The Development of INCAP Vegetable Mixtures

I. Basic Animal Studies[1]

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IT IS NOW widely accepted that an important and practical approach to supplying the needed dietary protein in areas where milk and other products of animal origin are costly or in short supply is the development of suitable combinations of vegetable protein sources to supply both essential and nonessential amino acids in the proportions required.

Research efforts to this end are following three slightly different lines. One is to add high-protein foods of vegetable origin as a supplement to the cereal diets of underdeveloped areas. This approach is illustrated by the development of Indian Multipurpose Food.[1] A second means is the development of a protein-rich vegetable mixture which contains in its formula cereal grains commonly consumed in the area where the mixture is to be used. Such a mixture may be essentially a "complete food", even when it is intended for the supplementary and mixed feeding of infants and young children and as a part of adult diets. A third way of making more efficient use of the available vegetable protein supplies of the area is to encourage use of two or three different vegetable foods in proportions which yield protein quality superior to that of any of the components.

In Central America, we have concentrated most of our attention on the last two possibilities. Vegetable protein mixtures of relatively low protein content but of fairly good nutritive value have been studied in the hope of finding ways of improving diets by educational measures alone.[2] For example, the value of mixtures of lime-treated corn (*Zea mays*) and cooked black beans (*Phaseolus vulgaris*) is shown in figure 1. The bars in the lower part of the figure indicate the proportions of protein from lime-treated corn and beans respectively. The curves from top to bottom represent the average gain of rats in 28 days, the protein efficiency ratio (PER) and the liver fat percentage of the rats fed the different combinations. At the same protein level in the diet, the growth obtained with lime-treated

[1] INCAP Publication I—173

En: . "Progress in Meeting Protein Needs of Infants and Preschool Children". Proceedings of an International Conference held in Washington, D. C. August 21-24, 1960, Washington, D. C., National Academy of Sciences-National Research Council, 1961, p. 35-48. Publication 843.

