FORMULATION AND TESTING OF WEANING AND SUPPLEMENTARY FOODS CONTAINING OILSEED PROTEINS

R. Bressani

Division of Agricultural and Food Science
Institute of Nutrition of Central America and Panama
Guatemala, Central America

The increasingly limited availability of food, particularly protein foods, in adequate quantity and of acceptable quality is a universal problem which has received and is receiving much attention throughout the entire world. The problem of the low supply of protein foods has been amply documented (1,2) and many conferences as well as research activities in many countries have proposed and implemented logical solutions (3-6). Among these, the utilization of oilseed protein in the form of formulated foods has awakened the interest of governments, international agencies, and some sectors of the food industry who, in several cases, have developed food mixtures of high-protein content.

In many instances, however, very little or no attention whatsoever has been given to the many factors which must be considered in the over-all program of weaning and supplementary foods, from their formulation and testing to their practical application. Because of the great urgency of the problem, many products of doubtful nutritive value will be reaching the market with claims which cannot be supported and with a great loss of economic efforts and little improvement on the present situation of protein nutrition.

The commercial success of protein-rich food mixtures can only be achieved with the sincere support of governments, public health officials, and the food industry. This support, however, can only be obtained when the mixtures have been scientifically formulated and after the nutritive and supplementary value of the product has been amply demonstrated through a proper impartial and thorough scientific justification. Such a procedure also serves to protect the consumers from products of little or no value.

The thought behind the development of protein-rich food mixtures is that they are needed as weaning foods or as supplements to diets poor in quantity and in quality of protein, particularly those diets consumed by children. Whether the purpose is to develop a weaning or a protein supplement, the nutritional quality of both should be the highest possible, first because it will be practically the only food, and second because it must supply the nutrients missing in the diets being consumed by populations of low economic resources. This paper presents a general discussion of the factors to be considered in the formulation of food mixtures based on oilseed protein, and of the biological testing of the product itself as a weaning food and as a supplement to human diets with a protein quality requiring improvement. The data presented constitute a review of the work carried out by INCAP on protein-rich foods, known as the Incaparina Formulations.

Chemical and Nutritional Characteristics of Diets to be Supplemented

As indicated above, the formulation and development of protein supplements made from vegetable protein sources should seriously consider the chemical and nutritional characteristics of the diets to be supplemented, particularly the diets consumed by the population groups requiring more and better quality protein. Numerous nutritional surveys carried out in the Central American area, for example (7-11), have indicated that these diets are made up in the northern countries of the area, mainly of corn consumed as gruel or tortilla and small amounts of cooked beans. The other components are vegetables, fruits, and various carbohydrate sources providing small amounts of protein. Several examples of poor protein quality diets are shown in Table I. In the corn-based diets, this cereal provided 60-70% of the protein and 70-80% of the calories. Similar amounts are provided by the other cereals in the diets where rice and wheat replace corn. The second important

I-451 En: Protein-Enriched Cereal Foods for World Needs. Ed. by Dr. Max Milner. Published by the American Association of Cereal Chemists, St. Paul Minnesota, 1969, p. 49-66.

TABLE I

Average Amounts of Foods in Rural Diets Consumed by Children[a]

FOOD ITEM	LOCALITY		
	SCB[b]	SMC[c]	SCBY[d]
	g.	g.	g.
Milk products	47	5	13
Eggs	5	4	3
Meat	9	14	6
Black beans	10	20	10
Fresh vegetables	41	33	46
Fruits	31	17	20
Roots	3	4	1
Lime-treated corn	119	178	174
Sugar	23	34	29
Fat	1	1	1

[a]See ref. 10.

[b]Santa Catarina Barahona.

[c]Santa María Cauqué.

[d]Santa Cruz Balanyá.

food item is beans providing 20-30% of the protein and similar amounts of calories. It is very important to point out that the rest of the food items are responsible for diluting the protein content of the diet, and decreasing the amounts of undigestible nutrients, making the over-all diet bulky in nature. A second important aspect is that the food items listed are consumed together in the three meals every day, becoming very monotonous. The chemical composition of these diets is shown in Table II. On a dry weight basis they contain less than 12% protein, about 2.5% crude fiber, 5-8% fat, 2-3% ash, and 70% carbohydrate. This table also shows the protein quality of these diets as indicated by the protein efficiency values which range from 1.1 to 1.6.

TABLE II

Chemical Composition of Diets

	LOCALITY		
	SCB[a]	SMC[b]	SCBY[c]
	%	%	%
Protein	11.9	12.3	11.5
Fat	7.6	4.8	7.3
Crude fiber	2.7	2.5	2.4
Ash	3.0	3.0	2.5
Moisture	7.6	4.8	7.3
Carbohydrate	67.2	72.6	69.0
PER	1.64	1.32	1.13

[a]Santa Catarina Barahona.

[b]Santa María Cauqué.

[c]Santa Cruz Balanyá.

