Nitrogen balance[b]						
Treatment (rice)	1	86**	159**	92**	31**	6*
Error	8					
CV (%)		32.2	0.6	1.2	1.2	1.9
LSD (5%)		0.46	0.8	1.4	1.3	1.8
Resistant starch[c]						
Treatment	5	27**	81**	104**	56**	31**
Processing (P)	(2)	26**	102**	258**	94**	50**
Nebacitin (N)	(1)	80**	182**	$<1^{ns}$	66**	44**
P × N	(2)	$<1^{ns}$	10**	2^{ns}	12**	5*
Error	24					
CV (%)		22.4	0.9	1.1	1.1	1.5

[a] * = $0.05 > P > 0.01$, ** = $P < 0.01$; ns = not significant, $P > 0.05$.
[b] Study on cooked IR36 and mutant milled rices (Table III).
[c] Study on raw, cooked, and cooked-parboiled mutant milled rices (Table V).

TABLE V
In Vitro and In Vivo Resistant Starch, Indigestible Energy, and Nitrogen Balance of Raw, Cooked, and Cooked-Parboiled Mutant Milled Rice With and Without Nebacitin (Nb)[a]

Property	Raw	Cooked	Cooked-Parboiled
In vitro resistant starch, % DM	1.8 b	1.5 a	1.5 a
In vivo resistant starch, % DM			
Total (+Nb)	1.9 a	2.8 b	3.2 b
Unfermented (−Nb)	0.6 a	1.1 a	2.0 b
Fermentable (by difference)	1.3 **	1.7 **	1.2 **
Indigestible energy, % of total			
Total (+Nb)	8.0 a	12.3 b	14.0 c
Unfermented (−Nb)	5.9 a	7.3 b	9.7 c
Fermentable (by difference)	2.1 **	5.0 **	4.3 **
Indigestible protein, % of intake			
Total (+Nb)	9.8 a	13.3 b	18.6 c
Unfermented (−Nb)	8.7 a	13.7 b	18.6 c
Fermentable (by difference)	1.1^{ns}	-0.4^{ns}	0.0^{ns}
Biological value, % of absorbed N			
+Nb	70.1 c	73.1 b	74.5 a
−Nb	71.6 c	77.7 a	75.9 b
Difference	−15 **	−4.6 **	−1.4 *
Net protein utilization, % of N intake			
+Nb	63.3 a	63.4 a	60.7 b
−Nb	65.4 b	67.0 a	61.8 c
Difference	−2.1 **	−3.6 **	-1.1^{ns}

[a] Values in the same line followed by the same letter are not significantly different at $P = 0.05$ by Duncan's (1955) multiple range test. Difference due to Nebacitin addition: * = $0.05 > P > 0.01$, ** = $P < 0.01$, ns = not significant, $P > 0.05$.

samples. Corresponding indigestible protein values for high-amylose rice are: 5.7–6.2% (total), 0–0.2% (unfermented), and 5.7–6.0% (fermentable) in raw rice; 11.0–13.6% (total), 9.7–10.2% (unfermented), and 0.8–3.9% (fermentable) in cooked rice; and 14.9–18.3% (total), 13.1–15.5% (unfermented), and 1.8–2.8% (fermentable) in cooked-parboiled rice (Eggum et al, *unpublished*). Unlike high-amylose rice, mutant rice had higher indigestible protein, which was mainly unfermented in raw rice, consistent with the poorer starch and energy digestibilities.

BV of protein was lowest for raw and highest for cooked mutant rice (Table V). Nebacitin treatment significantly reduced the BV for all three samples, particularly for cooked rice. By contrast, Nebacitin treatment increased the BV of raw high-amylose rice and had no effect on cooked and cooked-parboiled high-amylose rice (Eggum et al, *unpublished*).

NPU was highest for cooked mutant rice without Nebacitin and lowest for cooked-parboiled mutant rice without Nebacitin (Table V). Nebacitin treatment significantly reduced the NPU for raw and cooked rice only. In high-amylose rices, Nebacitin treatment also decreased NPU in all samples except raw rice (Eggum et al, *unpublished*).

The poorer digestibility of starch, energy, and protein in raw rice of the mutant as compared with high-amylose rice suggests differences in properties of the mutant and ordinary rice. Nebacitin treatment had no significant effect on the TD of *ae* mutant protein. SDS-PAGE showed that the 60-kDa waxy gene product was prominent in the fecal sample of rats fed raw, cooked, and cooked-parboiled mutant rice but not in fecal samples of those fed IR36 cooked rice (not shown).

Compared with isonitrogenous diet (Table III), values for the mutant cooked rice were higher in the isocaloric diet (Table V) for digestible energy and TD, comparable for NPU, but lower for BV. SDS-PAGE of fecal starch indicated the presence of proteins, as had been reported for lintnerized starch (Maniñgat and Juliano 1979). The *ae* mutant is richest (0.9%) in waxy gene product or granule-bound synthase (Villareal and Juliano 1989), so the complex may not be readily digested. It can only be extracted after starch gelatinization. Residual protein in *ae* rice starch granule preparation has about 6.5% lysine (IRRI, *unpublished data*), somewhat lower than the previously reported value of 8.6% lysine (Wang et al 1990). The lower TD probably contributed to lower NPU and digestible energy in the mutant rice. The results stress the importance of biological tests to confirm the results of chemical tests in the study of protein quality and digestibility of energy, starch, and protein.

The coefficient of variation was low (0.6–1.9%) for all rat assays, except for in vivo resistant starch (Table IV) due to variation among rats. However, the in vitro resistant starch assay was not used because of the difficulty, particularly in the critical dispersion step, of ensuring complete dispersion of only the nonretrograded starch for the amylolytic digestion.

Thus, the *ae* mutant (very high amylose) rice had protein quality similar to that of high-amylose rice, despite its higher lysine content, because of lower protein, energy, and starch digestibility. Starch-granule-bound protein probably contributed to poor protein and starch digestibility, even in raw mutant rice. Resistant starch levels were similar to those of high-amylose barley (Björck et al 1990) and lower than those of *ae* corn (Berry 1986). The lower amylose content of translucent grains is probably a contributing factor (Juliano et al 1990).

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