COMBINATIONS BETWEEN LIME-TREATED CORN AND COOKED BLACK BEAN PROTEINS

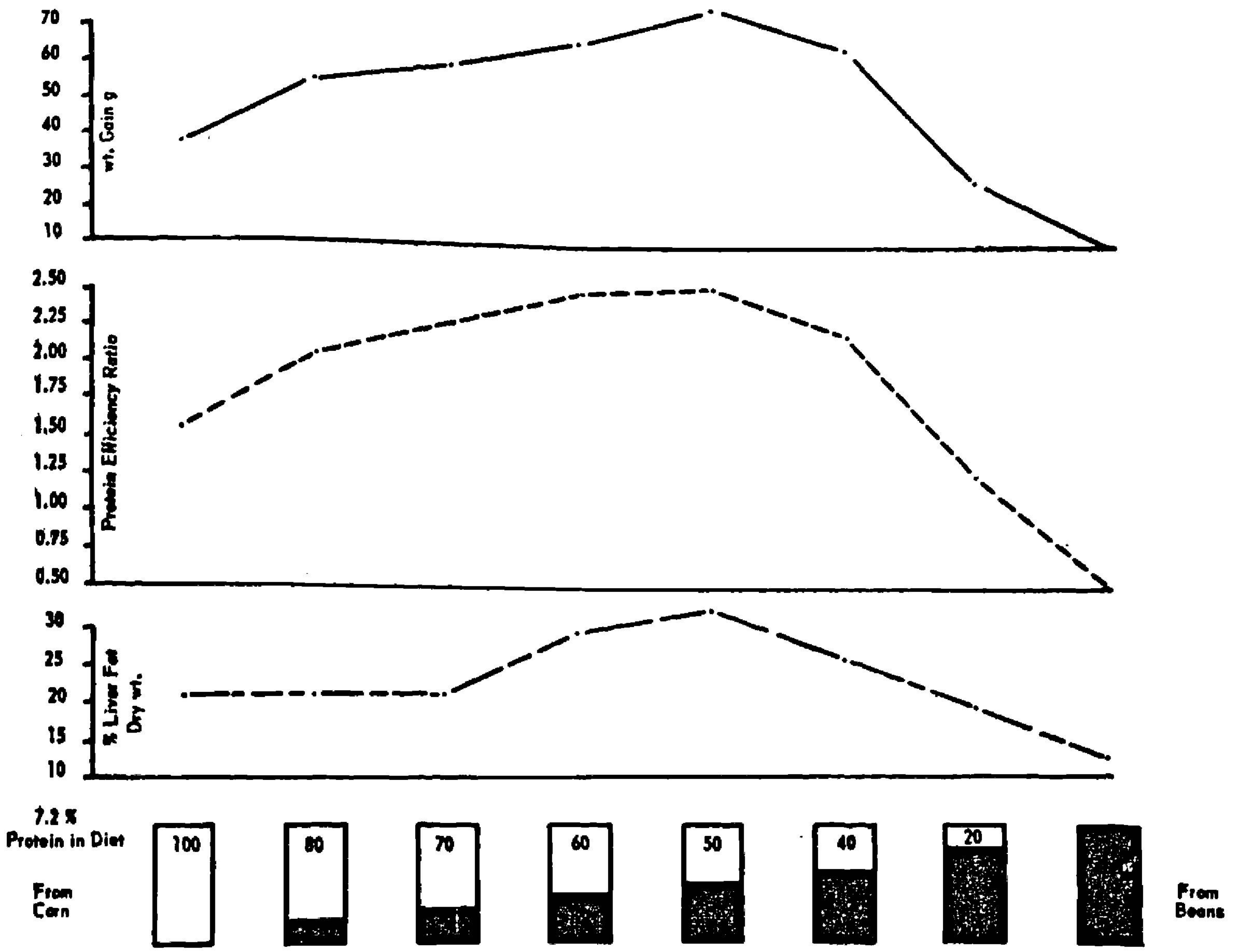

Figure 1—The bars in the lower part of the figure indicate the proportions of protein from lime-treated corn and beans respectively. The curves from top to bottom represent the average gain of rats in 28 days, the protein efficiency ratio (PER) and the liver fat percentage of the rats fed the different combinations.

corn is superior to that obtained with cooked black beans, even though essential amino acid limitations are less in black bean protein than in lime-treated corn. This effect is believed to be due to a lower digestibility of black bean compared with corn protein, and hence a decreased availability of the essential amino acids. The most efficient combination of the two foods is one in which approximately 50% of the protein of the diet comes from each. This finding is very consistent from experiment to experiment with both young and adult protein-depleted rats. The combination obtaining maximum growth produces more fat in the liver. Since lime-treated corn contains about 8.5% protein and black beans approximately 21.0%, the above mixture would have a protein content of 12% with a formula composition by weight of 71% lime-treated corn and 29% cooked black beans.

The amino acid pattern of lime-treated corn and black beans and the optimum combination of the two foods for rat growth is compared in table 1 with that of the FAO reference protein. It is evident that the main amino acid deficiencies in lime-treated corn have been corrected by the amount of black bean protein added; the mixture is now limiting in total S-amino acids and tryptophan. The higher fat content of the liver of the rats fed the 50-50% protein combination may be the result of a methionine deficiency in the mixture. In other experiments, methionine addition to the 50-50% mixture not only further improved growth and the PER ratio, but also lowered liver fat.[2]

TABLE 1
ESSENTIAL AMINO ACID PATTERNS

Amino Acid	Lime-treated Corn	Cooked Beans	50-50 Protein Combination Corn-Beans	FAO Ref. Prot.
		mg/g N		
Arginine	242	388	315	......
Histidine	249	242	246	......
Isoleucine	227*	350	288	270
Leucine	576	273*	426	306
Lysine	139*	568	353	270
Methionine } Cystine	195*	125*	161*	270
Phenylalanine	272	339	305	180
Threonine	228	332	280	180
Tryptophan	29*	76*	52*	90
Valine	298	517	408	270

* Limiting Amino Acids.

TABLE 2
ESSENTIAL AMINO ACID PATTERNS

Amino Acid	Rice	Cooked Beans	65-35 Protein Combination Rice-Beans	FAO Ref. Prot.
		mg/gm N		
Arginine	519	379	471	......
Histidine	200	237	213	......
Isoleucine	329	343	334	270
Leucine	483	269*	408	306
Lysine	258*	556	362	270
Methionine } Cystine	342	123*	230*	270
Phenylalanine	329	331	330	180
Threonine	224	324	260	180
Tryptophan	76*	74*	76*	90
Valine	378	506	423	270

* Limiting Amino Acids.

White rice and black beans are another common and similar type of vegetable mixture.[3] The results of an experiment with these foods are shown in figure 2. The bars in the lower part of the figure again represent the source and distribution of protein in the diet. Rice protein alone produced better growth than did protein from black beans alone. The nutritive value of the two foods combined is better than that of either rice or beans alone; again, the highest amount of liver fat was found when better growth was obtained. In contrast to the results with lime-treated corn and beans, there was no clearly superior combination observed in this experiment. As shown in figure 2, good combinations were those with rice contributing from 50% to 80% of the protein and black beans 50% to 20%. The most efficient combination was one in which rice contributed 80% of the protein to the diet and the cooked black beans the remaining 20%. The composition by weight of the 80-20% protein mixture is 92% rice and 8% cooked beans, and its protein