Since the main components of these diets are corn and beans, it is of interest to discuss such foods further. It has been well established that corn protein is deficient in lysine and tryptophan (12,13). Bean protein, on the other hand, is a good source of lysine but deficiencies in methionine and tryptophan have been demonstrated (14,15). Therefore, mixtures of the two foods will have different protein values as indicated in Fig. 1. As

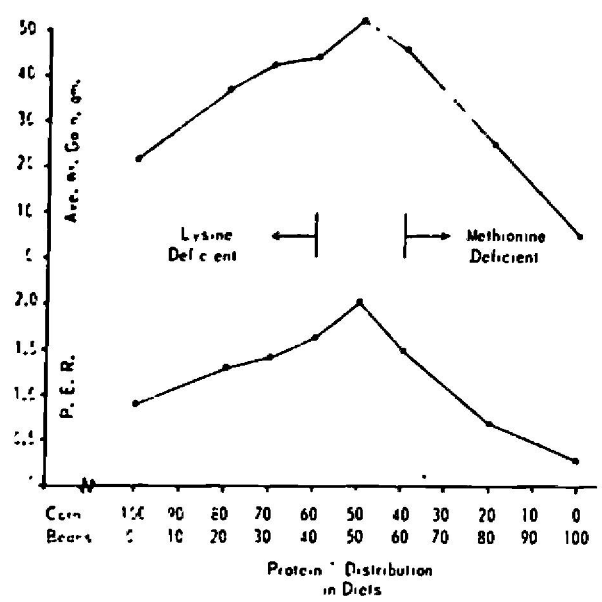

Fig. 1. Nutritive value of various combinations of lime-treated corn and cooked black beans.

can be seen, the combination giving the best protein value is one made of 72 g. of corn and 28 g. of beans or a corn-to-bean ratio of 2.6. Experimental work demonstrated that lysine was the first limiting amino acid as the amount of corn increases above 72 g. and methionine the first limiting amino acid if beans increase above 28 g. (16). Table III shows the

TABLE III

Average Daily Intake of Corn and Beans in Various Areas of Central America

	CORN	BEANS	CORN/BEAN RATIO
	g./day	g./day	
Guatemala			
Adults	423	58	7.3
Children	281	24	11.7
	295	26	11.3
	277	15	18.5
Honduras			
Adults	398	56	7.1
El Salvador			
Adults	374	60	6.2
Best ratio	72	28	2.6

average intakes of these two foods for adults in three countries of Central America and for children in three towns in Guatemala (7,8). In all cases, the ratio of corn to bean is greater than the optimum, which is 2.6.

Therefore, all these diets not only have the disadvantage of being low in total protein, but the first limiting amino acid is lysine. The logical conclusion is that the supplement to use for populations consuming such diets should be one with a high-protein concentration and with a relatively high content of lysine. In general, however, the supplement should have a high concentration of essential amino acid, with particularly high levels of lysine, methionine, tryptophan, and probably threonine.

Selection of Ingredients to be Used in Supplement

To meet the specifications indicated in the last paragraph of the above section, high-protein content can be achieved only through the use of protein concentrates. Since protein concentrates of animal origin are scarce, it is necessary to use available ones of vegetable origin. These very seldom provide the necessary amounts of essential amino acid particularly with respect to lysine and methionine. These high levels can, however, be obtained as indicated later.

The vegetable protein concentrates most commonly used have been derived from soybean, cottonseed, peanut, and sesame seed. The protein content of the flours made from these seeds, the protein quality of the products made by the best practical methods, and the main limiting amino acid are shown in Table IV. As is well known, the best material is

TABLE IV

Vegetable Protein Concentrates Commonly Used in Mixtures

VEGETABLE PROTEIN SOURCE	PROTEIN	AMINO ACID Main Deficiency	AMINO ACID Good Source
	%		
Soybean flour	50.0	Methionine	Lysine
Cottonseed flour	50.0	Lysine	--
Peanut flour	50.0	Methionine, lysine	--
Sesame flour	50.0	Lysine	Methionine

soybean, followed by cottonseed, sesame, and peanut. The main limiting amino acid is lysine for cottonseed and sesame and methionine for the other two. On the other hand, soybean is the best source of lysine, while sesame is the best source of methionine.

This information is therefore of importance in formulations which have been carried out in several ways, with the purpose of arriving at a combination of ingredients giving an essential amino acid pattern and biological value similar or equal to that of the high-protein quality foods. Because milk is a natural source of protein of high biological value, particularly for children, formulations of vegetable protein mixtures have been made to contain approximately 25% protein level, which would meet the first requirements as a good supplement. This can be accomplished by dilution of the protein in the protein concentrate through the use of low-protein-containing foods, such as cereal grain flours and carbohydrates.

Formulations of Supplement

There are at least three practical methods by which the protein in different foods can be combined to develop a mixture with a high-protein quality.

As a first approximation a formulation can be derived by mixing two or more protein foods according to their respective essential amino acid patterns in regard to a reference protein. Formulation of protein-rich foods by this method usually takes into consideration the fact that some proteins are limiting in one or two amino acids which must be supplied by a second protein. Although, theoretically, it should be possible to arrive at good mixtures following this technique, the result is generally not the one expected because the availability of the amino acids in the different food proteins is not usually considered. Furthermore, the only consideration commonly given in this type of formulation is with respect to the limiting amino acids, but not to excesses which could take place upon mixing and which could create unbalances decreasing even further the utilization of the protein in the mixture by the animal. In any case, the quality of the mixture formulated following the above technique has to be confirmed by biological testing with experimental animals.

