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All-Vegetable Protein Mixtures for Human Feeding VII. Protein Complementation Between Polished Rice and Cooked Black Beans [a,b]

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SUMMARY

Studies were carried out to determine the best combination of rice and cooked black beans when one partially replaced the other isonitrogenously. Experiments with young and with adult protein-depleted rats indicate that the best combination was one in which about 60% of the protein of the diet is from rice and 40% from black beans. The range of values was from 70 to 50% rice and from 30 to 50% cooked black beans. The median figures correspond to 80 g of rice and 20 g of beans. On an isoproteic basis, rice protein is superior to black bean protein, and supplementation of either protein source with known limiting amino acids improved the protein value. Cooked black beans supplemented with methionine and valine still did not induce as good growth as rice supplemented with lysine and threonine. When rice-black bean diets containing 50% protein from each source were supplemented stepwise with the limiting amino acids methionine, lysine, and leucine, the growth and feed and protein efficiencies of the rats improved; serum protein and liver fat tended to increase.

Rice is nutritionally better than most cereal grains, and is also one of the major staple foods contributing toward the nutrition of the world population. Large quantities are consumed throughout Central America, and in Nicaragua, Costa Rica, and Panama (Reh and Fernández, 1955; Sogandares and Barrios, 1955) it replaces corn as the major staple. Rice is deficient, however, in some of the essential amino acids and relatively low in total protein (Kik, 1956a). The most-limiting amino acids in polished rice are lysine and threonine (Deshpande *et al.*, 1955; Harper *et al.*, 1955; Hundley *et al.*, 1957; Kik, 1956b; Pecora and Hundley, 1951), and the addition of these two amino acids significantly improves its biological value. The nutritive value of rice in human diets is improved by consuming it with foodstuffs that have a complementary amino acid pattern.

In a previous study of corn masa flour and cooked beans, the best combination, as measured by rat growth, was that in which each contributed 50% of the total protein of the diets tested (Bressani *et al.*, 1962). Since beans are also a possible supplementary source of the limiting amino acids in rice and also of additional protein, the present work was designed to determine the relative quantities of rice and bean protein in a diet for optimum biological results.

EXPERIMENTAL METHODS

A 50-lb sample of polished rice from the lowlands of Guatemala was ground in a Wiley mill to pass a 40-mesh screen and stored at 4°C. The beans *(Phaseolus vulgaris)* were purchased in Tecpan, Guatemala, and cooked as previously described (Bressani *et al.*, 1962). Raw and cooked samples of each were analyzed for their essential amino acid content by microbiological methods referred to previously (Bressani and Rios, 1961; Bressani *et al.*, 1962). The protein content in the

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Table 1. Nitrogen distribution of experimental diets.[a,b]

Diet no.	Rice in diet (%)	Cooked beans in diet (%)	Nitrogen from: Rice (g)	Nitrogen from: Cooked beans (g)	Nitrogen distribution: Rice (%)	Nitrogen distribution: Cooked beans (%)
1	89.00	....	0.98	......	100	0
2	71.20	5.93	0.78	0.20	80	20
3	62.40	8.90	0.69	0.29	70	30
4	53.40	11.89	0.59	0.39	60	40
5	44.50	14.86	0.49	0.49	50	50
6	35.60	17.82	0.39	0.59	40	60
7	17.80	23.80	0.20	0.78	20	80
8	....	29.70	......	0.98	0	100

[a] Rice contained 1.10% of nitrogen; cooked black beans contained 3.50%.
[b] All diets were further supplemented with 4.0% mineral mixture (Hegsted *et al.*, 1941), 2.0% cod liver oil, 5.0% cottonseed oil, cornstarch to adjust to 100%, and 5 ml/100 g of a vitamin solution (Manna and Hauge, 1953).

materials and diets was determined by the macro-Kjeldahl method (A.O.A.C., 1950).