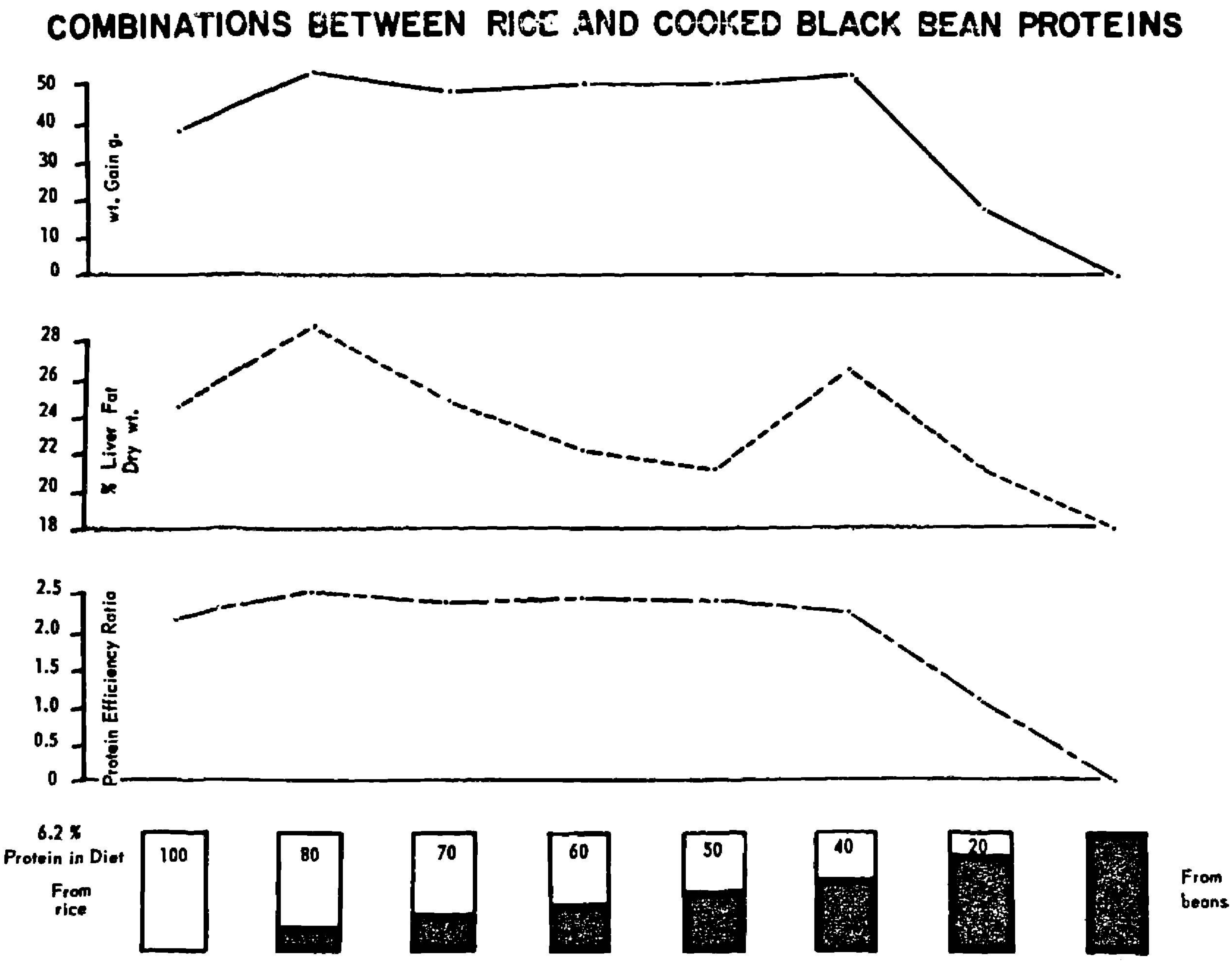

Figure 2—See legend, Figure 1.

content is only about 8%. The "rice-black bean mixture" recommended is the 65-35% protein distribution. It would have a formula composition of 84% white rice and 16% cooked black beans and a protein content of approximately 9.5%.

The amino acid pattern of the two components, the 65-35% mixture and the FAO reference pattern are shown in table 2. The rice-bean mixture appears to be limiting only in total S-amino acids and tryptophan. Methionine and threonine supplementation of the 65-35% mixture resulted in improved rat growth and PER ratio and lowered liver fat.[3] Apparently the threonine deficiency of rice [4, 5] was not completely corrected by the addition of cooked black beans.

Combinations similar to those previously described have also been worked out with corn and cowpea (*Vigna sinensis*).[6] The results of a typical experiment are shown in table 3. These two foods are efficiently combined when corn contributes 25% to 50% of the protein and cowpea from 75% to 50%. A vegetable mixture with 40% of the protein from corn and 60% of the protein from cowpea would have a composition by weight of 64% corn and 36% cowpea, with a total protein concentration of around 15%, which is higher than that of lime-treated corn and of rice and beans. The limiting amino acids in the corn-cowpea mixture are again methionine and tryptophan. All these mixtures share the disadvantage for child feeding of low protein content. The results indicate, however, that the common foods of the rural population of underdeveloped areas can be used more efficiently and supply better protein when combined so that their amino acids complement each other.

