Protein and Amino Acid Content of Rice as Affected by Application of Nitrogen Fertilizer[1]

R. M. PATRICK and F. H. HOSKINS, Department of Food Science, Louisiana State University, Baton Rouge 70803; and E. WILSON and F. J. PETERSON, Rice Experiment Station, Crowley, Louisiana 70526

ABSTRACT

Nitrogen fertilizer (N) applied to three major Louisiana rice varieties at specified stages in the physiological development of the rice plant produced a marked response in crude protein content. More protein was usually found in the grain when all the N was applied at seeding, or where half of the N was applied at seeding and half applied late in the season at the 2-mm. panicle stage. Protein content was increased by both the 80- and 120-lb. rates of N with the 80-lb. rate providing the greatest increase. The addition of fertilizer N produced large increases in yield for all varieties. There was a striking inverse relationship between grain yield and protein content between the 1968 and 1969 crops. In 1968, grain yield was higher and protein content lower than in 1969. The yield of protein was relatively constant for both years. Although some variation in amino acid content was evident, there was no marked effect of variety, rate and time of N application, or method of seeding on amino acid composition of the protein, even though there were considerable differences in protein content. Contents of lysine and threonine, first- and second-limiting amino acids in rice, were markedly reduced in the milling process.

Rice contains all the dietary essential amino acids, although the proportions are not ideally balanced and the total protein content is low. Because of the importance of rice in the diets of people in protein-deficient countries, there is a keen awareness of the necessity of improving the quantity and quality of rice protein.

Intensive research in the last few years has brought about higher rice yields as a result of improved varieties and better cultural practices. No such effort has gone into the search for improved quantity and quality of protein in rice since protein content is not a factor in determining economic value on domestic or world markets.

Few controlled studies have been reported on the protein and amino acid content of rice as affected by varietal difference and fertilizer nitrogen (N) rate and time of application. Over a period of years, Kik (1) found that the average protein content of rice grown in fertilized plots was not significantly different from that grown in nonfertilized plots. Sturgis et al. (2) also found that fertilizer treatments failed to significantly increase the percentage of protein N even though rice yields were usually increased. Highly significant differences were found in the protein content of major rice varieties. Location where rice is grown affected protein and amino acid content more than variety (3,4).

However, an increase in protein content of a particular variety, from whatever cause, did not necessarily reflect an improvement in the essential amino acid pattern. Generally as the protein content increased, the content of essential amino acids decreased. Environmental factors such as season and year have caused protein content of a variety to vary from 9.0 to 14.7% (5).

Analysis of protein content of six lines grown in the Philippines indicated that, on the average, protein increased from 7.2 to 9.5% as the fertilizer N level was

[1]Presented at the 56th Annual Meeting, Dallas, Oct. 1971.

Copyright © 1974 American Association of Cereal Chemists, Inc., 3340 Pilot Knob Road, St. Paul, Minnesota 55121. All rights reserved.

increased from 0 to 150 kg./ha. (6). The effect of different times of N fertilizer application on the protein content showed that later applications produced higher protein content than did early applications. The purposes of the study reported here were to determine: 1) the variation in protein content of both brown and milled rice as affected by rate and time of application of N fertilizer; 2) total protein yield as affected by the different treatments; and 3) the amino acid content of representative samples.

MATERIALS AND METHODS

Rice samples used in this study were obtained from the Rice Experiment Station at Crowley, La., and were harvested during 1968 and 1969. Plantings were part of an experiment designed to study the effect of variety, method of seeding, rate of N fertilizer, and method and time of application of N fertilizer on yield of rice. Varieties used were Saturn, Dawn, and Bluebelle. Saturn is a medium-grain variety, and Dawn and Bluebelle are long-grain.

All treatments were replicated four times using a split-plot arrangement in a random block design, with time of application constituting the main plot, and N rates the subplots. Separate but adjacent plots were used for each variety.

Nitrogen application methods consisted of subsurface placement of all N fertilizer before seeding (a method designed to provide maximum conservation of N), broadcast application of all N fertilizer prior to first flood (a method commonly used by farmers because of its convenience), and two methods which involved broadcast application of half the N fertilizer at first flood and half during the growing season to correspond with given physiological stages of development of the rice plant. Ammonium sulfate at rates of 0-, 80-, and 120-lb. N per acre was used as the N source. All plots received an amount of phosphate and potash considered adequate for good yields, 50 lb. each P_2O_5 and K_2O per acre. Specific treatments used in this study are outlined in Table I.

Preparation of Samples

Samples consisting of 1 kg. of cleaned, air-dried, rough rice were hulled with a McGill sheller. The hulled rice grains were separated from the loose hulls, and the resulting brown rice was milled and polished with a McGill No. 3 miller, according to official government inspection procedures (7). Percentage of hulls averaged 20% of the rough sample, and milling yields of 67.2, 70.6, and 71.8% were obtained for Dawn, Bluebelle, and Saturn, respectively.