For evaluation of the best combination of rice and beans, both rat growth and rat protein repletion experiments were carried out on female albino rats of the CD strain from the Charles River Laboratories. In all experiments, the rats were distributed by weight, 6 animals per group, in individual all-wire cages with raised screen bottoms, and fed *ad libitum* the diets whose partial composition is shown in Table 1. These diets were supplemented with 2.0% cod liver oil (Mead Johnson, Evansville, Ind.), 5.0% refined cottonseed oil (manufactured in Guatemala), and 4.0% Hegsted *et al.* (1941) mineral mixture, with cornstarch added to 100%. All diets contained a complete vitamin supplemnt (Manna and Hauge, 1953).

Food consumption and weight gains were measured every seven days for 28 days. The rats were then sacrificed and their livers removed for fat and protein determination and microscopic examination. In one experiment, serum protein values were measured by the density-gradient method of Lowry and Hunter (1945). In the rat repletion studies, the experimental period lasted 14 days and records were made every seven days.

In the second growth study, three of the previous diets were further supplemented with the amino acids listed in Table 2. Lysine and threonine were added to diet No. 1; lysine, methionine, and threonine to diet No. 5; and methionine and valine to diet No. 8. These were then respectively designated diets 1A, 5A, and 8A. The 9% casein diet of Harper *et al.* (1954) was used as a control (Table 3).

The third growth experiment was planned to determine whether the nutritive value of diet 5 could be improved by the stepwise addition of methionine, threonine, lysine, and finally leucine. In this experiment the amino acids replaced an equal amount of cornstarch; the levels of methionine and leucine were adjusted to those of rice protein, and those of lysine and threonine to the levels found in black beans. The amounts of amino acids added were corrected for the inactivity of some of the D-forms. At the end of the 28-day experimental period, the animals were sacrificed to permit analysis of liver tissue.

RESULTS

Table 2 shows the essential amino acid composition of the sample of rice used. The composition of the cooked beans was described previously (Bressani *et al.*, 1961). Comparison with the amino acid pattern of the FAO Reference Protein (FAO, 1957) suggests that tryptophan is the first limiting amino acid and lysine the second.

Table 2. Essential amino acid composition of Guatemalan rice compared with that of the FAO Reference Protein.

Amino acid	Rice (g AA/g N)	FAO (g AA/g N)
Arginine	0.530	....
Histidine	0.204	....
Isoleucine	0.336	0.270
Leucine	0.493	0.306
Lysine	0.263 (97) [a]	0.270
Methionine	0.214	0.270
Cystine	0.079	
Phenylalanine	0.336	0.180
Tyrosine	0.487	0.180
Threonine	0.229	0.180
Tryptophan	0.078 (87) [a]	0.090
Valine	0.386	0.270

[a] Percentage of FAO value given in parentheses.

Table 3. Weight gains, protein and feed efficiency, and fat content of the livers of young rats fed several rice and cooked-bean combinations, and repletion weight gains of adult rats fed the same diets.

	Source of protein		Growth [a]				Protein repletion [f]	
							Av. weight	
Diet no.	Rice (%)	Cooked beans (%)	Av. weight gain (g)	Feed efficiency [c]	Protein efficiency [d]	Liver fat [e] (%)	Initial (g)	Gain (g)
1	100	0	40	6.95	2.3	23.0	170	53
2	80	20	51	5.63	2.7	24.3	170	62
3	70	30	52	5.69	2.7	24.1	171	66
4	60	40	53	5.92	2.6	25.6	170	68
5	50	50	51	5.86	2.6	21.8	171	67
6	40	60	38	6.61	2.3	14.2	170	57
7	20	80	18	11.22	1.3	15.9	171	41
8	0	100	—4	....	....	16.2	171	23
9[b]	....	....	79	3.61	3.4	23.7	......	....

[a] Average initial weight per group: 52 g. Duration of experiment: 28 days.
[b] 9% casein diet (Harper *et al.*, 1954).
[c] Feed efficiency: average feed consumed in g/average gain in weight in g.
[d] Protein efficiency: average gain in weight in g/average protein consumed in g.
[e] Dry-weight basis.
[f] Duration of repletion: 14 days.