TABLE 3
COMBINATIONS BETWEEN CORN AND COWPEA PROTEINS

Protein distribution in Diet % From Corn	% From Cowpea	Ave. Wt. Gain, gm	F.E.[1]	P.E.[2]
100	0	54	5.21	1.22
75	25	78	3.96	1.59
50	50	102	3.43	1.84
25	75	104	3.51	1.82
0	100	78	4.41	1.41

[1] Average food consumed per average weight gained.
[2] Average weight gained per average protein consumed.

When compared with the FAO reference protein,[7] the deficient amino acids in all of these mixtures were methionine and tryptophan. In studies of amino acid supplementation of these three mixtures, it was found that, after correcting the first amino acid deficiency, tryptophan addition did not bring about an improvement of the nutritive value of the mixtures. This suggests that the level of tryptophan in the FAO reference protein is probably higher than needed.

Because of the relatively low protein content in these food combinations, vegetable mixtures of higher protein content were sought. The first of these to receive extensive biological testing was known as Vegetable Mixture 8. It consisted of 50% lime-treated corn flour, 35% sesame flour, 9% cottonseed flour, 3% Torula yeast and 3% Kikuyu leaf meal.[8-10] Vegetable Mixture 8 contained 25% crude protein, and the comparison of its essential amino acid pattern with that of the FAO reference protein indicated deficiencies in lysine, methionine and tryptophan. The protein score based on the FAO reference pattern was approximately 67%.

Rat growth experiments indicated that the mixture was palatable and that it gave good growth and efficient feed utilization. Representative results with rats are shown in table 4. The addition of 0.45% lysine or skim milk substituted for part of the corn in the mixture improved the feed efficiencies but not the growth of the rats. When the protein in Vegetable Mixture 8 was diluted to 15% with cornstarch, the addition of lysine improved both the growth and feed efficiencies.

TABLE 4
RAT TRIALS OF VEGETABLE MIXTURE 8
(12 Rats Per Group, 8 Weeks With 25.2% Protein)

	Trial Mixture	Feed Efficiency	Initial Wt. gm	Final Wt. gm
2A	8	2.71	44	223
2B	8 + 0.45% lysine	2.14	44	230
2C	Modified 8 *	2.08	44	233
2D	Modified 8 * + 0.45% lysine	1.90	44	232
2E	Modified 8 * 9% skim milk #	2.29	44	236
2F	Modified 8 * 9% skim milk # + 0.45% lysine	1.98	44	235

* Cottonseed meal replaced by additional sesame.
# Substituted for corn masa.

Representative results with chicks are shown in table 5. Addition of lysine did improve growth and feed utilization by chicks. Further studies with chicks indicated that grain sorghum, buckwheat, rice or whole ground corn could replace lime-treated corn in the mixture without altering its nutritive value.[10]

TABLE 5
CHICK TRIAL OF INCAP VEGETABLE MIXTURE 8
(24 chicks per group, 5 weeks with 25% protein)

Mixture [1]	Feed Efficiency [3]	Initial Wt. gm	Final Wt. gm
8	2.80	42	200
Modified 8 [2]	2.84	42	217
8 + 0.2% Lysine	2.19	42	298
8 + 0.4% Lysine	2.05	42	369

[1] With minerals and vitamins added to meet chick requirements.
[2] Whole ground corn substituted for lime-treated corn (masa).
[3] Grams fed per grams gained.

As a result of the work on Vegetable Mixture 8, several facts became clear. First of all, the work showed that an all-vegetable mixture of good protein quality could be developed; second, that it could cure severe forms of protein malnutrition; and third, that it was possible to achieve good acceptance by the needy populations of technically underdeveloped areas.

Although Vegetable Mixture 8 proved to be good, it was not sufficiently economical for the Central American area because of the short supply of sesame seed, which raised its cost. A less expensive mixture eliminating sesame seed was needed. Cottonseed flour was selected as the sole concentrated protein source for several reasons. During the development of Vegetable Mixture 8, experience was acquired in its use; cottonseed flour, when properly processed, has good protein quality, and cottonseed oil meal is readily available in most of the Central American countries. Although cottonseed flours for human feeding have been known for some time, they have been little used for the purpose. One disadvantage is, of course, the presence of gossypol pigments and the frequent destruction of protein quality during processing.[11]