After hulling and/or milling, replicates of each treatment were packaged, labeled, and stored in airtight cans. Samples from 1968 were stored at −20°C. until after the 1969 harvest when all samples were prepared for simultaneous analyses.

Four replicates from each treatment were thoroughly mixed, forming a composite sample representing each treatment. A subsample of each composite was hand picked to remove any hull residue, then thoroughly ground in a Waring Blendor for 4 min. This method of grinding produced samples of which 95% passed through a 16-mesh sieve, and 57% passed through a 32-mesh sieve.

Crude Protein Determination

Approximately 2 g. of the well-mixed ground sample was used for determination of total N by routine Kjeldahl Gunning-Arnold methods (8).

TABLE I. TREATMENTS USED IN EXPERIMENT

Varieties
- Saturn (brown and milled)
- Dawn (brown and milled)
- Bluebelle (brown and milled)

Rate of nitrogen fertilizer used
- 0 lb. per acre
- 80 lb. per acre
- 120 lb. per acre

Method and time of application of nitrogen fertilizer
- T_1 Subsurface drilling of all N at seeding.
- T_3 Broadcast application of all N prior to first flood.
- T_6 Half N broadcast at first flood and half applied at elongation of basal internode, or first joint.
- T_9 Half N broadcast at first flood and half applied when panicle was 2 mm. in length.

Duplicate determinations were made on each sample, but in different runs to minimize errors. Duplicate values that did not check within 2% were repeated. The conversion factor of 5.95 X N was used for calculating crude protein content. Analyses are reported at 12% moisture content.

Amino Acid Analyses

Amino acid contents of 25 selected samples representing various varieties and treatments were determined by conventional procedures with a Beckman Model 116 amino acid analyzer. Approximately 200 mg. of finely ground rice sample was weighed into hydrolysis tubes and 3 ml. of 6N HCl was added. The acid-sample mixture was frozen under vacuum, thawed, and refrozen under vacuum to remove entrapped air. Samples were hydrolyzed under vacuum at 110°C. for 22 hr. Hydrolyzed solutions were filtered, washed with 40 ml. of 0.1N HCl, and evaporated on a rotary evaporator at a temperature of 50° to 55°C. A few ml. of deionized water was added three times to the residue and re-evaporated to remove HCl. Samples were then dissolved in 2.2 pH buffer, tightly sealed, and frozen for 1 month until analyzed. Values for isoleucine, methionine, serine, threonine, and valine were corrected for their incomplete hydrolysis or degradative loss during acid hydrolysis by multiplying by the factors 1.078, 1.034, 1.082, 1.036, and 1.081, respectively (9). Tryptophan and cystine could not be determined by this method of hydrolysis. Results were expressed as g. amino acid per 16.8 g. N.

RESULTS AND DISCUSSION

Protein Content

Protein contents of brown and milled rice of Saturn, Dawn, and Bluebelle varieties as affected by rate, time, and method of N fertilizer application are reported in Figs. 1 through 6. In almost every case, protein content was increased by the addition of N fertilizer. Protein content for milled rice ranged from 5.7% for Dawn variety receiving 80 lb. N per acre in 1968 to 7.6% for Bluebelle variety receiving 120 lb. N in 1969. For brown rice, protein content ranged from 6.6% for Dawn receiving 80 lb. N per acre in 1968 to 8.4% for Bluebelle receiving 120 lb. N in 1969. Bluebelle variety generally showed the highest protein values for both brown and milled rice in 1968 and 1969, while Dawn variety usually had the lowest protein values. Irrespective of variety, adding either 80 or 120 lb. N per acre to the crop generally increased protein content more than did any of the other treatments used, although increases were not always consistent. Saturn variety was especially responsive to fertilizer N with increases resulting from both 80- and 120-lb. N rates, with the exception of one case where no increase was noted. There was much less effect of N rate on protein content of Dawn and Bluebelle milled rice. The milling process removed a considerable percentage of the increased protein resulting from N fertilization, indicating that much of the increased protein was present in the outer layers. Other investigators have found that the degree of milling is important in determining protein loss in the rice kernel (3,10,11).

Results from this investigation confirmed that protein content varies widely from year to year. Without exception, protein content for comparable treatments of all varieties was higher in 1969 than in 1968, often as great as 1% or more. The effect of year was so marked that rice from plots receiving no N in 1969 had higher protein contents than the high N treatments in 1968. Kymal (3) found that protein content of rice varied from year to year when the same variety was grown in the same location. Juliano et al. (12) have shown a difference of 4% protein in the same rice planted in different seasons.

Time and method of application of N influenced protein content of Saturn (Figs. 1 and 2). For brown Saturn rice both treatments T_1 and T_9 (half the N applied at seeding, other half applied late in the season when panicle was 2 mm.