A second method for the formulation of mixtures results from the data of studies on supplementation, such as those shown in Fig. 2. In this example, 6 g. of fish protein

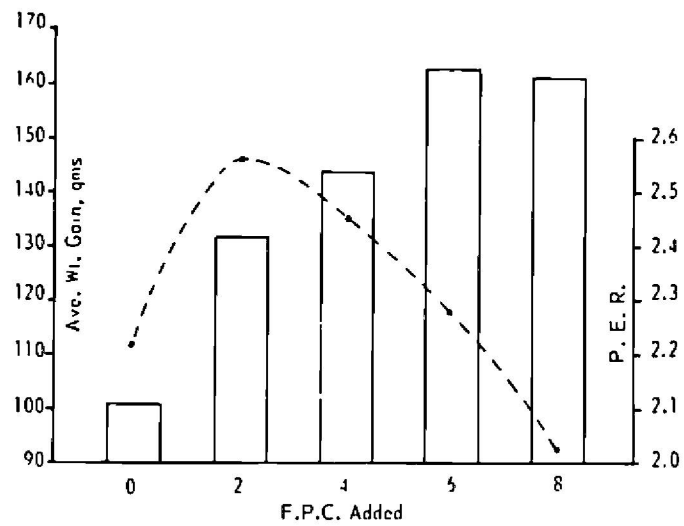

Fig. 2. Supplementation of cottonseed flour with fish protein concentrate.

concentrate efficiently supplemented 20 g. of cottonseed flour at the 10% protein level in the diet (17). If the level of total protein desired in the mixture is 30%, for example, the two basic figures should be multiplied by 3, resulting in a formula with 18% FPC and 60% cottonseed flour. The difference of 22% can be made up of other foodstuffs such as cereal grain flours, carbohydrates such as starch and sugar, and other nutrients required to supplement vitamins, and finally, flavoring agents.

This method of formulation has several advantages, such as the ease in changing the level of protein without altering protein quality, the choice of several types of filler as indicated above, and the mixture of the two protein concentrates alone, that can be used to supplement quality protein diets.

The third method of formulating protein-rich mixtures has been developed from data obtained from biological tests in which the amino acid pattern of one protein complements the amino acid pattern of a second protein. When mixing two or more proteins, four types of products can be formed (2,18). Theoretical lines representing these four types are shown in Fig. 3. Type I results from the mixture of two proteins of different or similar protein concentration, but with similar amino acid deficiency. The nutritive value of the protein in the mixture is equal to that of either component.

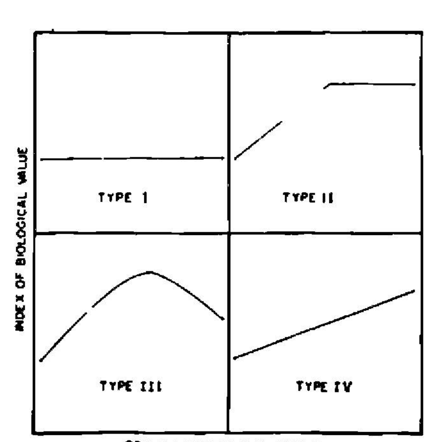

Fig. 3. Theoretical responses in protein quality upon mixing two food protein sources.

Type II is a combination that results from the mixture of two proteins with a deficiency of the same amino acid; however, one of the proteins contains a higher level of the particular amino acid than the other protein. The nutritive value of the mixture remains equal to that of the component containing the higher level of the amino acid up to the point where its concentration is decreased by the protein in the other component.

The third type is a combination where there is a synergistic supplementation or complementation of the amino acids in the component proteins being mixed. The quality of these mixtures is higher than the quality of either component. The limiting amino acids in one component are balanced by the excesses in the second component and vice versa.

Type IV is that in which there is a large difference in quality between components, so that the nutritional value of the mixture is determined by the amount of the higher quality component present in the mixture.

From this information mixtures of different protein concentration and quality can be formulated. An example of type I is shown in Fig. 4 for mixtures between peanut and

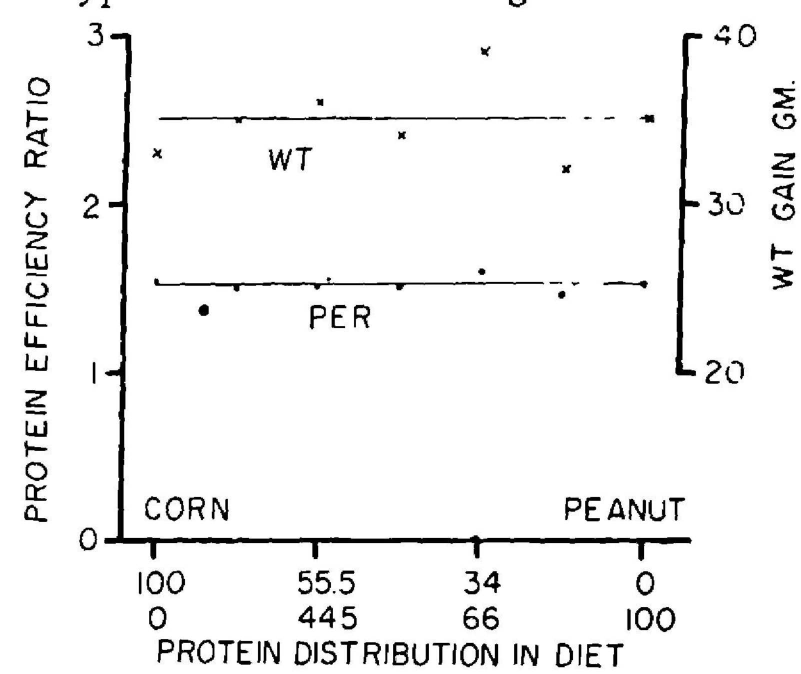

Fig. 4. Nutritive value of various combinations of peanut flour and corn.

corn proteins (18,19). Since both have equal amino acid deficiency, that of lysine, there is no question of higher protein quality. Therefore, mixtures between these two proteins can be formulated to have different protein concentration as determined by the amount of peanut flour in the mixture, but the quality of any mixture will be similar.

