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# All-Vegetable Protein Mixtures for Human Feeding VI. The Value of Combinations of Lime-Treated Corn and Cooked Black Beans [a,b]

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## SUMMARY

**Raw and lime-treated corn and raw and cooked beans were analyzed for nutrient content including essential amino acids. No major treatment changes were found. Eight different corn and bean combinations were tested isoproteically and isocalorically in both growing and protein-depleted adult rats. Corn protein alone gave better results than bean protein. Replacement of part of the lime-treated corn protein by cooked-bean protein improved growth up to the point where each dietary component contributed 50% of the total protein of the diet (72% corn and 28% beans by weight); thereafter growth was decreased with increasing amounts of bean protein in the diet.**

**When the diets were supplemented with the limiting amino acids found to be deficient by comparing their amino acid pattern with that of the FAO Reference Protein, methionine proved to be the most limiting, particularly in those diets with a higher content of bean protein. The addition of lysine and methionine together improved protein efficiency further. Whenever adequate growth was obtained, higher amounts of fat were found in the liver.**

As previous INCAP publications have stressed, corn and beans are the most important staple foods consumed by the rural population of Central America (Castillo and Flores, 1955; Flores and Reh, 1955). The incidence of protein malnutrition among children in this area is high (Béhar *et al.*, 1958). It was hoped that a corn masa (Bressani and Scrimshaw, 1958; Bressani *et al.*, 1958a) and cooked black bean combination would be found that would be suitable for preventing this disease and also for treating mild cases of it.

Cereal grain–legume seed combinations are nutritionally better than is either ingredient alone (F.A.O., 1954; Baptist, 1956). However, optimum combinations of legume seeds and cereal grains have not been reported. Since corn is deficient in lysine and tryptophan (Sauberlich *et al.*, 1953; Bressani *et al.*, 1958b) but adequate in methionine, and beans are good sources of lysine and tryptophan and deficient in methionine (Russell *et al.*, 1946; Tandon *et al.*, 1957), the proteins of these two staples should complement each other efficiently.

Baptist (1956) found that rats fed a combination of cereal grain with legume seeds grew as well as those fed the stock ration of the colony. Tongur and Orlova (1956) obtained good results by mixing 60 parts of buckwheat, 20 parts of soybean, and 16 parts of rice Desikachar *et al.* (1956) and Chitre and Vallury (1956) demonstrated that a legume and rice combination improved rat growth. More recently, Brock *et al.* (1955) reported that a combination of mealie-meal (ground

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corn) with cowpea can initiate cure in children with kwashiorkor.

In view of these findings, it was of practical interest to study the nutritive value of several combinations of corn masa with cooked black beans. The best mixture was further evaluated by supplementing the diets with the limiting amino acids.

## EXPERIMENTAL METHODS

One hundred pounds of white starchy corn *(Zea mays)*, cultivated in the highlands of Guatemala, were made into masa using the lime-treatment procedure described by Bressani and Scrimshaw (1958), a procedure involving cooking about 60 min at 96–98°C. A similar quantity of whole black beans *(Phaseolus vulgaris)* from Tecpan, Guatemala, were washed with water to remove foreign matter and cooked 60 min in the autoclave at 16 lb pressure and 121°C in the proportion of 100 g of whole beans to 2000 ml of distilled water. Both were then dried with hot air at 80°C and ground to pass 40 mesh. Representative samples of both raw and cooked material were analyzed by the A.O.A.C. method (1950) for moisture, nitrogen, ether extract, crude fiber, and ash. The carbohydrate content and metabolizable energy were obtained by calculation. The essential amino acid composition was determined by microbiological techniques with *Leuconostoc mesenteroides* P-60, using Difco media (Difco Laboratories, Detroit, Michigan) for lysine, methionine, leucine, isoleucine, arginine, cystine, phenylalanine, and tyrosine, and the media of Steel *et al.* (1949) for histidine and valine. The former medium was also used with *Lactobacillus arabinosus* 17-5 for the assay of tryptophan, and the latter with *Streptococcus faecalis* 8043 for the determination of threonine.

