

Properties and Protein Quality in Growing Rats of a Low-Glutelin Content Rice Mutant

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ABSTRACT

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Protein quality of cooked milled rice of low-glutelin (high-prolamin) content 1 mutant was compared with that of japonica parent Nihonmasari in growing rats. Biological value (BV) of the mutant was lower, but digestible energy and true protein digestibility (TD) were high and similar to those of the parent, resulting in lower net protein utilization of the mutant rela-

tive to the parent. Protein of the mutant had lower lysine content than did the protein of the parent, which explains its lower BV, although its prolamin content was lower than that of the parent. Cooking did not adversely reduce TD for both samples, in contrast to earlier studies of japonica and indica rices.

A low-glutelin (high-prolamin) mutant (NM67) was recently reported by Iida et al (1993). It was obtained from Nihonmasari by treatment with 0.2% ethyleneimine and screened by sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins. The mutant has lighter glutelin bands and heavier prolamin bands than the parent. The low-glutelin (high-prolamin) character is controlled by a single dominant gene. Out of 41 F₃ lines homozygous for the low-glutelin gene from the cross between NM67 and Nihonmasari, low-glutelin content (LGC) 1 mutant was one of seven lines homozygous for normal green leaf and had yield and protein content similar to that of Nihonmasari. Protein bodies (PB) in rice endosperm consist of glutelin-rich crystalline segmented PBII and prolamin-rich spherical PBI (Bechtel and Juliano 1980, Tanaka et al 1980). Cooking milled rice reduces the digestibility of core protein of PBI which ends up as fecal protein particles (Tanaka et al 1975a,b, 1978; Resurreccion et al 1993). True protein digestibility (TD) of cooked milled rice is 85–90% both in rats (Eggum et al 1977) and in man (WHO 1985), in contrast to 100% for raw rice in rats (Eggum et al 1977, Juliano 1993).

With the lower glutelin (PBII) and higher prolamin (PBI) contents of LGC 1 mutant, Iida et al (1993) predicted that the mutant will have only 72% of the TD of Nihonmasari. Our recent N balance study of the protein quality of cooked milled rice of glutelin and prolamin subunit mutants of Kinmaze showed that all three prolamin mutants had TD similar to that of Kinmaze and only the glutelin mutant had higher TD (Eggum et al 1994). In view of this unpredictability of TD from glutelin to prolamin ratio, the N balance of cooked milled rice of LGC1 mutant and Nihonmasari was determined in growing rats.

MATERIALS AND METHODS

Milled rice of the low-glutelin mutant and Nihonmasari were from the 1994 crop at the Institute of Radiation Breeding, Ohmiya, Ibaraki, Japan. They were sent by air parcel to PhilRice Los Baños, and stored at –20°C until used. Raw milled rice was analyzed for alkali spreading value (Little et al 1958); translucency was measured with a Riken Sanno rice meter (brown rice model); $L^*a^*b^*$ color was measured with a Minolta chromameter CR-110; and cooked rice hardness was measured after cooking in an electric cooker at water to rice ratio of 1.3 (Eggum et al 1994). A representative sample was ground in a Udy cyclone mill with 60-

mesh screen, and the flour was analyzed for apparent amylose content (Juliano et al 1981), gel consistency (Cagampang et al 1973), and microKjeldahl N and prolamin, using 60% 2-propanol as prolamin solvent (Lookhart et al 1991). Rice (600 g) was cooked in an electric rice cooker in a water to rice ratio of 1.3. The samples were left for an additional 10 min in the cooker after power shut-off before they were cooled, frozen at –20°C, and freeze-dried. The freeze-dried rices were ground in the Udy mill with 40-mesh screen and sent to Tjele, Denmark, for the rat assays.

Nitrogen Balance Studies

At the Danish Institute of Animal Science, two groups of five Wistar male rats weighing ≈ 70 g were used for the N balance experiment, with a preliminary period of four days and a balance period of five days (Eggum et al 1989, 1994). The rats were housed individually in Plexiglas cages with stainless steel mesh bottoms (allowing separate collection of feces and urine) in a controlled environment of 25°C and 60% RH. Lighting was controlled by alternating 12-hr periods of light and darkness. Each animal received daily 10 g of dry matter and 100 mg of N throughout the preliminary and balance period. Body weight and diet intake were recorded at the end of the period. During the balance period, urine and feces were collected separately. Diet and feces were analyzed by calorimetry and for Kjeldahl N, and urine was analyzed for Kjeldahl N. Metabolic N and endogenous N were determined by adding ether-extracted, freeze-dried egg (100% digestible) equivalent to 4% protein to the N-free (corn starch) diet. True protein digestibility (TD), biological value (BV), net protein utilization (NPU), and digestible energy were calculated. Rat data were subjected to analysis of variance.

