Protein Quality of Rice As Affected by Application of Nitrogen Fertilizer

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Six Varieties of rice (Zenith × AdT_3-Stg 6511071, Zenith × AdT_3-Stg 6511076, Bonnet 73, Saturn, Starbonnet, and Nortai) were harvested after receiving 0, 90, and 180 kg of ammonium sulfate per ha. Yields per hectare increased 26 to 40% with fertilizer level. Protein contents of all varieties (as a percentage of the milled rice) also increased with the level of fertilizer. Lysine levels (as milligrams per gram of N) were not affected by the treatments. Protein efficiency ratios (PER's) and net protein ratios (NPR's) were determined on selected rice samples. Relative nutritive ratios (RNR's) were lower in one variety (Zenith × AdT_3-Stg 6511071) than in the other varieties but were unaffected by level of fertilization.

The possibility of increasing the protein content of rice by genetic upgrading or by agronomic practices has long interested plant geneticists and nutritionists who are concerned with improving the protein/calorie ratios of diets commonly eaten in Asia. It is generally conceded, however, that increased protein content should not be gained at the expense of reduced yields or of uneconomic agronomic practices. Furthermore, because the nutritive quality of rice protein is somewhat less than ideal, maintenance of protein quality should be assured during crop treatment or genetic manipulation. The purpose of the study reported here was to determine whether N fertilizer applications resulted in (1) higher yields of rice, (2) higher protein content in rice, (3) changes in lysine content of rice, (4) changes in rice protein quality, and finally (5) increases in total utilizable protein (bioavailable protein) per field unit.

MATERIALS AND METHODS

Samples of milled rice were collected from the 1973 crop grown at the (USDA) Rice Production and Weed Control Research Center at Stuttgart, Ar. Several varieties, shown in Table I, that received different amounts of N fertilizer (as ammonium sulfate) were selected for determinations of crude protein and lysine content and for animal assays of protein quality.

Total nitrogen was determined by an automated Kjeldahl procedure on a Technicon Autoanalyzer. The automated procedure has a history of high correlation (above 0.9) of data with the micro rotary Kjeldahl method used by AOAC (1965). Very minor variations were made in the official micro rotary method. Crude protein content was calculated with 5.95 × N as the conversion factor (Jones, 1931). Hydrolysates for lysine determinations were prepared by mixing 190.5 ± 0.5 mg of the ground sample with 1 mL of 6 N hydrochloric acid in 15 × 150 mm Pyrex test tubes. The tubes were heat sealed and placed in a gravity convection oven at 100 ± 2 °C for 22 h. After being cooled, the samples were filtered through Whatman No. 2 filter paper and washed with small portions of pH 2.2 sodium citrate buffer to a final volume of 40 mL. Lysine content were obtained by ion-exchange column chromatography on a Beckman 121 automatic amino acid analyzer by use of principles developed by Moore et al. (1958). All analyses were made in duplicate.

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Table I. Yield and Lysine and Crude Protein Contents of Rice Samples

variety	fertilizer, kg/ha	"milled" yield,[a] kg/ha	protein % of milled rice	lysine, mg/g of N
Zenith × AdT_3-Stg 6511071	0	5121	8.15	182
	90	6810	8.21	188
	180	6387	9.40	177
Zenith × AdT_3-Stg 6511076	0	3853	7.50	198
	90	4658	8.21	196
	180	5280	8.92	200
Bonnet 73	0	5365	5.59	223
	90	6998	5.83	224
	135	7203	6.31	215
	180	7538	6.84	207
Saturn	0	5744	6.19	221
	90	6836	6.31	217
	180	7232	7.74	198
Starbonnet	0	4712	6.13	223
	90	5770	6.07	235
	180	6152	7.50	213
Nortai	0	5851	5.83	204
	90	6761	6.19	192
	180	7538	6.49	200

[a] Milled rice yield was estimated to be 90% of brown rice.

Because of the low protein content of the rice samples (5.59–9.40%), we could not carry out the standard AOAC assay (Derse, 1962) for protein efficiency ratio (PER) at dietary levels of 10% protein. Therefore, for comparison of as many varieties and treatments as possible, we carried out animal assays with diets containing approximately 1.0% N or 6% protein equivalent. Thus, each diet was formulated to provide 6% protein from the ground rice sample, 1% vitamin mix, and 4% Jones and Foster Salt Mix (Jones and Foster, 1942). Corn oil (maximum 10%) and cornstarch were added to 100%.