In evaluations of the nutritive value, both young and protein-depleted adult rats of the Wistar strain, obtained from the INCAP colony, were used. In three growth trials, 36, 48, and 48 weanling rats were distributed by weight, using 3 males and 3 females per experimental diet. The animals were placed in individual, all-wire screen cages with raised screen bottoms and provided food and water ad libitum, and weight gains and food consumption were measured every 7 days for 28 days.

At the end of the experiments, the animals in each group were sacrificed and their livers removed and analyzed for fat by ether extraction and for nitrogen by the micro-Kjeldahl procedure. A fragment of liver tissue was fixed in 10% neutral formalin solution for staining with hematoxylin-eosin, Gomori's reticulin fiber, and Sudan IV stains (Lillie, 1948). The amount of fatty change in the liver was estimated as follows: Grade I, no sudanophilic material seen; II, a few hepatic cells showing fat droplets (usually cells located in the periportal area of the hepatic lobule); III, same as Grade II but more severe; IV, fatty change diffuse and more severe.

In the depletion-repletion experiments, adult albino rats, weighing around 200 g, were depleted of protein by feeding a nitrogen-free diet made up of 86% cornstarch, 5% Hegsted mineral mixture (Hegsted *et al.*, 1941), 5% refined cottonseed oil (manufactured in Guatemala), 2% cod liver oil, and 2% cellulose (Nutritional Biochemical Corp., Cleveland, Ohio), supplemented with 5 ml per 100 g of a vitamin solution suggested by Manna and Hauge (1953). After losing 25% of their initial body weight, the rats were distributed by weight among the experimental groups and handled as previously described for the young rats. Two experiments with eight groups each were carried out in which body weight and food consumption were measured at 7 and 14 days.

In the growth studies, the animals were fed the experimental diets described in Table 1, which also shows the amount of cooked beans and lime-treated corn flour necessary to give isonitrogenous and isocaloric diets. The table also shows the protein percentage distribution from masa and cooked beans; the other ingredients of the diets were 2% cod liver oil (courtesy of Mead-Johnson & Co., Evansville, Indiana), 5% cottonseed oil, 4% Hegsted mineral mixture (Nutritional Biochemical Co., Cleveland, Ohio) (Hegsted *et al.*, 1941), 2% Alfacel, and cornstarch to adjust to 100%. All rations were further supplemented with a complete vitamin solution (Manna and Hauge, 1953).

Two amino acid supplementation studies were also carried out. In the first, each of the eight diets tested previously was supplemented with limiting essential amino acids to the levels indicated by the amino acid pattern of the FAO Reference Protein (1957). The second, the diet in which 50% of the protein came from beans and 50% from corn masa, was supplemented with methionine, lysine, isoleucine, and threonine for determination of the most limiting amino acid. In the amino acid supplementation studies, correction was made for the form of the amino acid added; but no attempt was made to make all diets completely isonitrogenous, because the quantities of nitrogen contributed by these additions were so small.

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

| Diet no. | Masa (% in diet) | Cooked beans (% in diet) | Nitrogen from: Corn masa (g) | Nitrogen from: Cooked beans (g) | Nitrogen distribution: Corn masa (%) | Nitrogen distribution: Cooked beans (%) |
|---|---|---|---|---|---|---|
| 1 | 87.00 | ........ | 1.15 | ...... | 100 | 0 |
| 2 | 69.59 | 7.33 | 0.92 | 0.23 | 80 | 20 |
| 3 | 60.89 | 11.00 | 0.80 | 0.34 | 70 | 30 |
| 4 | 52.19 | 14.67 | 0.09 | 0.46 | 60 | 40 |
| 5 | 43.50 | 18.34 | 0.57 | 0.57 | 50 | 50 |
| 6 | 34.79 | 22.00 | 0.46 | 0.69 | 40 | 60 |
| 7 | 17.40 | 29.34 | 0.23 | 0.92 | 20 | 80 |
| 8 | ........ | 36.67 | ...... | 1.15 | 0 | 100 |

[a] % N in corn masa, 1.32; % N in cooked beans, 3.14.