The actual research work on Vegetable Mixture 9 was started at INCAP in November 1957. Since then a large amount of information has been accumulated and is being published.[12-14, 16] The first experiments on Vegetable Mixture 9 were designed to learn whether the protein quality of cottonseed flour was similar to the protein quality of sesame meal. The results of one experiment carried out with rats are shown in figure 3. Ten per cent protein was used in the diet of this

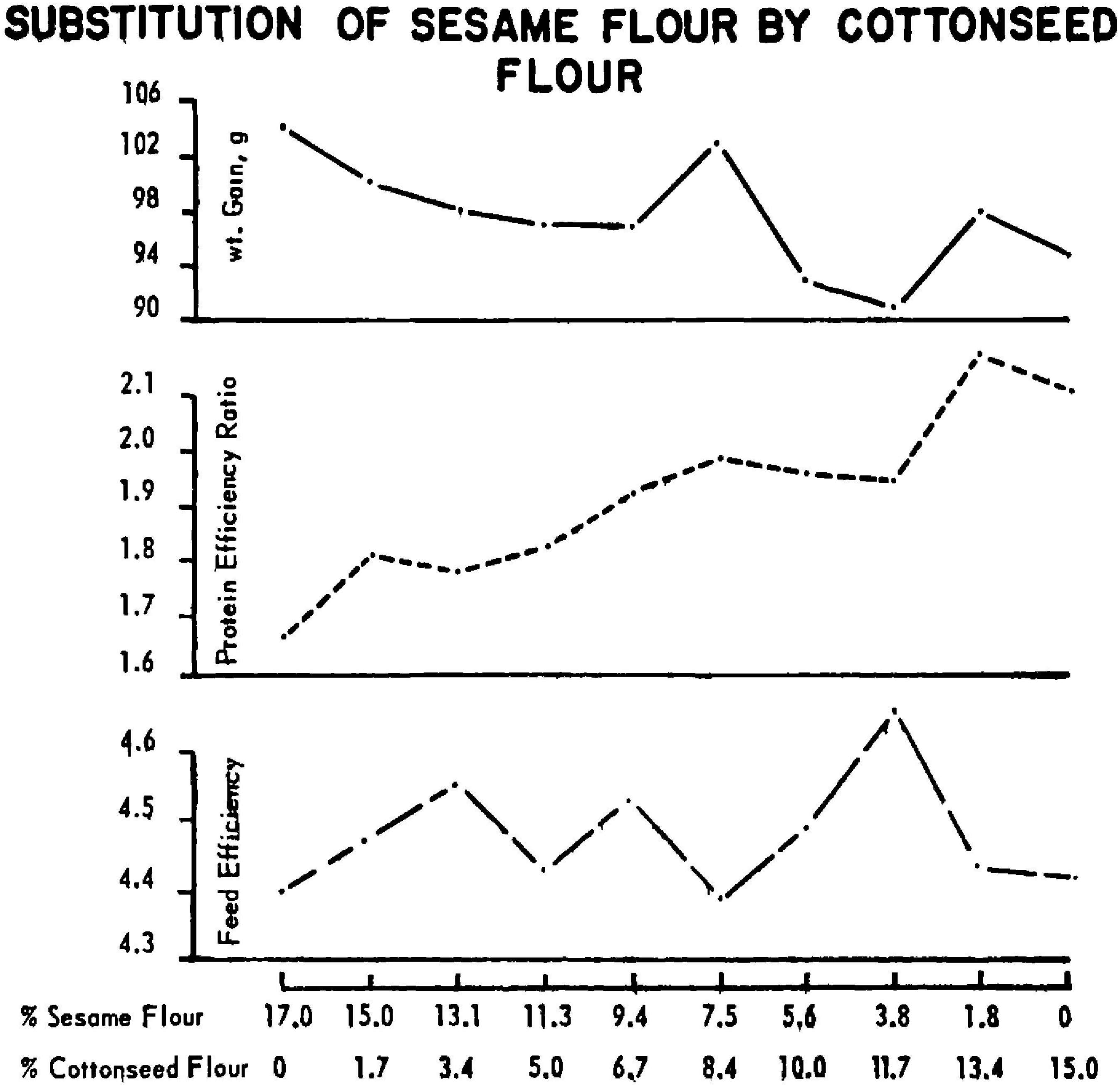

Figure 3—See legend, Figure 1.

experiment. The actual amounts of sesame flour and cottonseed flour used are shown in the lower part of the figure. It is evident that there was little difference in growth and feed efficiency as cottonseed flour replaced sesame flour, although the protein efficiency improved. Experiments carried out with chicks also gave similar results.[13]

The next step was to determine the most efficient combination of corn and cottonseed flour protein. The results of one experiment in rats is shown in figure 4. The bars in the lower part of the figure indicate the protein contribution to the diet from cottonseed flour and from corn. The upper curve is the weight gain of the rats after 28 days. As the protein contribution from corn increases, there is a decrease in weight gain up to the 10-90% protein proportions between the two ingredients. An increase is found at the 15% to 85% protein ratio. Weight gains then decrease as the protein contribution from corn is increased, and the feed and protein efficiencies behave in a similar manner. It is interesting to note that the 15% to 85% ratio is no better than the 0 to 100% ratio between corn and cottonseed flour. The result was, however, consistent in other rat as well as chick trials.