The diets were fed for 21 days to Charles River CD male weanling rats (Sprague Dawley strain from Charles River Breeding Laboratories, Wilmington, Ma) after a 1–3-day adjustment period during which they received a commercial chow diet. Rats were weighed weekly, scattered food was carefully recovered, and food intakes were determined. We calculated protein intakes by using the protein content of the diets as determined by N analysis. There were 6 rats/dietary group. One group of six rats was fed a protein-free diet as a control for determination of net protein ratios (NPR's). For standardizing the calculations for PER's and for determining relative nutritive values from the NPR's, we used a casein-based diet containing about 6% protein, 1% vitamin mix, 4% Jones

Table II. Protein Efficiency Ratio[a] (PER), Net Protein Ratio[b] (NPR), and Relative Nutritive Ratio[c] (RNR) of Rice Samples

variety	fertilizer, kg/ha	PER	NPR	RNR
Zenith × AdT_3-Stg 6511071	0	1.30	3.35 ± 0.11	72
	90	1.32	3.31 ± 0.12	71
	180	1.29	3.35 ± 0.15	72
Zenith × AdT_3-Stg 6511076	0	1.72	3.78 ± 0.12	82
	90	1.82	3.87 ± 0.04	84
	180	1.47	3.52 ± 0.15	76
Bonnet 73	135	1.78	3.92 ± 0.06	85
	180	1.94	4.02 ± 0.10	87
Saturn	90	1.88	3.99 ± 0.02	86
	180	1.76	3.89 ± 0.14	84
Starbonnet	180	1.84	3.89 ± 0.16	84
Nortai	180	1.72	3.85 ± 0.10	83
casein		2.50	4.63 ± 0.18	100

[a] (3-wk ΔW)/(3-wk protein intake). Corrected to casein (2.50). [b] (3-wk ΔW + wt loss on N-free diet)/(3-wk protein intake). [c] Reference protein was casein set at 100.

Table III. Yields of Crude Protein and Utilizable Protein[a]

variety	fertilizer, kg/ha	crude protein, kg/ha	utilizable protein, kg/ha
Zenith × AdT_3-Stg 6511071	0	417	300
	90	559	397
	180	600	432
Zenith × AdT_3-Stg 6511076	0	289	237
	90	382	321
	180	471	358
Bonnet 73	135	455	387
	180	516	449
Saturn	90	431	371
	180	560	470
Starbonnet	180	461	387
Nortai	180	489	406

[a] Crude protein × (RNR/100).

and Foster Salt Mix (Jones and Foster, 1942), 4% celluflour, and 10% corn oil, with cornstarch added to 100%. Even at this low level of dietary protein, diets could not be formulated for some of the rice samples. The varieties and N fertilizer levels used for the feeding experiments are shown in Tables II and III and were chosen because they contained sufficient protein for the 6% protein diets.

RESULTS AND DISCUSSION

Fertilization with N increased the yields of all rice varieties (Table I). The percent crude protein as calculated from N content also increased with amounts of fertilizer N. Protein content differed among varieties. Both of the Zenith × AdT_3 varieties were superior in this respect to Bonnet 73, Saturn, Starbonnet, and Nortai. The total yield of crude protein increased with increments of applied N, as shown by Patrick et al. (1974) with Dawn, Bluebelle, and Saturn varieties. The crude protein yield of Zenith × AdT_3-Stg 6511076 was 417 kg/ha at "0" applied N and 600 kg/ha at 180 kg/ha (Table III).

Because lysine is the first limiting amino acid in rice, it is also measured and expressed in relation to crude protein content by many investigators. Several investigators have reported in rice an inverse relationship between the lysine content of protein and the protein content itself (Juliano et al., 1964; Cagampang et al., 1966; Houston et al., 1969). Our results, expressed as milligrams of lysine per gram of N, do not show any consistent pattern of change with N fertilization or with variety. Similar results were obtained by Eggum and Juliano (1975) and Dutta and Barua (1978), although the latter claimed an inverse relationship between all amino acids and protein content of their rice samples from Assam. In fact, our results can be recalculated to show that the increase in crude protein content due to N fertilization is generally accompanied by a concomitant increase in total lysine of the same magnitude.