[b] All diets were supplemented with 4% mineral mixture (Hegsted *et al.*, 1941); 2% cellulose; 2% cod liver oil; 5% cottonseed oil and with cornstarch to adjust to 100 g. All diets received 5 ml of a vitamin solution (Manna and Hauge, 1953) per 100 g diet.

## RESULTS

Table 2 shows the chemical composition of the corn and the black beans in their raw and cooked states, and Table 3 their essential amino acid content. For comparison, the amino acid pattern of the FAO Reference Protein is also included in Table 3.

When the amino acids are expressed as g of amino acid per g of nitrogen, a change in arginine and tryptophan content is noted between the raw corn and masa. Other amino acids remain the same or are slightly higher in lime-treated corn than in raw corn. Small changes were found between the raw and cooked black beans in arginine, isoleucine, leucine, lysine, phenylalanine, and tryptophan content. The comparison of the amino acid pattern of lime-treated corn with that of the FAO Reference Protein indicated that the limiting amino acids were tryptophan, lysine, methionine, and isoleucine; in cooked black beans, methionine was most deficient, followed by tryptophan and possibly leucine.

Fig. 1 shows the results of the rat growth experiments. It can be seen that at the same protein percentage of the diet, growth in all experiments was better with lime-treated corn alone than with cooked beans alone. The maximum growth response was obtained when corn masa contributed 40–60% of the total protein with bean protein supplying the remainder.

Fig. 1 also shows the similar results of the protein depletion-repletion experiments. At equal protein levels, lime-treated corn as the sole protein source induced better weight gains during repletion than did cooked beans. Replacement of part of the lime-treated corn protein by cooked-bean protein improved repletion weight gains.

Fig. 1 also shows the fat content of the livers of the growing rats fed the several corn-bean combinations. It is evident that the fat content in the livers was greater when growth was better. Histopathological studies of the liver of the rats fed the several corn-bean combinations showed no consistent change in the hepatic structure. The hepatic cells were found to be normal, except for a variable degree of fatty change in the majority of the animals studied. The fatty change occurred mainly in the periportal areas, and the fat change in each cell varied from very slight (fine intracytoplasmic droplets) to severe (coarse