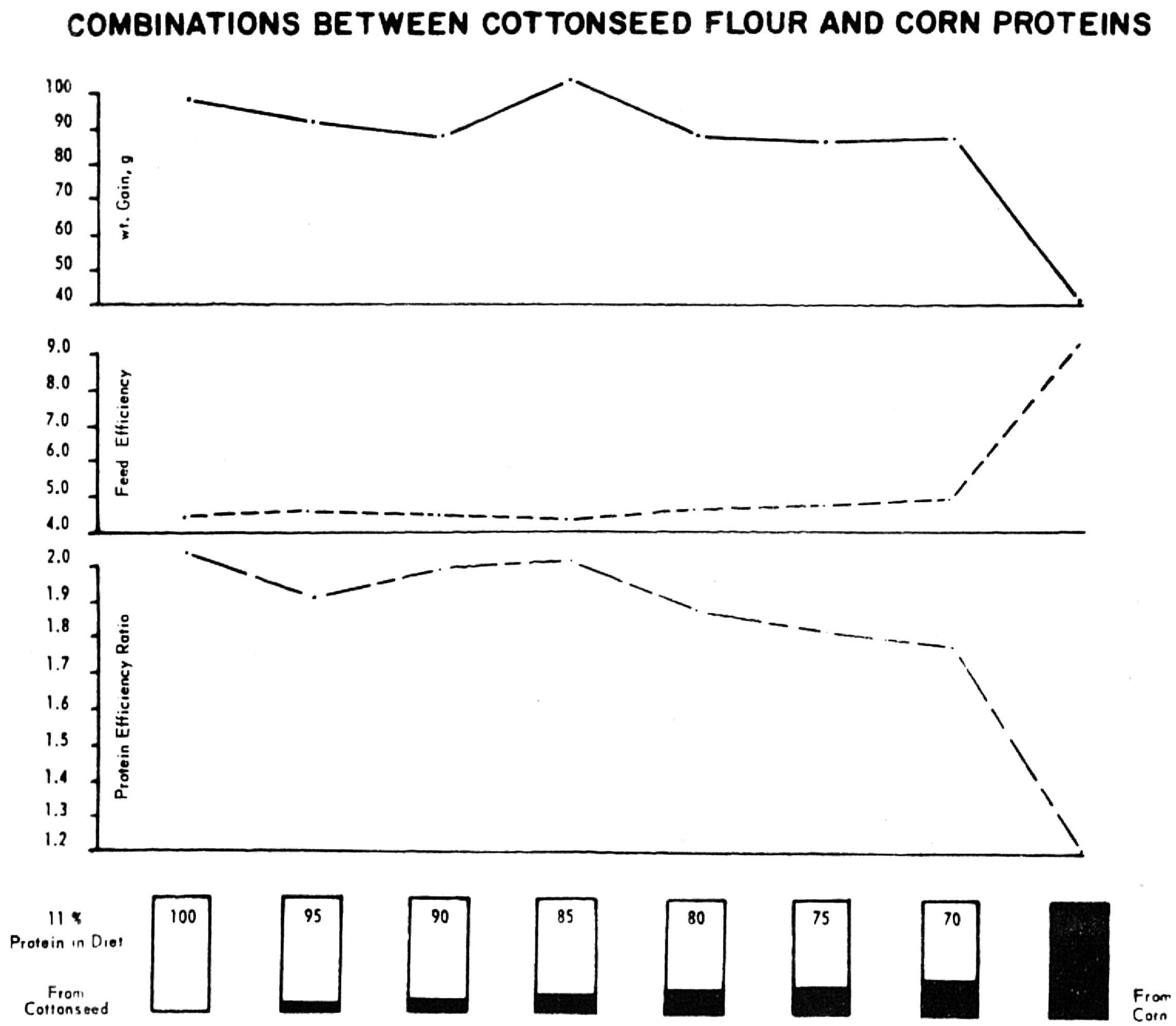

Figure 4—See legend, Figure 1.