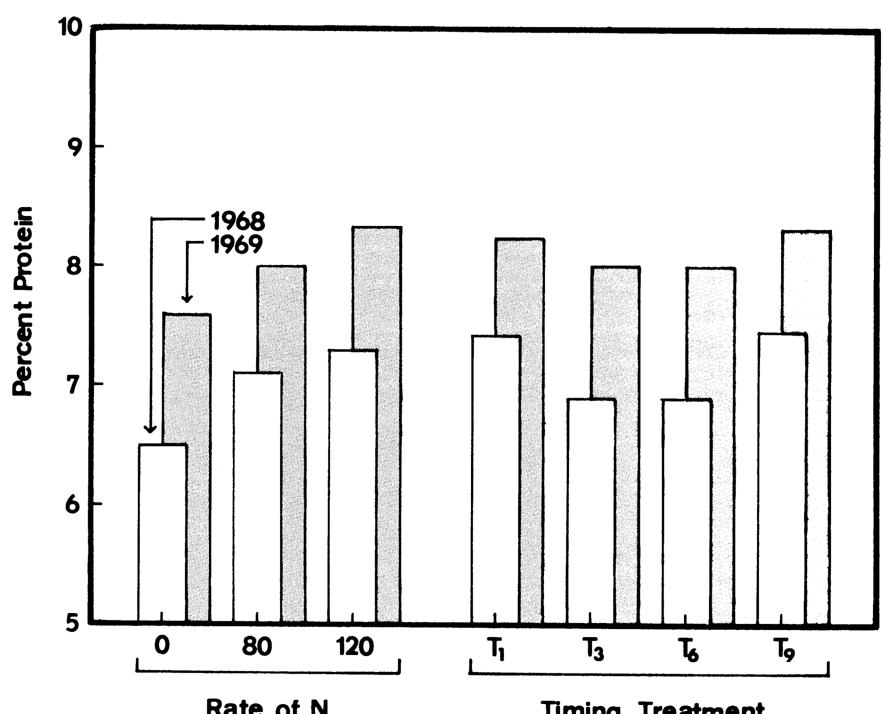

Fig. 1. Protein content of Saturn brown rice as affected by rate (0, 80, and 120 lb. per acre) and time of nitrogen application in 1968 and 1969.

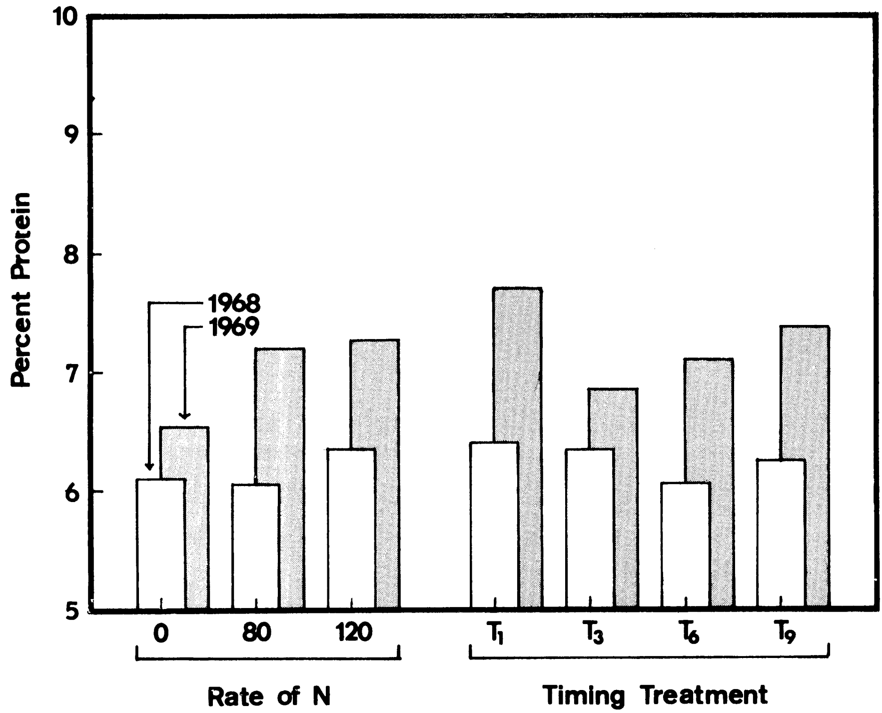

Fig. 2. Protein content of Saturn milled rice as affected by rate (0, 80, and 120 lb. per acre) and time of nitrogen application in 1968 and 1969.

long) appeared slightly superior to the other two treatments; however, the difference was not statistically significant at the 5% level of probability. For milled Saturn rice, applying all the fertilizer at seeding (T_1) was statistically superior to other application treatments in 1969. Differences in protein content of Dawn for the various N-timing treatments are shown in Figs. 3 and 4. Protein was significantly higher in brown rice for the late season application (T_9), especially in 1968. Most of this increased protein was removed in the milling process. For Bluebelle, application of N at seeding (T_1) produced a higher protein content than did the other treatments (Figs. 5 and 6). Increase in protein content was statistically significant for milled rice and approached significance for brown rice. When all three varieties were considered, it was evident that more protein was found in the grain where all the N was applied at seeding (T_1) or where half the N was applied late in the season at the 2-mm. panicle stage (T_9). Late season application of N has been suggested as a means of increasing protein content of the grain, but little experimental verification of this effect is available.