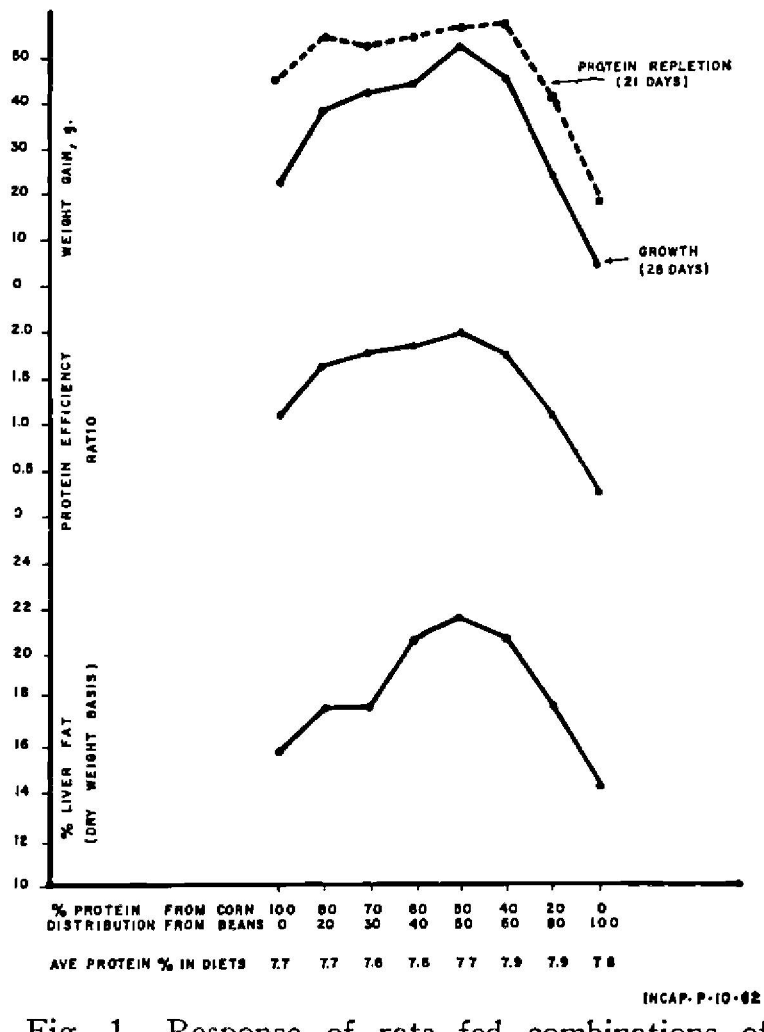


Fig. 1. Response of rats fed combinations of lime-treated corn and cooked black beans.

Table 2. Chemical composition of corn and black beans before and after cooking.

| Nutrient | Raw ground corn | Lime-treated corn masa | Raw black beans | Cooked black beans |
|---|---|---|---|---|
| Moisture (g %) | 14.20 | 11.50 | 12.40 | 10.50 |
| Protein (g %) | 9.18 | 8.25 | 18.50 | 19.62 |
| Ether extract (g %) | 4.00 | 3.30 | 1.80 | 0.50 |
| Crude fiber (g %) | 3.10 | 2.30 | 4.80 | 5.60 |
| Ash (g %) | 1.20 | 2.70 | 4.20 | 3.50 |
| Carbohydrate (g %) | 68.32 | 71.95 | 58.30 | 60.28 |
| Metabolizable calories/100 g | 346 | 351 | 323 | 324 |

unique drops). No pathological findings were encountered in the portal tracts, central veins, or hepatic lacunae.

**Amino acid supplementation.** The difference between the amino acid pattern of each combination of lime-treated corn and cooked beans and that of the FAO Reference Protein (1957) was calculated and the amount representing this difference was added to each combination of corn and beans. The only exception was methionine, which, on the basis of previous results (Bressani *et al.*, 1954; 1958), was added at a level of 180 instead of 270 mg/g N.

In the first of two experiments, 6 rats were used per group, and eight corn-bean combinations were supplemented with amino acids. Diets 1, 5, and 8 of the previous experiments served as negative controls. The results are in Table 4. The addition of lysine, tryptophan, and isoleucine to diet 1, in which all the protein was contributed by corn, improved growth and protein efficiency significantly. The supplementation of diets 2 and 6 resulted in similar growth responses; the amino acid addition to diet 5 gave only a small response in weight gain. Diet 7, with lime-treated corn contributing 20% and cooked beans 80% of the total protein, gave a poor growth response compared to the other dietary corn-bean combinations, even though it was supplemented with methionine and tryptophan. In the diet with all of the protein contributed by cooked beans, supplementation with methionine, tryptophan, and leucine improved growth response significantly. The weight gain, however, was much below that obtained when the diet contained only unsupplemented corn protein. Table 5 also shows the fat content of the liver of the rats in this experiment. The fat increased as the proportions of lime-treated corn decreased to 60% of the protein of the diet and the cooked beans increased to 40%. The fat decreased as the amount of cooked beans in the diet increased beyond 40%. In general, the amino acid supplements to the diets did not decrease the fat content of the liver of the experimental animals.