The amino acid patterns of corn, cottonseed flour and the 15-to-85% mixture are shown in table 6. Comparison with the FAO pattern shows deficiencies in isoleucine, lysine, sulphur-containing amino acids and tryptophan. Addition of cottonseed flour to corn improves but does not wholly correct the lysine and tryptophan deficiency in the cereal grain. The amino acid proportions in the 15-to-85% mixture, using tryptophan as the base, are similar to those of the FAO pattern. From these experimental results, calculations on protein and amino acid

TABLE 6
ESSENTIAL AMINO ACID PATTERNS

Amino Acid	Corn	Cottonseed Flour	15-85 Protein Combination Corn-Cottonseed	FAO Ref. Prot.
		mg/gm N		
Arginine	262	719	647	......
Histidine	231	113	132	......
Isoleucine	213*	231*	228*	270
Leucine	572	413	437	306
Lysine	126*	256*	235*	270
Methionine / Cystine	189*	169*	172*	270
Phenylalanine	276	294	291	180
Threonine	214	294	281	180
Tryptophan	32*	75*	68*	90
Valine	281	331	323	270

* Limiting Amino Acids.

content of corn and cottonseed flour, and calculations on other chemical components in cottonseed flour, Vegetable Mixture 9 was formulated.

The corn-cottonseed protein combination chosen for Vegetable Mixture 9 was the one in which corn provides 20% of the protein and cottonseed flour 80%. This meant that the maximum protein content in the final mixture would be from 26% to 28%. Vegetable Mixture 9 was then formulated as follows: 56% ground corn, 38% cottonseed flour, 3% Torula yeast and 3% dehydrated leaf meal. Experimental results also indicated that sorghum grain could replace all or part of the corn and, for economic reasons, the experimental formula was made of 28% ground corn, 28% sorghum grain, 38% cottonseed flour, 3% Torula yeast and 3% dehydrated leaf meal.

The above formula was then subjected to extensive testing in chicks, rats and dogs.[12-15] A representative chick trial is shown in table 7. Chick growth and feed efficiencies are about equal when the mixture is made with 28% corn and

TABLE 7
REPRESENTATIVE CHICK GROWTH TRIAL WITH INCAP VEGETABLE MIXTURE 9
(35 days—20 chicks per group)

Diet	Protein in Diet %	Final Weight gm	Feed Efficiency
V.M. 9	23.5	479 [1]	2.31
V.M. 9 with 56% Corn	23.8	460 [1]	2.25
V.M. 9 with 56% Sorghum	24.1	479 [1]	2.27
"Ace-Hi"	23.9	587 [1]	2.01
V.M. 9	23.0	310 [2]	2.45
" + 0.3% DL-Met	23.0	361 [2]	2.26
" + 0.2% L-Lys HCl	23.0	472 [2]	2.14
" + both A.A.	23.0	490 [2]	2.04

[1] 55 gm initial weight. [2] 45 gm initial weight.

28% sorghum, 56% corn or 56% sorghum. The response is, however, only 82% of that obtained with a commercial chick feed containing animal protein. As shown in the lower part of the table, supplementation with lysine improved growth of the chicks fed Vegetable Mixture 9, and methionine addition had a small effect.

A representative rat repletion study is shown in table 8. Good repletion was observed in the rats fed Vegetable Mixture 9 at the 10% protein level in the diet. This was further improved by lysine addition, but methionine addition had no effect.

TABLE 8
REPRESENTATIVE RAT REPLETION STUDY
AMINO ACID SUPPLEMENTATION OF
INCAP VEGETABLE MIXTURE 9
(10% Protein in diet, 6 rats per group)

A.A. Added	gm%	Repletion Gain in 14 days
None	0	51 gm
L-Lysine HCl	0.1	60
" "	0.2	71
" "	0.3	62
DL-Methionine	0.1	53
" "	0.2	51
" "	0.3	53

The conclusion, based on a large number of experiments with rats and chicks[12-14] as well as supplementary biological studies in dogs and pigs[15] is that Vegetable Mixture 9 has a relatively high nutritive value and is completely free of any adverse effects.

It was stated previously that these protein-rich mixtures are intended for the supplementary and mixed feeding of infants and young children and as a part of an adult diet. It is known from nutritional surveys carried out in Central America[17-19] that the protein intakes of the habitants of rural areas are low in both quality and quantity. It becomes important, therefore, to find the amounts of the vegetable mixtures needed to complement the protein consumed by the rural population of the area. The amount of the vegetable mixture will be dependent upon the quality of the protein consumed, which consists most commonly of rice or of lime-treated corn and cooked black beans. The importance of this problem is evident from the findings of several investigators [20], [21] that amino acid imbalances may result from the addition of proteins which are noncomplementary.

Preliminary experiments along these lines have been initiated at INCAP by comparing the nutritive value of the bean and lime-treated corn mixture with the rice and bean and the corn and cottonseed mixtures. The amino acid patterns of the three mixtures and that of the FAO reference protein are given in table 9. All three mixtures are deficient in methionine and tryptophan as compared with the FAO reference protein. If proportions rather than amounts are used to compare the mixtures, and the proportions are calculated on the basis of the isoleucine content because all three mixtures have a value that is similar to that of the FAO pattern, the main deficiency in the three mixtures is methionine. Theoretically, the least deficient combination is that with 15% of protein from corn and 85% from cottonseed flour. On the basis of the methionine deficiency, the decreasing order of nutritive value is: Corn and cottonseed, rice and beans and corn and beans.