Protein content of milled rice was highly correlated with protein content of brown rice, although the correlation coefficient ($r=+0.827$) was not as high as has been reported. Because of the close association found between protein contents of brown and milled rice, some investigators use only brown rice for evaluating protein content (13).

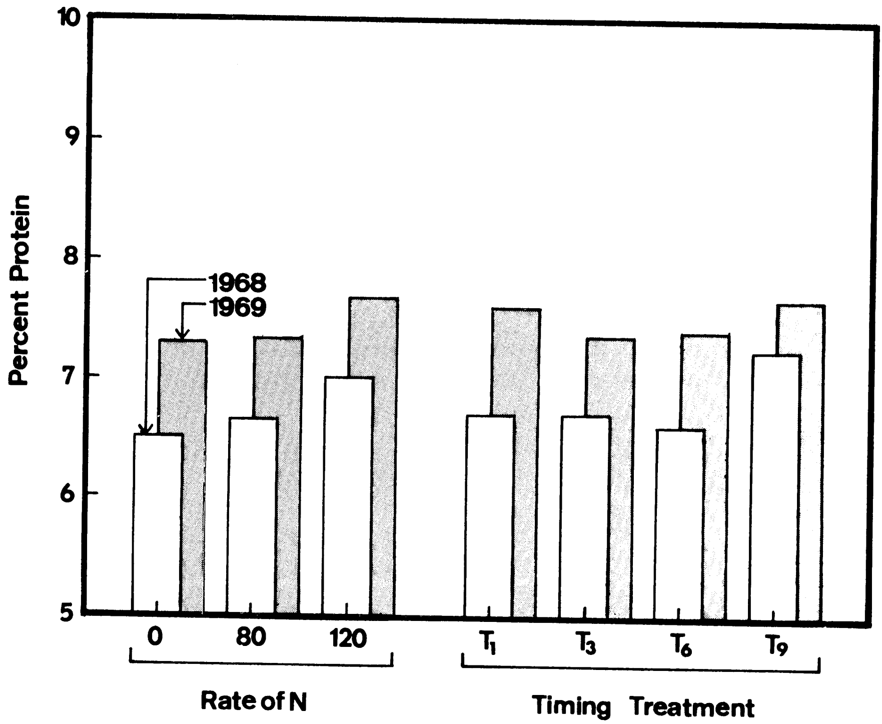

Fig. 3. Protein content of Dawn brown rice as affected by rate (0, 80, and 120 lb. per acre) and time of nitrogen application in 1968 and 1969.

Grain and Protein Yields

A complete discussion of grain yields is not within the scope of this report since this information is available elsewhere (14). Brief mention of grain yields will be made, however, since the yield affected the protein content.

As expected, the addition of N fertilizer produced large increases in yield with the biggest increase coming from the 80-lb. rate of N. An additional yield increase usually resulted from the 120-lb. application. The time of application also affected yield, with the treatment receiving N at seeding (T_1) producing higher yields than most of the top-dress treatments. The yield of rice was considerably higher in 1968 than in 1969. Adverse environmental conditions, such as a cool growing season and increased insect damage, limited grain yield in 1969.

Protein yields for brown and milled rice of Saturn, Dawn, and Bluebelle varieties were markedly increased with each increment of applied N (Table II). For Saturn and Bluebelle, protein yields were significantly increased with the 80-lb. rate of N. The 120-lb. rate brought an additional, although nonsignificant, protein yield increase. For Dawn, protein yield was significantly increased by both levels of N. The time of application of N had no significant effect on protein yield for any variety.

Protein yield of milled rice for all varieties was markedly less than protein yield of brown rice due primarily to milling loss. As indicated previously, percent protein in the 1969 rice was mich higher than that in 1968; however, there was little or no difference in yield of protein for the 2 years. In most cases where grain yield was higher, as in 1968, protein content was lower, thus giving a fairly constant per acre protein yield for the 2 years.

Although the reason for higher protein in 1969 is not definitely known, it is likely that the relatively adverse environmental conditions in that year had a pronounced effect on both yield of rice and protein content. The lower grain yield in 1969 probably resulted in N being present in the plant in a larger concentration than was needed. In 1968, the extra N was utilized in grain production and consequently did not show up in increased protein content.

When all varieties and treatments were considered, however, no general inverse relationship was shown between grain yield and protein content. Surprisingly, Saturn milled rice in 1969 showed a highly significant positive correlation between yield and protein content. This was not a typical response and occurred only in 1969 when yields were generally low. However, the correlation for Saturn in 1968 was also positive and approached significance. Saturn may be quite different from most other varieties in responding to increased N supply by both an increase in yield and an increase in protein content.