An example of type II combination is given by mixtures of cottonseed flour and corn as shown in Fig. 5 (18,20). Cottonseed protein can be diluted up to 70 to 80% with

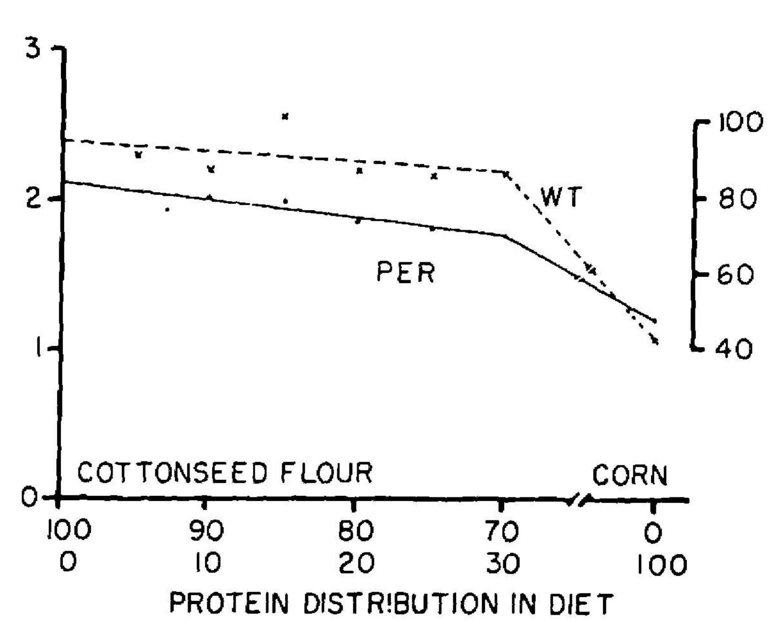

Fig. 5. Nutritive value of various combinations of cottonseed flour and corn.

corn protein without decreasing the protein quality of the cottonseed flour. These results are the basis for INCAP Vegetable Mixture 9 (20). Cottonseed protein is low in lysine, but it contains more of this amino acid than corn protein. Cottonseed flour, however, cannot supply all the lysine needed to meet the deficiency of this amino acid in corn and balance the other essential amino acids as well.

An example of a mixture in the type III group is shown in Fig. 6. Black bean protein and cottonseed combined in a protein ratio of 40:60 give a good protein efficiency ratio of 2.34 (21,22). In this situation the essential amino acids of one protein source

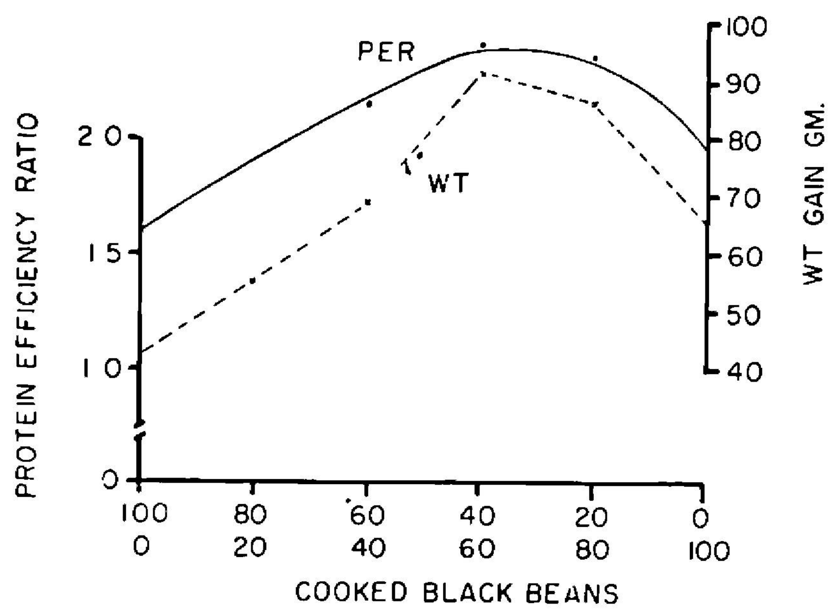

Fig. 6. Nutritive value of various combinations of cottonseed flour and black beans.

complement very closely the essential amino acid pattern of the other protein component, resulting in a product with a higher protein quality than either food source fed alone.

Examples for the type IV group are shown in Fig. 7. There is no complementary effect between cottonseed and sesame flour and the protein value is proportionate to the