Lysine and other essential amino acids in rice are measured to determine whether or not the nutritional value of the rice protein has been affected by fertilizer treatment or genetic variation. Protein efficiency ratios (PER's) and net protein ratios (NPR's) measured in growing rats provide more direct measurements of nutritional quality and are shown in Table II. Levels of N fertilizer had no consistent effect on these indices of protein quality, except that protein quality was lower for Zenith × AdT_3-Stg 6511071 than for Zenith × AdT_3-Stg 6511076 across all N fertilizer levels. The data show that "forcing" higher yields and increasing the protein content of the rice grain by increased N fertilization did not lower protein quality.

If one of the goals of research on rice varieties and cultivation is to achieve higher yields of protein, the results will be most useful if they are expressed in terms of nutritionally utilizable protein, that is, in relation to a nutritionally defined reference protein such as casein. Relative nutritive ratios, derived from net protein ratios, can be used for this calculation, whereas the commonly used protein efficiency ratio, although more familiar to investigators and laymen alike, cannot be used to calculate a quantitative relationship to a reference protein. Results of these calculations are shown in Table III. Casein does not give maximum PER's or NPR's, but protein quality values at or above that of casein are used to define high-quality proteins in the U.S. Therefore, we calculated "utilizable protein" by using 100 as the relative nutritive ratio for casein (see Table II), in contrast to the value of 75 used by Bressani et al. (1971).

As would be expected from the data already discussed, utilizable protein production per hectare increased with the level of N fertilizer simply because protein quality did not change as protein content increased. The relative constancy of protein quality probably was related to the fact that the lysine content of the total protein mixture did not change as a result of N treatment, as previously shown by Patrick et al. (1974). On the other hand, the rice with the lowest lysine concentration (Zenith × AdT_3-Stg 6511071) also had the lowest RNR of the group of rices tested.

LITERATURE CITED

Association of Official Agricultural Chemists (AOAC), "Official Methods of Analysis", Vol. 31, 1965, p 102.
Bressani, R., Elias, L. G., Juliano, B. O., *J. Sci. Food Agric.* **19**, 1028 (1971).
Cagampang, G. B., Cruz, L. J., Espiritu, S. G., Santiago, R. G., Juliano, B. O., *Cereal Chem.* **43**, 145 (1966).
Derse, P. H., *J. Assoc. Off. Agric. Chem.* **45**, 418 (1962).
Dutta, L., Barua, J. N., *Indian J. Nutr. Diet.* **15**, 42 (1978).
Eggum, B. O., Juliano, B. O., *J. Sci. Food Agric.* **26**, 425 (1975).
Houston, D. F., Allis, M. E., Kohler, G. O., *Cereal Chem.* **46**, 527 (1969).

Jones, D. B., *U.S. Dep. Agric., Circ.* **183**, 22 (1931).
Jones, J. H., Foster, C., *J. Nutr.* **24**, 245 (1942).
Juliano, B. O., Bautista, G. M., Lugay, J. C., Reyes, A. C., *J. Agric. Food Chem.* **12**, 131 (1964).
Moore, S., Spackman, D. H., Stein, W. H., *Anal. Chem.* **30**, 1185 (1958).
Patrick, R. M., Hoskins, F. H., Wilson, E., Peterson, F. J., *Cereal Chem.* **51**, 84 (1974).

Received for review November 2, 1979. Accepted May 15, 1980.

Comparison between a Spectrophotometric and a High-Pressure Liquid Chromatography Method for Determining Tryptophan in Food Products

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A comparison is made between a spectrophotometric method and a high-pressure liquid chromatography (LC) method for tryptophan content of a variety of foods. The spectrophotometric method has an RSD of 2.53%. The LC method has an RSD of 2.03% with a recovery of 95.5 ± 2.4% for spiked samples. The mean tryptophan content of 18 samples by the spectrophotometric method was 0.38%, and that by the LC method was 0.35%.

Among the major challenges facing the food chemist today is the need for more accurate and cost-effective methods for nutrient analysis. Spectrophotometry and/or high-pressure liquid chromatography (LC) when coupled with appropriate sample preparation and workup procedures meet these criteria.