Table 3 shows the gains in weight as well as the feed and protein efficiency in the first rat-growth trial, and the results from the depletion-repletion experiment. In both experiments there was an increase in weight gain until 60% of the protein of the diet was contributed by rice and 40% by cooked black beans. Feed and protein efficiencies followed the same trends as weight gains. Rice alone gave better growth and repletion gains than black beans at the same protein level in the diet. The fat content of the liver was high when rice contributed higher amounts of protein to the diet and decreased as bean protein replaced more than 50% of rice protein. The highest fat and lowest protein content of the liver occurred when weight gains were greatest. The opposite tendency was noted in the rats fed more than 50% black bean protein.

The upper part of Table 4 shows the results of the third biological trial. The average weight gains of the rats were similar to those of the previous experiment. Addition of 0.40% L-lysine HCl and 0.50% DL-threonine to the diet in which all of the protein was from rice (No. 1) induced a highly significant improvement in growth and feed and protein efficiency. There was also a marked decrease in liver fat and an increase in serum proteins. The addition of 0.25% L-lysine HCl, 0.14% DL-methionine, and 0.45% DL-threonine to the diet where rice and beans each contributed 50% of the total protein of the diet (No. 5), resulted in improved growth, increased feed and protein efficiency, and decreased liver fat. A small decrease in serum proteins was also observed. The levels of lysine, methionine, and threonine added were those of diet No. 1 supplemented with the levels of lysine and threonine described above. The growth response with the supplemented diet No. 5 was not, however, as high as that obtained with all-rice protein diet No. 1 supplemented with lysine and threonine. Finally, when a diet containing only black bean protein was supplemented with 0.22% DL-methionine and 0.30% DL-valine, there was a marked increase in weight gain and in feed and protein efficiency of the rats.

The lower half of Table 4 shows the results of the fourth growth experiment. In this trial, a diet containing 50% rice protein and 50% bean protein was supplemented stepwise with methionine, threonine, lysine, and leucine. Methionine addition alone, increased weight gains and both feed and protein efficiency; liver fat also increased, while serum proteins were unchanged.

The addition of 0.10% DL-threonine to the methionine-supplemented diet caused no further change in weight or liver fat. It did, however, improve feed and protein efficiency and increase serum proteins. The addition of 0.19% L-lysine further improved feed and protein efficiency and growth. Adding 0.21% L-leucine to the amino acid supplement increased not only weight gain and feed and protein efficiency, but also serum protein, although it seemingly decreased liver fat.

DISCUSSION

Nutritionists have always advocated the use of legume seeds as protein supplements for cereal grains. In this study, cooked black beans improved the nutritive value of the diet when bean protein replaced rice protein isonitrogenously. The improvement effect reached a peak and was followed by a progressive decrease as bean protein became the major component of the diet. The maximum response occurred when rice contributed about 60% of the protein of the diet, with a range from 70 to 50%, and black beans about 40%, with a range from 50 to 30%. The 60/40 mixture corresponds to about 80 g of rice and 20 g of beans. These results are similar to those reported previously for the combination of beans and corn masa (Bressani *et al.*, 1961).

Measured on an isoproteic basis, the protein of rice is superior nutritionally to that of black beans. This may be due to a lower amino acid availability in beans as well as to the known deficiency of total sulfur-containing amino acids (Bressani and Elías, 1961; Russell *et al.*, 1946). It is also possible that the conditions of cooking beans further lowered the nutritive value of their protein. Most of the studies of essential amino acids in legume seeds of the species *Phaseolus* indicate sulfur-containing amino acids to be limiting (Bressani and Elías, 1961; Russell *et al.*, 1946).

Since the fat content of the liver is one indicator of the quantity and quality of the protein in a diet, the results suggest that, although replacement of rice by bean protein induced better growth up to a point, the amino acid balance was still not adequate for the effective mobilization of liver fat. The amino acid supplementation experiments indicate that this was due to amino acid deficiencies in the diets tested.