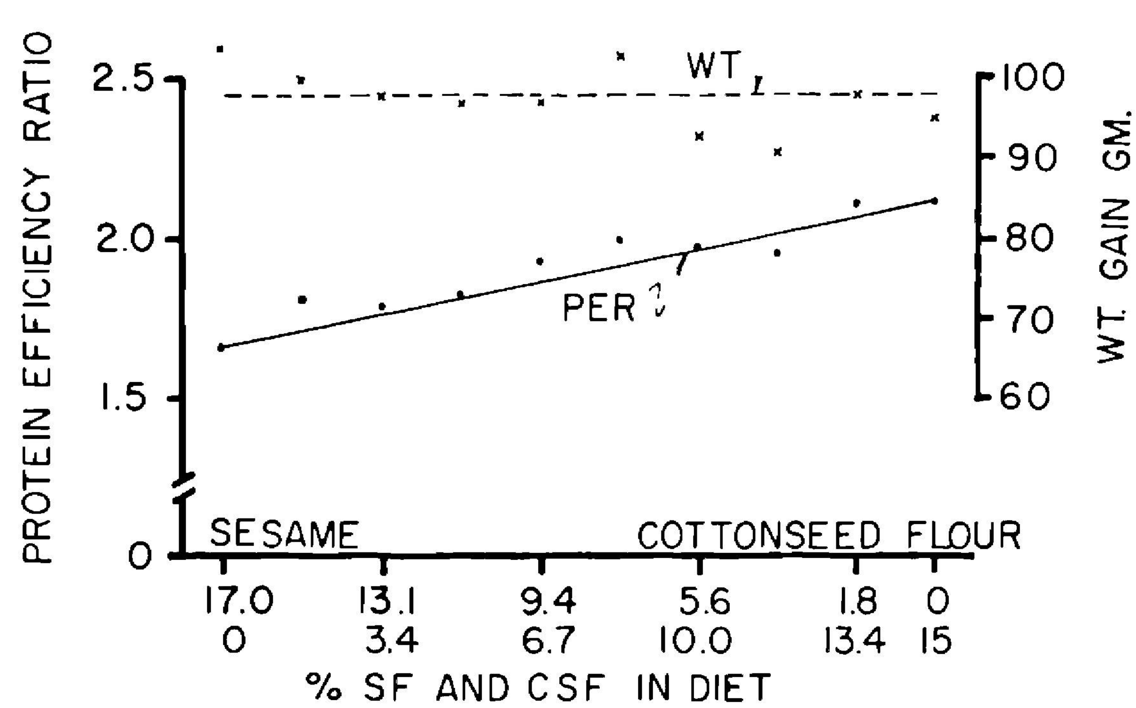

Fig. 7. Nutritive value of various combinations of cottonseed flour and sesame flour.

amount of each ingredient in the mixture (18,23). Mixtures within this group usually result from the combination of foods having highly different protein values but a common essential amino acid deficiency, although in differing degrees. In the particular case shown, sesame protein is much more deficient in lysine than cottonseed protein; however, sesame is a better source of methionine than cottonseed.

Evaluation of Quality of Protein Supplement

A. Experimental animals

1. Growth studies. There are several methods available for determination of protein quality of the supplement. The method most commonly used is the protein efficiency ratio (PER), which determines the amount of weight gained by the animal per unit of protein consumed. It has been shown that weight gain correlates highly with nitrogen deposited in the body. Therefore, PER is a good index of protein utilization or biological value. The test is usually carried out in comparison with a reference protein, which is casein, at a protein concentration in the diet of 10%. Representative results of several vegetable protein mixtures are shown in Table V (20,24-26).

TABLE V

Protein Efficiency Ratio of Several Vegetable Protein Mixtures

PROTEIN SOURCE	PROTEIN IN DIET	AV. WT. GAIN[a]	PER
	%	g.	
INCAP Mixture 9	10.3	84	2.03
Skim milk	10.3	117	2.88
INCAP Mixture 14	9.6	123	2.79
Skim milk	12.0	129	2.73
INCAP Mixture 15	10.8	87	2.22
Casein	11.9	116	2.52

[a]28 days.

The main disadvantage in this method is that not all proteins give maximum PER at the same protein level in the diet, which for casein is 10%. Therefore, if possible, tests should be carried out at several levels of dietary protein. The results of such studies allow for a better evaluation of the quality of the protein, suggest that the protein is deficient in essential amino acids, and provide information on the presence of adverse physiological factors.

Representative examples of comparisons between a mixture and casein fed at different levels of protein in the diet are shown in Table VI (24). It is evident that the

TABLE VI

Protein Quality of a Cottonseed-Corn Mixture and Skim Milk at Various Levels of Protein Intake

VEGETABLE MIXTURE			SKIM MILK		
Protein in Diet	Av. Wt. Gain	PER	Protein in Diet	Av. Wt. Gain	PER
%	g.		%	g.	
5.2	17	1.14	5.7	36	2.34
10.6	89	2.04	10.3	117	2.88
15.5	125	1.85	14.3	127	2.24
19.6	129	1.67	19.6	148	1.94
24.5	147	1.42	23.5	133	1.48

vegetable mixture is lower in quality than casein, even though the main components of the protein mixture were blended at the point of optimum amino acid complementation. These results suggest amino acid deficiencies in the mixture which can be detected through supplementation. As a first approximation to this step in deciding which are the limiting amino acids, attention should be given to the biological studies which served as a basis for formulation. A representative example is shown in Fig. 8 for mixtures based on soybean

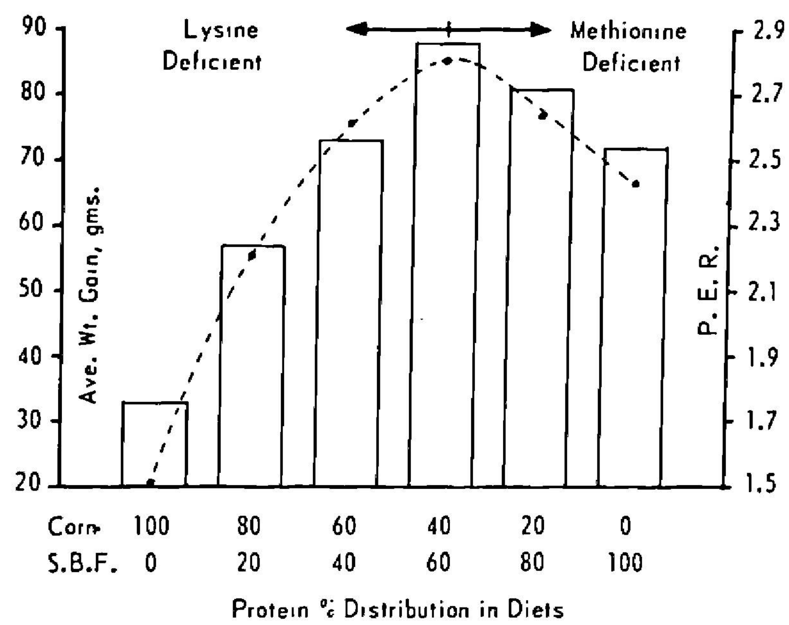

Fig. 8. Nutritive value of various combinations of corn and soybean flour.

protein concentrate and corn (25). The first is a rich source of lysine but relatively low in methionine, while corn is low in lysine although it does not contain excesses of methionine. Therefore, sulfur-containing amino acids are probably the amino acids that are low in the mixture.