The second amino acid supplementation experiment was designed to determine the limiting

Table 3. Essential amino acid composition of corn and beans before and after treatment.

| Amino acid | Raw corn (g/g N) | Masa (g/g N) | Raw beans (g/g N) | Cooked beans (g/g N) | F.A.O. Ref. Prot [a] (g/g N) | Score [b] Masa (%) | Score [b] Cooked beans (%) |
|---|---|---|---|---|---|---|---|
| Arginine | 0.262 | 0.242 | 0.408 | 0.387 | .... | .... | .... |
| Histidine | 0.231 | 0.249 | 0.244 | 0.242 | .... | .... | .... |
| Isoleucine | 0.213 | 0.227 | 0.366 | 0.350 | 0.270 | 84 | .... |
| Leucine | 0.572 | 0.575 | 0.285 | 0.274 | 0.306 | .... | 90 |
| Lysine | 0.126 | 0.138 | 0.584 | 0.567 | 0.270 | 51 | .... |
| Methionine | 0.114 | 0.119 | 0.088 | 0.083 | 0.190 | 72 | 47 |
| Cystine | 0.075 | 0.076 | 0.045 | 0.043 | 0.080 | | |
| Phenylalanine | 0.276 | 0.271 | 0.360 | 0.338 | 0.180 | .... | .... |
| Tyrosine | 0.199 | 0.195 | 0.181 | 0.171 | 0.180 | | |
| Threonine | 0.214 | 0.228 | 0.296 | 0.331 | 0.180 | .... | .... |
| Tryptophan | 0.032 | 0.028 | 0.080 | 0.076 | 0.090 | 31 | 84 |
| Valine | 0.281 | 0.297 | 0.493 | 0.516 | 0.270 | .... | .... |
| Nitrogen (%) | 1.47 | 1.32 | 2.96 | 3.14 | .... | .... | .... |

[a] Amino acid levels of F.A.O. Reference Protein.
[b] Amino acid adequacy in percent of masa and of cooked beans according to F.A.O. Reference Protein.

amino acids in a diet in which 50% of the protein came from cooked beans and 50% from corn masa. Amino acids were added as in the previous experiment. The results are shown in Table 4. Methionine addition alone improved weight gain and protein efficiency over the unsupplemented diet. Supplementation with methionine and lysine improved protein efficiency, whereas isoleucine and threonine added to the diet supplemented with methionine and lysine did not further improve growth or protein efficiency.

## DISCUSSION

The high incidence of protein malnutrition in Central America is due to the low consumption of good-quality protein during the critical age of 2–5 years (Béhar *et al.*, 1958). Therefore, if a good corn and bean diet combination were fed during this period, prevention of protein malnutrition should be possible.

The amino acid patterns of the two foodstuffs under study complemented each other in the range of 80–50% of protein from lime-treated corn and 20–50% from beans. At isoproteic levels, lime-treated corn proteins are nutritionally better than cooked-bean proteins, even though the comparison of the amino acid pattern of each protein with that of the FAO Reference Protein suggests that corn proteins are low in at least three amino acids, while beans are low in only one or two.

The lesser growth observed with the diet in which all the protein was contributed by

Table 4. Response of rats to amino-acid-supplemented lime-treated-corn and cooked-black-bean diets.

| Diet no. | Source of protein: Lime-treated corn (%) | Source of protein: Cooked beans (%) | Protein in diet (%) | Average weight gain [a] (g) | Feed efficiency [b] | PER [c] | Fat content of liver [d] (%) |
|---|---|---|---|---|---|---|---|
| Experiment 1 [e] | | | | | | | |
| 1 | 100 | 0 | 9.25 | 29 | 8.21 | 1.05 | 16.34 |
| 1A | 100 | 0 | 8.81 | 74 | 4.59 | 2.47 | 17.51 |
| 2A | 80 | 20 | 9.00 | 58 | 5.26 | 2.15 | 20.28 |
| 3A | 70 | 30 | 9.43 | 61 | 5.64 | 1.91 | 25.35 |
| 4A | 60 | 40 | 9.06 | 61 | 5.33 | 1.89 | 20.69 |
| 5 | 50 | 50 | 9.25 | 51 | 5.76 | 2.10 | 21.14 |
| 5A | 50 | 50 | 9.12 | 59 | 5.34 | 2.03 | 16.75 |
| 6A | 60 | 40 | 8.94 | 61 | 5.26 | 2.10 | 21.58 |
| 7A | 20 | 80 | 9.00 | 45 | 5.91 | 1.88 | 11.81 |
| 8 | 0 | 100 | 9.50 | —3 | .... | .... | 16.03 |
| 8A | 0 | 100 | 9.25 | 23 | 10.26 | 1.04 | 14.18 |
| Experiment 2 [f] | | | | | | | |
| 5–1 | 50 | 50 | 9.60 | 64 | 5.09 | 2.05 | .... |
| 5–2 | 50 | 50 | 9.30 | 71 | 4.92 | 2.18 | .... |
| 5–3 | 50 | 50 | 9.66 | 75 | 4.28 | 2.42 | .... |
| 5–4 | 50 | 50 | 9.60 | 72 | 4.67 | 2.26 | .... |
| 5–5 | 50 | 50 | 9.68 | 70 | 4.79 | 2.16 | .... |