Amino Acid Analyses

Samples selected for amino acid analyses represent both brown and milled rice,

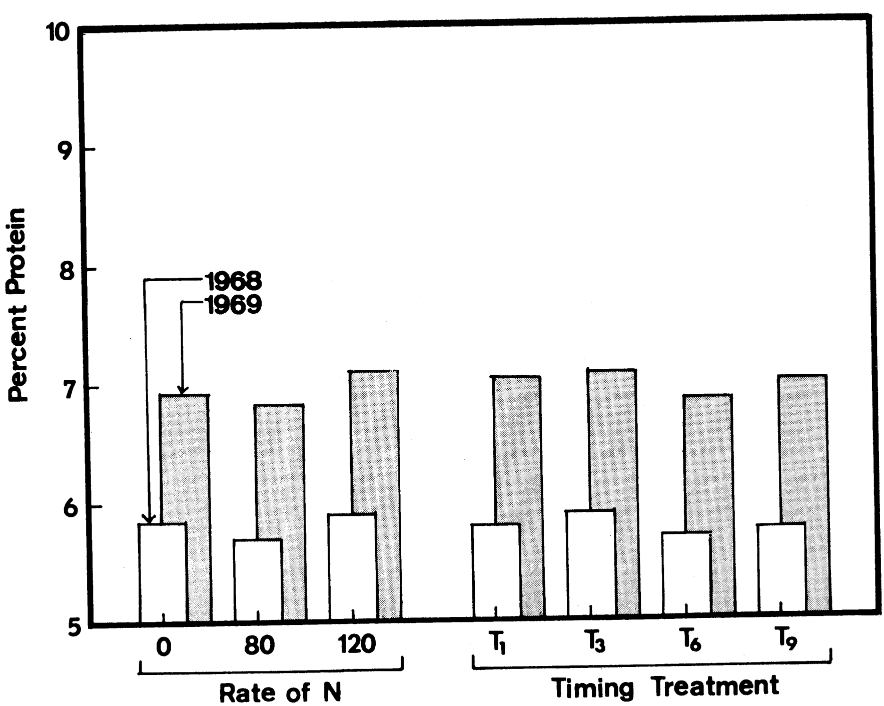

Fig. 4. Protein content of Dawn milled rice as affected by rate (0, 80, and 120 lb. per acre) and time of nitrogen application in 1968 and 1969.

and were selected to show the effect of variety, year, and rate and time of fertilizer N application on amino acid composition. Amino acids are reported as actual N recovery. Nitrogen recovered accounted for 73.3 to 99.2% of total N. Most values ranged from 75 to 88%. Tryptophan and cystine were not included. Considerable

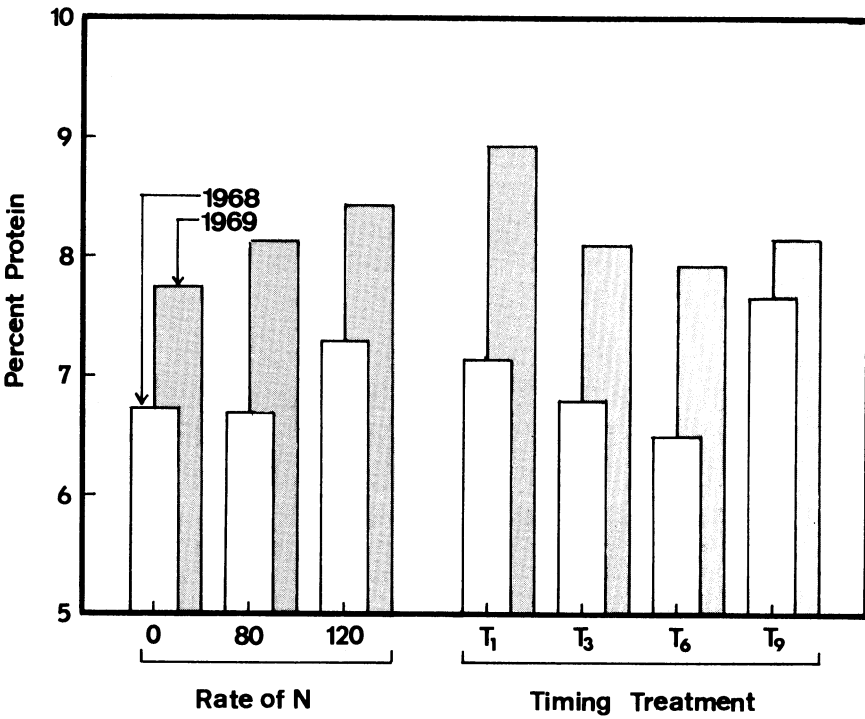

Fig. 5. Protein content of Bluebelle brown rice as affected by rate (0, 80, and 120 lb. per acre) and time of nitrogen application in 1968 and 1969.