TABLE VII

Limiting Amino Acids in a Soybean-Corn Protein Mixture (INCAP No. 14)

AMINO ACID	AMOUNT	AV. WT. GAIN[a]	PER
		g.	
None	0	110	2.56
DL-Methionine	0.20	139	2.93
DL-Methionine L-Threonine	0.20 0.20	142	3.05
DL-Methionine L-Threonine L-Lysine HCl	0.20 0.20 0.20	164	3.39

[a]Average initial weight: 48 g.

Table VII presents results of studies designed to test this hypothesis. The PER values resulting from the addition of methionine indicate that this is the first limiting amino acid. A further increase results when lysine and threonine are added in the presence of methionine (25). The value of this information is of great practical importance, since such vegetable-protein mixtures are being designed to supplement diets not only poor in total protein content, but diets which have deficiencies of lysine and of methionine, and possibly other amino acids as well.

2. Nitrogen balance. All other possible information on the quality of the mixture

should be obtained to be able to make appropriate recommendations for its use by the population for which it is intended. For this reason, at INCAP, the protein quality of such foods is also tested by nitrogen balance in young dogs, using as index of quality the nitrogen retention of the animals fed low, intermediate, and high levels of dietary protein and comparing those results with a reference protein. A representative example is shown in Fig. 9 for Vegetable Mixture 15 based on soybean and cottonseed flours and corn (25,26). From such results, information is obtained on its protein digestibility, amount of protein needed for maintenance purposes, and biological values in comparison with a reference protein.

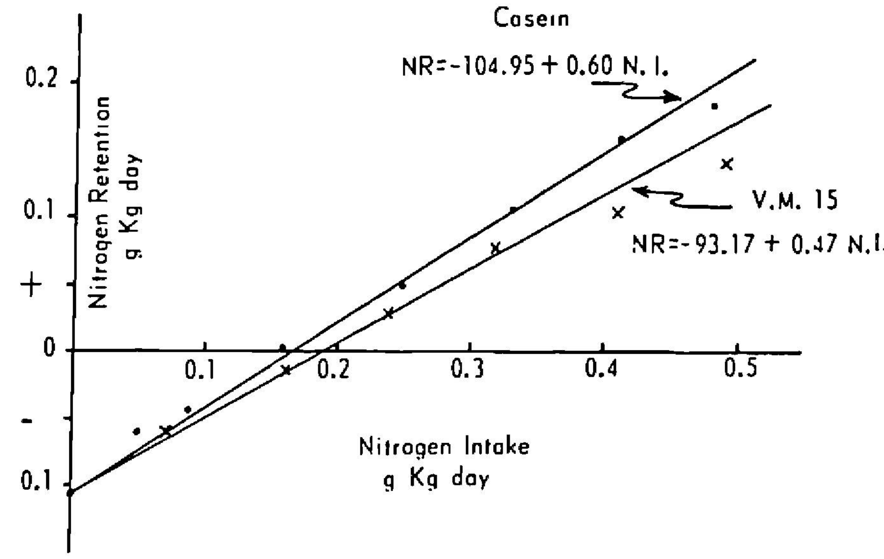

Fig. 9. Nitrogen balance index in dogs fed various intakes of protein from INCAP Formula 15 and casein.

3. Toxicity tests. It is well recognized (27) that most plant proteins contain adverse physiological factors which in many cases are eliminated or destroyed totally or partially by the method of preparation of the protein concentrate. However, it is common to find that small amounts of these substances remain in the concentrate, which are usually without effect if their intake is limited.

Since the protein mixtures are to be used as daily supplements, the intake of these substances, although small, will be continuous and there is the possibility that their accumulation in body tissues may cause adverse physiological effects. This could be particularly true of substances such as gossypol. Therefore, toxicity studies should be carried out.

TABLE VIII

Growth and Breeding Performance of Rats Fed a Cottonseed Flour-Protein Mixture[a]

	MIXTURE 9	CONTROL
Final weight (420 days)		
Males[b] (g.)	522	547
Females[c] (g.)	343	314
Total number of rats in six breedings	372	289
Av. no./mother	11.3	10.3
Av. wt. at birth (g.)	6.1	6.5
Av. weaning wt. (g.)	40.9	37.2
Mortality of born rats (%)	7.9	15.9

[a]38% Cottonseed flour, INCAP Mixture 9.

[b]Average initial weight: 52 g.

[c]Average initial weight: 48 g.

An example is indicated in Table VIII in which a cottonseed flour-based formula was fed for 14 months to rats during growth and reproduction (28). The results of such studies indicate that small amounts of gossypol, even though ingested daily for the period indicated, did not affect the growth, lactation, and performance of the animals, which were able to eliminate such a compound as it was ingested.

When the ingredients used in the formulation of vegetable protein mixtures are products which are not considered normal human foods, a safe procedure is to test these new foods in other experimental animals. A specific example is the testing to which INCAP Vegetable Mixture 9, one of the Incaparina formulas, was subjected, since its main protein source is cottonseed flour. Representative results of tests carried out with Mixture 9 in chicks are shown in Table IX. The results indicate no adverse physiological effects which could have occurred owing to the presence of gossypol in the mixture (29).

These studies should be carried out for all such mixtures, particularly for those made from materials which can become easily contaminated with toxic substances, as in the case of peanut protein concentrate, contaminated with the aflatoxins by *Aspergillus flavus* (30).