As reported by Harper *et al.* (1954, 1955), Rosenberg and Culik (1957), and

Table 4. Growth of rats fed different combinations of rice and black beans, and response to amino acid supplementation of diets.

Diet no.[a]	Protein distribution: Rice (%)	Protein distribution: Black beans (%)	Av. weight gain (g)	Feed efficiency[b]	Protein efficiency[c]	Liver fat[d] (%)	Serum proteins (%)
Trial no. 3[e]							
1	100	0	38	7.50	2.21	24.5	5.14
1A	100	0	134	3.16	4.58	13.9	5.80
2	80	20	54	5.81	2.54	28.8	5.17
3	70	30	49	6.12	2.37	24.8	5.29
4	60	40	51	5.74	2.44	22.3	5.23
5	50	50	51	5.86	2.43	21.2	5.53
5A	50	50	95	3.93	3.38	16.4	5.30
6	40	60	54	5.83	2.24	26.5	5.13
7	20	80	18	10.67	1.08	21.2	5.00
8	0	100	0	....	......	18.0	4.47
8A	0	100	60	5.13	2.54	21.0	5.40
Trial no. 4[f]							
5a	50	50	63	5.30	2.66	13.9	5.08
5b	50	50	73	4.90	2.87	18.4	5.12
5c	50	50	76	4.76	2.94	18.5	5.33
5d	50	50	97	3.82	3.32	14.5	5.20
5e	50	50	105	3.82	3.48	13.9	5.63

[a] Diets were supplemented with amino acids as follows: 1A: 0.40% L-lys. HCl, 0.50% DL-threo. 5A: 0.25% L-lys. HCl, 0.14% DL-met., 0.45% DL-threo. 8A: 0.22% DL-met., 0.30% DL-val., 5a: no supplement. 5b: 0.14% DL-met., 5c: 0.14% DL-met., 0.10% DL-threo., 5d: 0.14% DL-met., 0.10% DL-threo., 0.19% L-lys. HCl. 5e: 0.14% DL-met., 0.10% DL-threo., 0.19% L-lys. HCl, 0.21% L-leu.

[b] Feed efficiency: average feed consumed in g/average gain in weight in g.

[c] Protein efficiency: average gain in weight in g/average protein consumed in g.

[d] Dry-weight basis.

[e] Average initial weight per group: 48 g. Duration of experiment: 28 days.

[f] Average initial weight per group: 45 g. Duration of experiment: 28 days.

others (Kik, 1956b; Pecora and Hundley, 1951), growth improves significantly and liver fat decreases when a rice protein diet is supplemented with lysine and threonine. The results reported here also indicate that lysine and methionine are required to bring about a decrease in liver fat in those diets in which part of the protein is contributed by beans. Threonine is no longer limiting, for no response was observed when this amino acid was added to the diet (no. 5). The highest accumulation of liver fat and greater growth both resulted from the diets where most of the protein was contributed by rice. Presumably the amino acids in the diet were employed by the animal to synthesize body proteins for growth to a greater extent than for the mobilization of liver fat.

The amino acid pattern of each diet showed that beans contribute proportionately higher amounts of lysine and threonine, while there is a progressive decrease in methionine as bean protein replaces rice protein in the diet. The greater rat growth observed may be due to relative increases in lysine and threonine, an effect that is reversed as methionine becomes limiting. The point of maximum growth shows a lysine-to-threonine-to-methionine ratio of 1.4:1:0.83 when threonine is taken as a unit. Rosenberg and Culik (1957) also found that growth response from the amino acid supplementation of rice is maximum when the lysine-to-threonine ratio is 1.4:1.

For populations consuming most of their proteins from rice and beans, additional foods containing proteins of good quality are necessary to supply the needed amino acids. This can be done by adding protein concentrates such as to supply both the limiting amino acids of the mixture and extra protein.

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