TABLE II. PROTEIN YIELD (lb./acre) of SATURN, DAWN, AND BLUEBELLE RICE AS AFFECTED BY RATE, METHOD, AND TIME OF APPLICATION OF NITROGEN[a]

	Saturn		Dawn		Bluebelle	
Treatment	Brown	Milled	Brown	Milled	Brown	Milled
0 N	172a	142a	157a	125a	184a	149a
80 lb. N	286b	224b	267b	191b	273b	215b
120 lb. N	320b	243b	302c	211c	309b	238b
T_1 All N applied preplant[b]	319a	253a	289a	207a	278a	221a
T_3 All N prior to first flood[b]	298a	222a	275a	196a	300a	238a
T_6 Half N at first flood, half at first joint[b]	296a	222a	283a	201a	278a	219a
T_9 Half N at first flood, half at 2 mm. panicle[b]	306a	237a	290a	201a	308a	228a
1968 crop	302a	225a	278a	194a	273a	210a
1969 crop	275a	198a	262a	194a	285a	226a

[a]Values are averages for 1968 and 1969. Values in any given group followed by the same letter do not differ significantly at 5% level of probability.

[b]80-lb. N and 120-lb. N treatments only.

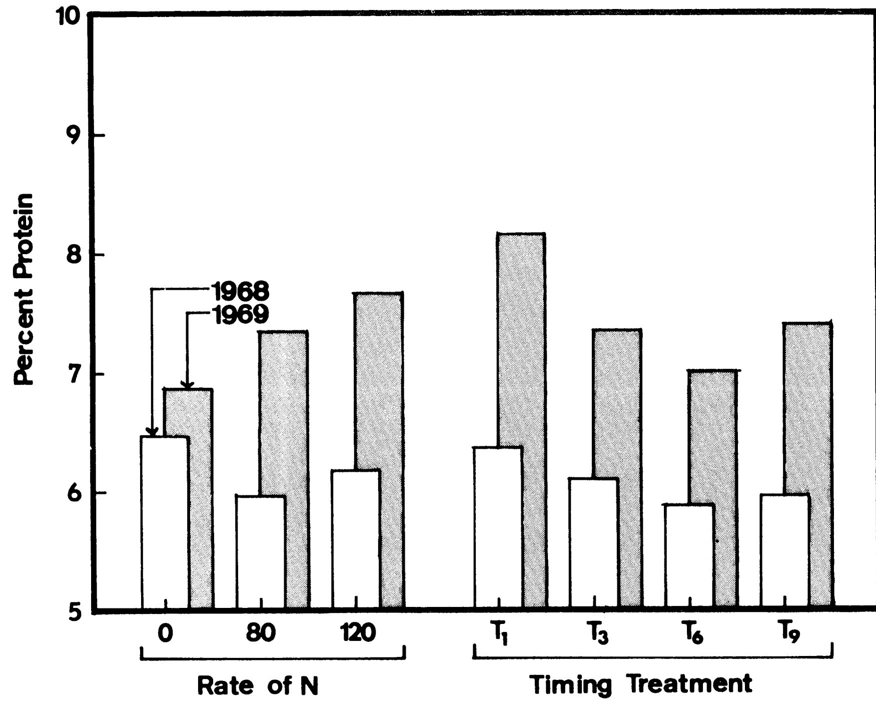

Fig. 6. Protein content of Bluebelle milled rice as affected by rate (0, 80, and 120 lb. per acre) and time of nitrogen application in 1968 and 1969.

variation in N recovery has been reported, and the recovery values in this study were not consistently as high as was reported by Houston et al. (15).

Although some variation in amino acid content was evident, there did not appear to be a marked effect of variety, rate of N, or time of application on amino acid composition, even though there were considerable differences in the protein content. Our results are in agreement with those obtained by Houston et al. (15), who found that despite a 60% variation in protein content, there was relatively little variation in amino acid composition.

Effect of Nitrogen Levels on Amino Acids

In general, increased levels of N fertilizer did not increase the concentration of individual amino acids of milled rice (Table III and Fig. 7), including lysine and threonine, the two most limiting amino acids. However, glutamic acid was usually considerably higher with increased N fertilization.

Effect of Year on Amino Acids

As pointed out earlier, there was a large and consistent increase in protein percentage in 1969 compared to 1968. The percentage of individual amino acids in the protein was also generally greater in 1969 (Table IV). Based on relatively few samples, aspartic acid, valine, and tyrosine were significantly higher in 1969, and the difference in several others, including lysine, approached significance. Threonine showed little or no difference between years.