TABLE IX

Representative Growth Response of Chicks Fed a Cottonseed Flour Mixture

PROTEIN TEST	AV. FINAL WEIGHT	FEED EFFICIENCY	PROTEIN IN DIET
	g.		%
INCAP Mixture 9	479[a]	2.31	23.5
Control	587	2.01	23.9
INCAP Mixture 9	299	2.76	17.5
INCAP Mixture 9	310[b]	2.45	23.0
INCAP Mixture 9 + lysine (0.3%)	494	2.07	23.0

[a]Av. initial weight: 55 g.

[b]Av. initial weight: 45 g.

4. Supplementation of poor quality diets. As indicated earlier, there is a need to consider the ingredient composition of the human diet to be supplemented, as well as its quality, as a prerequisite for selection and formulation of protein-rich foods. Once the formula has been developed and tested by itself, it is recommended that its quality, as a supplement to the basic human diet for which it was intended, be tested. An example of such testing is shown in Table X for INCAP Vegetable Mixture 9 and a rural diet consumed by Guatemalan children (9,11). The diet was supplemented with 1, 2, 3, and 4 g. of INCAP Mixture 9, or milk. The data presented indicate that the vegetable mixture does not improve the protein quality of the rural diet, although higher weight gains are obtained because of an increase in the intake of protein. On the other hand, skim milk does improve the quality of the rural diet as judged by an increased PER over the basal diet. At the same time, there is an increase in weight gain, again because of an increase in protein intake. These results are interpreted to mean that Mixture 9 could not improve the quality of the diet because both are deficient in the same amino acid, which in this case is lysine (31). On the other hand, skim milk is a good source of lysine, explaining its effect on the protein quality of the diet.

Similar results have been obtained with other diets (9), and only when the quality of such diets is very low does Mixture 9 increase its quality. An example is indicated in Table XI. In this case supplementation of the diet with Mixture 9 brought about a significant increase in weight gain of the animals, and in the quality of the protein ingested. Milk protein caused even better results.

TABLE X

Supplementary Value of INCAP Mixture 9 and Skim Milk to Child's Average Diet[a]

	MIXTURE 9		SKIM MILK	
SUPPLEMENT	Av. Wt. Gain[b]	PER	Av. Wt. Gain[b]	PER
g./day	g.		g.	
None	62	1.61	--	--
1	72	1.68	87	2.00
2	81	1.67	113	2.16
3	97	1.67	138	2.21
4	105	1.67	157	2.22

[a]Santa Catarina Barahona.

[b]Av. initial weight: 52 g.

TABLE XI

Supplementary Value of INCAP Mixture 9 and Skim Milk to Child's Average Diet[a]

	MIXTURE 9		SKIM MILK	
SUPPLEMENT	Av. Wt. Gain[b]	PER	Av. Wt. Gain[b]	PER
g./day	g.		g.	
None	14	0.58	--	--
1	38	1.12	53	1.39
2	51	1.25	68	1.52
3	70	1.40	82	1.55
4	82	1.40	88	1.52

[a]Santa Maria Cauque.

[b]Av. initial weight: 52 g.

Mixtures based on soybean protein could also be ineffective as a supplement, particularly for diets deficient in sulfur-containing amino acids. As a conclusion, therefore, it is necessary that the amino acid content of such mixture be as high as possible and well balanced.

5. Improvement of the amino acid balance of vegetable mixtures. The discussion presented in the previous section is enough to indicate the need to increase the biological value of the vegetable mixtures to be effective and efficient supplements to poor-quality diets. Increased biological value can be obtained by including, in the formulation, sources of amino acids in the form of protein, or by adding the limiting amino acids of the mixture.

An example of the first situation is shown in Table XII, where Mixture 15 was mixed with skim milk (26). The combination of 60% of the protein from the mixture and 40% from the skim milk gives a formulation of higher quality than either protein source alone. The formula with 25% protein would contain 12 g. of cottonseed flour, 12 g. of soybean flour, 28 g. of skim milk, 3 g. of yeast, 36 g. of corn, and 9 g. of other ingredients.

The second approach is to find the limiting amino acids in the mixture. A representative example is presented in Table XIII for INCAP Formula 9 based on 38% cottonseed

TABLE XII

Protein Complementation between Vegetable Mixture 15 and Skim Milk

PROTEIN DISTRIBUTION IN DIET			
VM15	Skim Milk	AV. WT. GAIN[a]	PER
		g.	
100	0	118	2.42
80	20	136	2.52
60	40	156	2.98
40	60	135	3.00
20	80	148	2.86
0	100	142	3.03

[a]Initial average weight: 48 g.

TABLE XIII

Improvement of the Protein Quality of INCAP Mixture 9

AMINO ACID	AMOUNT	AV. WT. GAIN[a]	PER
		g.	
None	0	78	1.92
L-Lysine HCl	0.25	109	2.46
L-Lysine HCl	0.25		
DL-Methionine	0.10	114	2.59
DL-Threonine	0.25		

[a]Av. initial weight: 48 g.

protein, 58% ground whole corn, 3% yeast, and 1% calcium carbonate. A large increase in protein quality results when lysine is added. A further increase is brought about when the mixture is supplemented with lysine, methionine, and threonine. Because of these results, lysine enrichment has been recommended and is being carried out for the Incaparina Formula 9 in commercial production in Central America (31).

The improvement of the quality of the mixture is of great significance when used as a supplement to poor-quality diets. A representative example is presented in Fig. 10, showing the weight of swine fed a diet similar to that consumed by children alone, supplemented with Mixture 9, with and without lysine addition. Obviously, the best supplementary effect resulted when INCAP Mixture 9 was itself supplemented with lysine, the amino acid deficient in the basal diet and in the mixture (32).