One of the nutritionally essential amino acids, tryptophan, has been analyzed by a variety of methods in the past. These methods have been reviewed by Friedman and Finley (1971). One of these methods, studied thoroughly by Spies (1967), which utilizes Pronase hydrolysis, derivatization with *p*-(dimethylamino)benzaldehyde, and spectrophotometric measurement appears to be well suited for food products. Tryptophan has also been measured by LC in biological samples by a variety of methods. These include separation on copolymer packings (Lefebvre et al., 1977, 1978; Kroeff and Pitrzyk, 1978), derivatization and colorimetric and/or fluorometric detection (LaPage et al., 1979; Hsu and Currie, 1978; Margolies and Brauer, 1978; Lammens and Verzele, 1978; Van Beeumen et al., 1978; Jornvall et al., 1978; Furukawa et al., 1977), direct fluorometric detection (Anderson and Purdy, 1977, 1979; Geeraerts et al., 1978; Krstulovic et al., 1977a,b; Meek and Neckers, 1977; Graffeo and Karger, 1976), and others (Rustun, 1978; Hancock et al., 1979; Riley et al., 1979; Krstulovic et al., 1978; Molnar and Horvath, 1978; Knudson et al., 1978; Grushka et al., 1977). Of these methods, reverse-phase chromatography coupled with direct fluorometric detection appeared to be the most viable method for food products.

Amino acids other than tryptophan are routinely analyzed by using ion-exchange chromatography following acid hydrolysis (Wall and Gehrke, 1976). Under these conditions tryptophan is labile and generally has to be analyzed separately. This is generally done by using basic hydrolysis or acid hydrolysis while protecting the tryptophan with an antioxidant followed by basic ion-exchange analysis on an amino acid analyzer, ultraviolet spectrophotometry, or fluorometry (Peters and Berridge, 1970; Berridge et al., 1971; Wapnir and Stevenson, 1969; Wilinson et al., 1976; Eftnik and Ghiron, 1976; Hassan, 1975; Lewis et al., 1976; Spackman et al., 1958). What was sought, therefore, was a method to complement the amino acid analyzer ion-exchange method and quantitate tryptophan accurately and efficiently. The spectrophotometric method was a modification of that of Spies (1967). The LC method developed utilized the hydrolysis developed by Spies and separation and quantitation similar to those used by Krstulovic et al. (1977a) for serum samples.

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EXPERIMENTAL SECTION

Apparatus. Spectrophotometer: Linear Absorbance, Model 6120A (Coleman, Norwalk, CT 06856). Pump: Model 110A, Constant Flow (Altex, Berkeley, CA 94710). Column: μ-Bondapak C_{18} (Waters Associates, Milford, MA 01757) or Lichrosorb RP-18 (Altex). Injector: Autosampler LC 420 equipped with a 20-μL loop (Perkin-Elmer, Norwalk, CT 06856). Detector: Spectrofluoromonitor LC650-10 (Perkin-Elmer); excitation at 295 nm with a 12-nm slit width and emission at 320 nm with a 12-nm slit width.

Materials. *Phosphate Buffer (pH 7.5).* Sodium phosphat dibasic (Na_2HPO_4) (4.40 g) and potassium phosphate monobasic (KH_2PO_4) (4.40 g) were dissolved in and diluted to 1 L with water. The pH was checked and adjusted to 7.5 if necessary.

Pronase Solution (4 mg/mL). Pronase (Calbiochem B grade, 45 × 10^3 PUK/g; Calbiochem, La Jolla, CA 92037) (100 mg) was placed in a 25-mL volumetric flask, and the flask was brought to volume with phosphate buffer. The mixture was prepared just prior to use. NOTE: Since the solution remains turbid, it was shaken before addition to each sample.

Sulfuric Acid (21.2 N). Concentrated H_2SO_4 (142 mL) was added in 25-mL portions to 85 mL of distilled water while the flask was swirled under cold tap water. The solution was cooled to room temperature before use.

p-(Dimethylamino)benzaldehyde (DAB). DAB (0.94 g) was dissolved and brought to 250-mL volume with 21.2 N sulfuric acid. The solution was prepared just prior to use.

Sodium Nitrite (0.048%). Sodium nitrite (24 mg) was dissolved and brought to 50-mL volume with distilled water.

Tryptophan Standards. For a stock solution (1 mg/mL), 100 mg of L(−)-tryptophan was dissolved and brought to 100-mL volume with phosphate buffer solution. An

0021-8561/80/1428-0896$01.00/0