TABLE III. AMINO ACID COMPOSITION OF COMPARABLE MILLED RICE SAMPLES AS AFFECTED BY RATE AND TIME OF NITROGEN APPLICATION[a] (g. Amino Acid per 16.8 g. N)

Amino Acid	0-N	80-N, T_1	120-N, T_1	120-N, T_9
Lysine	3.29	3.39	3.00	3.27
Histidine	2.59	2.71	2.31	2.82
Arginine	7.80	5.87	6.85	8.22
Aspartic acid	7.36	7.50	7.00	7.86
Threonine	3.12	3.35	3.06	3.17
Serine	4.62	4.29	4.01	4.44
Glutamic acid	9.70	13.98	13.09	13.58
Proline	3.71	3.80	3.65	3.86
Glycine	4.12	4.05	3.81	4.80
Alanine	4.56	4.47	4.30	4.86
Valine	5.01	5.30	5.11	5.53
Methionine	2.12	2.12	1.66	2.42
Isoleucine	3.34	3.60	3.31	3.51
Leucine	6.10	6.12	5.72	6.29
Tyrosine	1.99	1.56	1.18	1.91
Phenylalanine	3.65	3.49	3.32	3.73
N recovery, %	82.60	85.10	80.90	89.80
Crude protein, %	6.23	6.13	6.65	6.10

[a]Data used are averages of the following treatments used in comparison: Saturn milled, water-seeded, 1968; and Bluebelle milled, water-seeded, 1968.

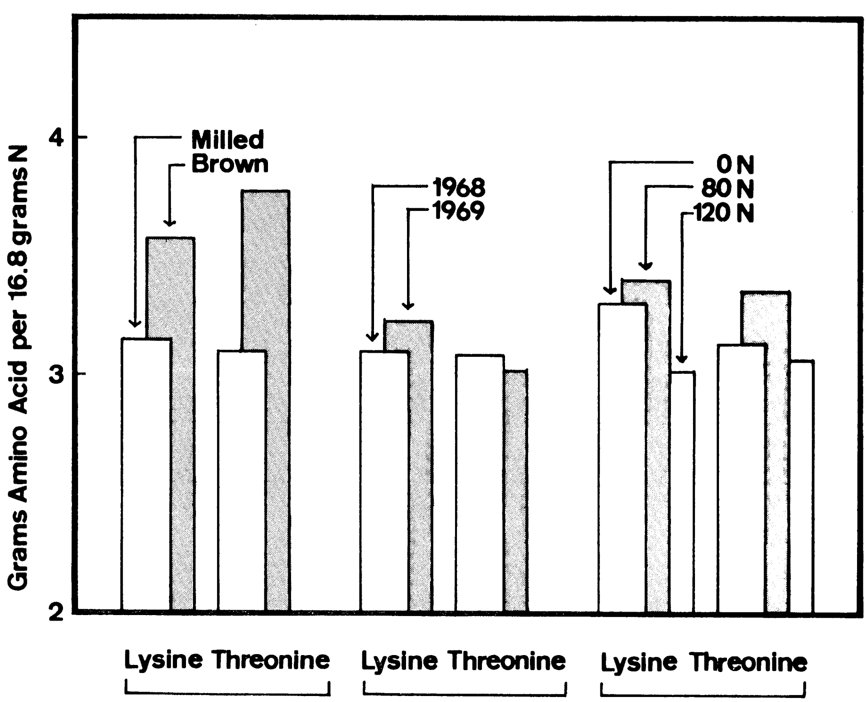

Fig. 7. Lysine and threonine contents of rice as affected by various treatments.

TABLE IV. EFFECTS OF YEAR AND MILLING ON AMINO ACID COMPOSITION OF RICE
(g. Amino Acid per 16.8 g. N)

	Effect of Year[a]		Effect of Milling[b]	
Amino Acid	1968	1969	Brown	Milled
Lysine	3.09	3.22	3.57*	3.14
Histidine	2.40	2.61	2.65	2.45
Arginine	6.96	7.68	7.45	7.32
Aspartic acid	7.03	7.88*	7.56	7.18
Threonine	3.08	3.02	3.78	3.09
Serine	4.10	4.04	4.51	4.32
Glutamic acid	13.11	13.59	13.49	11.38
Proline	3.69	3.82	3.87	3.68
Glycine	3.85	4.11	4.31	3.96
Alanine	4.45	4.68	4.83	4.43
Valine	5.20	5.59*	5.15	5.06
Methionine	1.79	2.19	1.84	1.89
Isoleucine	3.40	3.72	3.38	3.32
Leucine	5.98	5.20	6.00	5.91
Tyrosine	1.26	2.07*	1.54	1.59
Phenylalanine	3.47	3.83	3.50	3.50
N recovery, %	82.40	86.80	86.90	81.74
Crude protein, %	6.37	7.77*	7.09	6.44

[a]Treatments used in comparison were: Saturn milled, water-seeded, 120-N, T_1; Bluebelle milled, water-seeded, 120-N, T_1; and Dawn milled, water-seeded, 120-N, T_9.

[b]Data used are averages of the following treatments: Saturn, water-seeded, 0-N, 1968; Bluebelle, water-seeded, 0-N, 1968; Saturn, water-seeded, 120-N, T_1, 1968; and Bluebelle, water-seeded, 120-N, T_1.