TABLE 9
AMINO ACID PATTERNS OF VEGETABLE MIXTURES

Amino Acid	50-50 Protein Combination Corn-Beans	65-35 Protein Combination Rice-Beans	15-85 Protein Combination Corn-Cottonseed	FAO Ref. Prot.
		mg/gm N		
Arginine	315	471	647	......
Histidine	246	213	132	......
Isoleucine	288	334	228*	270
Leucine	426	408	437	306
Lysine	353	362	235*	270
Met. + Cys.	161*	230*	172*	270
Phenylalanine	305	330	291	180
Threonine	280	260	281·	180
Tryptophan	52*	76*	68*	90
Valine	408	423	323	270

* Limiting Amino Acids.

Table 10 shows the actual nutritive value from an experiment with rats in which the three mixtures were compared with each other. The PER was highest with rice and beans, followed by corn and cottonseed flour and corn and beans in that order. In any case, all three mixtures were of fairly good protein quality as judged by the PER. Experiments are under way to determine the effects of different proportions of the low- and high-protein-containing mixtures, not only the optimum combination of two ingredients, but other combinations as well. The results will provide a basis for recommending the quantity of Vegetable Mixture 9 needed to complement efficiently the common lime-treated corn and bean diet or the rice and bean diet of the rural population of the Central American countries.

The importance of knowing the protein nutritive value of combinations of natural foods is illustrated by the last figures in table 10. The particular mixture was

TABLE 10
COMPARISON OF THE NUTRITIVE VALUE OF FOUR VEGETABLE PROTEIN MIXTURES IN RATS

Vegetable Mixture	Protein Combination Ratio	Ave. wt. Gain 21 Days	F.E.	P.E.P
Rice + Black Bean	1.85/1.00	95	3.72	2.65
Lime-treated Corn + Black Bean	1.00/1.00	92	3.58	2.19
Lime-treated Corn + Cottonseed Flour	1.00/5.67	94	3.45	2.35
Lime-treated Corn + Cottonseed Flour + Cowpea	1.00/5.67/1.00	97	3.38	2.47

formulated on the basis of results obtained in previous studies of corn and beans, corn and cowpea and corn and cottonseed flour mixtures. It contained the protein from each component in the ratio of 5.67 of cottonseed flour to 1.00 each of corn and cowpea. The results show this formula to have a relatively high PER and feed efficiency. It is being subjected to further biological testing.

It is apparent from these results that consuming two or more selected vegetable foods in such proportions that their amino acid compositions complement each other is one effective way of making more efficient use of the available food supply and improving the nutritional status of the human population of underdeveloped areas. Furthermore, economically feasible vegetable protein mixtures of good nutritive value can be developed to meet the increasing demand for better quality of protein in areas where protein malnutrition is an important problem.

SUMMARY

One approach to the serious problem of protein malnutrition among young children in Central America has been the study of combinations of local vegetable foods in proportions to give a protein quality superior to that of any single ingredient. Such combinations as 84% rice and 16% cooked black beans (65% of protein from rice and 35% from beans), 71% lime-treated corn and 29% cooked black beans (50% of protein from each food), 64% corn and 34% cowpea (mixtures with 40% of the protein from corn and 60% from cowpea) have improved protein quality, but all are somewhat deficient in methionine and are too low in total protein content for effective feeding of young children.

In an attempt to devise a practical mixture with higher protein content, 35% sesame flour and 9% cottonseed flour were combined with 50% lime-treated corn, 3% Torula yeast and 3% Kikuyu leaf meal. This formula, identified as INCAP Vegetable Mixture 8, contains 25% protein and gives good growth and feed utilization in rats and chicks, although it is improved by lysine addition. When cottonseed flour replaced the sesame flour in this formula, there was little difference in growth and feed efficiency and some apparent improvement in protein efficiency. Because of the lower cost and greater availability of cottonseed flour in Central America, biological studies have since been concentrated on INCAP Vegetable Mixture 9 containing 28% corn, 28% sorghum, 38% cottonseed flour, 3% Torula yeast and 3% leaf meal. The sorghum was introduced to lower the cost, but any proportion of corn and sorghum may be used without significantly affecting the nutritive value of the mixture. A large number of experiments with rats and chicks and supplementary studies in dogs and pigs demonstrate that this mixture has a relatively high nutritive value and is completely free of any adverse effects. On the basis of these results both Mixtures 8 and 9 were recommended for experimental feeding trials in human subjects.

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