Unreplicated amino acid analysis of diets with internal standards was conducted at Tjele, Denmark, using the procedure of Mason et al (1980). Tryptophan was determined using the method of Bech-Andersen (1991). Nitrogen recoveries were 85–90% and were recalculated to 95% recovery. Standard deviation (in grams per 16 g of N) was 0.2 for lysine, 0.1 for cysteine, and methionine, and 0.05 for tryptophan. Amino acid score was calculated from the content of lysine, the first limiting essential amino acid, based on 5.8% lysine (WHO 1985) as 100%. Protein quality was calculated by multiplying TD by amino acid score (FAO/WHO 1990).

RESULTS AND DISCUSSION

The milled rice of the LGC1 mutant was comparable to that of the parent Nihonmasari in terms of 100-grain weight (2.1–2.2 g) and dimensions (length 4.8 mm, width 2.6–2.8 mm); color L^* (72.7) a^* (–0.2–0.0) b^* (11.5–11.6); translucency (84.5–86.0%); crude protein content; apparent amylose content (AC); alkali spreading value; and Instron cooked rice hardness (Table I). Both

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TABLE I
Mean Properties ± Standard Deviation of Milled Rice of Nihonmasari and Its Low Glutelin Content 1 (LGC1) Mutant

Property	Nihonmasari	LGC1 Mutant
Apparent amylose content (% dry basis)	18.1 ± 0.2	17.1 ± 0.9
Alkali spreading value	7.0 ± 0.0	7.0 ± 0.0
Instron cooked-rice hardness (kg/cm ²)	1.1 ± 0.1	1.0 ± 0.1
Crude protein (% N × 6.25)	5.8 ± 0.1	5.6 ± 0.3
Prolamin content (% of total protein)	9.3 ± 0.1	6.4 ± 2.5
Amino acid content (g/16 g of N)		
Histidine	2.39	2.22
Isoleucine	4.08	4.30
Leucine	7.50	8.66
Lysine content	3.52	2.85
Methionine + cysteine	4.53	4.58
Phenylalanine + tyrosine	8.36	8.79
Threonine	3.23	3.04
Tryptophan	1.20	1.17
Valine	5.76	5.78
Amino acid score (%) ^a	60.7	49.1
Balance in growing rats		
Digestible energy (% of total)	98.5 ± 0.5	97.8 ± 0.4
True protein digestibility (% of N intake)	99.8 ± 0.6	99.7 ± 0.6
Biological value (% of absorbed N)	84.7 ± 1.0	77.4 ± 0.9
Net protein utilization (% of N intake)	84.6 ± 1.0	77.2 ± 1.0
Protein quality (%) ^b	59.8	48.0

^a Based on 5.8 g lysine/16 g N as 100% (WHO 1985).

^b Amino acid score × TD/100 (FAO 1990).

have low AC, typical of japonica rice. Both had low protein content, but the mutant had lower, rather than higher, prolamin content than the parent. However, LGC1 protein had lower lysine content ($P < 0.05$), resulting in lower amino acid score. Leucine content was higher in the LGC1 protein.

Digestible energy (DE) and TD of cooked milled rice were high (Table I): TD was similar for the LGC1 mutant and the parent, but DE was higher in the parent ($P < 0.05$). BV was significantly lower ($P < 0.01$) in the mutant, consistent with its lower lysine content and amino acid score. As a result, NPU of the mutant was significantly lower ($P < 0.01$). Protein quality based on amino acid score and TD was also lower in the mutant because of lower lysine content. The LGC1 mutant had lower protein digestibility than the parent in earlier rat study on raw rice (T. Nishio, Institute of Radiation Breeding, Ohmiya, Ibaraki, Japan, *personal communication*, 1996).

The very high TD of the cooked samples (99.7–99.8%) (Table I), particularly the LGC1 low glutelin mutant (99.7%) is surprising and contrasts with the TD data for both japonica and indica rice (Eggum et al 1977, 1993a, 1994) and higher than the 72% predicted by Iida et al (1993). However, the original study on fecal protein particles from rice PBI was from Japanese rice (Tanaka et al 1975a). Kinmaze cooked rice has TD of 95.5% at 6.75% protein (Eggum et al 1994), whereas Nihonmasari had TD 99.8% at 5.83% protein (Table I). Both japonica rice have low AC. Cooked low-AC IR24 indica milled rice (7.1% protein) had TD of 94.4% and waxy IR29 had TD of 97.4% (Eggum et al 1993a). The TD value for an earlier sample of IR29 was 91.5% (Eggum et al 1977). Tanaka (Kyoto Prefectural Univ., *personal communication*, 1988) observed indica rice protein to have lower in vitro pepsin digestibility (70%) than did japonica rice protein (80%) (Tanaka et al 1975b). The IR36 amylose extender mutant had low TD even of raw rice (Eggum et al 1993b), probably because of the high level of starch synthase protein bound to the starch granules (Villareal and Juliano 1989). Differences in the molecular properties of waxy gene allele *a* (indica) and *b* (japonica) proteins (Villareal and Juliano 1989, 1993) may contribute to differences in their relative digestibility

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