Effect of Milling on Amino Acids

There was considerable loss of crude protein during the milling process. Because of the heterogeneous distribution of the proteins in the rice kernel, with this loss of protein there was also a corresponding loss of certain amino acids (Table IV and Fig. 7). Not only do the bran and outer endosperm of the rice kernel contain higher percentages of protein, but the protein also contains higher percentages of the water-soluble albumin than does the milled kernel (11,16,17). As the protein fraction albumin is reported relatively high in lysine, it would be expected that total protein in the outer layers would contain a higher percentage of lysine than does the predominant protein, glutelin, found largely in the endosperm. In this investigation, content of several amino acids, including lysine and threonine, was markedly reduced during the milling process (Table IV and Fig. 7). Houston et al. (15) found similar results for lysine in Calrose, a California short-grain rice.

Relation of Amino Acids to Protein Content

The relation between amino acids and protein was studied for 25 samples. Correlation coefficients between each of the amino acids and protein content were determined, but no significant relationship was observed.

Although not statistically significant, a decrease in lysine content accompanied an increase in protein content for both milled and brown rice. Histidine and leucine followed the same pattern. There was a tendency for threonine, glutamic acid, valine, isoleucine, and tyrosine to increase as the protein content increased. Several investigators have reported significant decreases in lysine as protein content increased (3,12,15,17). In an extensive study of rice protein and amino acid composition of high- and low-protein rices conducted at the International Rice Research Institute (6), protein differed only in lysine content. It should be kept in mind that even though the percentage of an individual amino acid in the protein may decrease slightly as a result of N fertilization, a large increase in percentage of protein in the grain as a result of the N application will result in the grain having a larger content of the amino acid. Such was the case in this study where there were definite differences in percent protein due to several of the treatments (time of N application and year), but no significant change in percentage of lysine in the protein.

Acknowledgments

The author expresses appreciation to W. F. McKnight and the School of Home Economics for permitting use of facilities for amino acid analyses, and special thanks to Charlotte Gandy for assistance in using the equipment.

Literature Cited

1. KIK, M. C. Nutritive studies of rice. Arkansas Agr. Exp. Sta. Bull. 508 (1951).
2. STURGIS, F. E., MIEARS, R. J., and WALKER, R. K. Protein in rice as influenced by variety and fertilizer levels. La. Agr. Exp. Sta. Bull. 466 (1952).
3. KYMAL, P. K. Variation in amino acid content of rice varieties. Ph.D. thesis. Louisiana State University, Baton Rouge (1955).
4. BANDEMER, S. L.,and EVANS, R. J. Nutrients in seeds. The amino acid composition of some seeds. J. Agr. Food Chem. 11: 134 (1963).
5. INTERNATIONAL RICE RESEARCH INSTITUTE. Annual report. Los Banos, Laguna, Philippines (1963).
6. INTERNATIONAL RICE RESEARCH INSTITUTE. Annual report. Los Banos, Laguna, Philippines (1969).
7. U. S. PRODUCTION AND MARKETING ADMINISTRATION. Rice inspection manual (revised). AMS GR Instruction No. 918-2 (1962).
8. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (10th ed.). The Association: Washington, D. C. (1965).
9. KOHLER, G. O., and PALTER, R. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44: 512 (1967).
10. HOGAN, J. T., NORMAND, F. L., and DEOBALD, H. J. Method for removal of successive surface layers from brown and milled rice. Rice J. 67(4): 27 (1964).
11. HOUSTON, D. F., IWASAKI, T., MOHAMMAD, A., and CHEN, L. Radial distribution of protein by solubility classes in the milled rice kernel. J. Agr. Food Chem. 16: 720 (1968).
12. JULIANO, B. O., BAUTISTA, G. M., LUGAY, J. C., and REYES, A. C. Studies on the physicochemical properties of rice. J. Agr. Food Chem. 12: 131 (1964).
13. JULIANO, B. O., IGNACIO, C. C., PANGANIBAN, V. M., and PEREZ, C. M. Screening for high-protein rice varieties. Cereal Sci. Today 13: 299 (1968).
14. WILSON, E., and PETERSON, F. J. Timing and midseason nitrogen at developmental stages of the rice plant. 61st Annual Progess Report, Rice Exp. Sta., La. State Univ. (1969).
15. HOUSTON, D. F., ALLIS, M. E., and KOHLER, G. O. Amino acid composition of rice and rice by-products. Cereal Chem. 46: 527 (1969).
16. JULIANO, B. O., compiler. Physicochemical data on the rice grain. Int. Rice Res. Inst. Tech. Bull. 6 (1966).
17. CAGAMPANG, G. B., CRUZ, L. J., ESPIRITU, S. G., SANTIAGO, R. G., and JULIANO, B. O. Studies on the extraction and composition of rice proteins. Cereal Chem. 43: 145 (1966).

[Received January 30, 1973. Accepted June 22, 1973]