[a] Average initial weight for Experiment 1, 62 g; and for Experiment 2, 49 g.
[b] Feed efficiency: average food consumed in g/average weight gain in g.
[c] Protein efficiency ratio: average weight gain in g/average protein consumed in g.
[d] Dry-weight basis.
[e] Diets with "A" were supplemented with amino acids as follows: 1A: L-lys. HCl, 0.19%; DL-tryp., 0.07%; DL-isoleu., 0.098%. 2A: L-lys. HCl, 0.07%; DL-tryp., 0.061%; DL-isoleu., 0.04%; DL-met., 0.016%. 3A: DL-tryp., 0.055%; DL-met., 0.023%; DL-isoleu., 0.01%. 4A: DL-tryp., 0.050%; DL-met., 0.032%. 5A: DL-tryp., 0.044%; DL-met., 0.039%. 6A: DL-tryp., 0.038%; DL-met., 0.047%. 7A: DL-tryp., 0.028%; DL-met., 0.064%. 8A: DL-tryp., 0.017%; DL-met., 0.080%; L-leu., 0.039%.
[f] The amino acid supplements for the diets in this experiment were: 5–1, none; 5–2, DL-met., 0.04%. 5–3 DL-met., 0.04% + L-lys. HCl, 0.07%. 5–4 DL-met., 0.04% + L-lys. HCl, 0.07% + DL-threo, 0.19%. 5–5, DL-met., 0.04%+ L-lys. HCl, 0.07%+ DL-threo., 0.19%+ DL-isoleu., 0.18%.

beans may have been due to a poor over-all amino acid balance or to a lower digestibility of bean protein, resulting in a decreased availability of the amino acids for the growing rat.

Other investigators (FAO, 1954; Castro and Pechnik, 1951; Baptist, 1956; Tongur and Orlova, 1956; Desikachar *et al.*, 1956; Chitre and Vallury, 1956) have generally found that combinations of two sources of protein give a product of higher protein value than either alone. However, if the protein of lower nutritive value contributed 50% or more of the total protein of the diet, the net growth responses were similar to those obtained with the single protein of poorer nutritive value. Results were opposite when a protein of better nutritive value furnished 50% or more of the total dietary protein.

The amount of fat in the liver reflects the level of protein in the diet (Harper *et al.*, 1954, 1955). In the experiments reported in this paper, the accumulation of fat in the liver was higher when growth was better. This suggests that the proportions of amino acids in some of the diets were adequate for good growth but not for the mobilization of fat from the liver. If animal growth improves when diets with non-evident deficiencies and imbalances are fed, the effect of these soon becomes apparent in biochemical and pathological alterations. The results indicate that some of the diets were capable of inducing good growth but were still deficient in nutrients essential for the mobilization of fat from the liver. Harper *et al.* (1955) and other investigators (Deshpande *et al.*, 1955; Rosenberg and Culik, 1957) have found a similar effect in rice-fed rats.

The amino acid supplementation results showed that methionine was the most limiting amino acid in the diet in which the protein was half from corn and half from beans. Methionine improved both protein efficiency and growth; the latter was further improved by the addition of lysine. For practical reasons the most efficient diet combination contained 50% of protein from corn and 50% from cooked beans, which is equivalent to 72% corn and 28% beans by weight.

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