B. Protein quality evaluation in children

Once the evidence obtained from animal experiments is satisfactory, the quality of the protein in the mixture should be tested in human subjects, preferably children, who

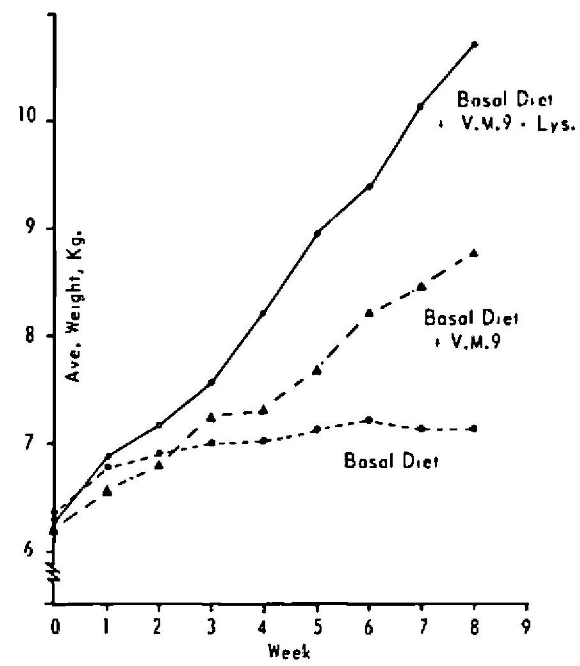

Fig. 10. Supplementary effect of INCAP Mixture 9 with and without lysine addition to a basal children's diet in Guatemala.

have the greatest need for such foods and are more sensitive to this testing. The trials in humans should consist of some measure of protein utilization, the most common of which is the nitrogen balance method. Most investigators working in this area are satisfied in testing the quality of such products at one level of protein intake and comparing the results obtained with those from milk feeding at equal levels of intake. This practice is not recommended particularly if the tests are carried out at a physiological level of protein intake. The explanation for this can be found in the relationship existing between the pattern of amino acids in the protein and their utilization by the organism, which follows the law of diminishing returns. At low levels of protein intake, the interaction between amino acid pattern and utilization is more critical than at high levels of intake. Because of this, testing should be carried out at various levels of intake, particularly in the region of the response curve, which is linear. The slope of such responses is directly related to protein quality, and the higher the slope, the higher the quality of the product. This technique was proposed and very well tested by Allison and Anderson (33). Representative examples are shown in Fig. 11 for various mixtures developed at INCAP (34).

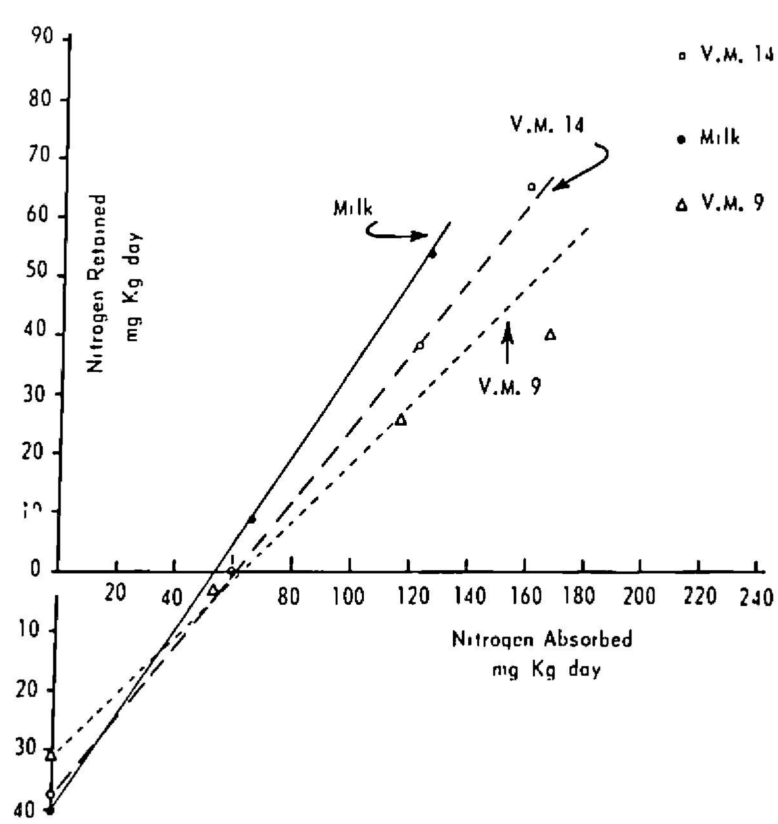

Fig. 11. Nitrogen balance index in children fed formulated vegetable protein mixtures and milk.

In these studies the mixtures were fed at intakes of protein from 0 to 2.0 g./Kg. of body weight/day, maintaining the intake of calories and other nutrients at adequate levels. As can be seen, the slopes are different for the different supplements and from milk or egg protein. The slope gives an indication of the biological value of the food, which can be calculated from such information. Furthermore, the data indicate the nitrogen intake level at which nitrogen equilibrium takes place and calculations can also be made of the true protein digestibility.

Finally, the supplementary value of the product should be tested. This can be done by feeding the basic diet and obtaining in such a way its nitrogen retention. The supplement is then fed together with the diet at the recommended level for supplementation, and nitrogen balance is again measured. A good example of this is shown in Table XIV for a

TABLE XIV

Supplementary Value of a Soybean-Corn Mixture to a Diet Consumed by Normal Children[a]

		NITROGEN		
TREATMENT	CASE	Intake	Absorbed	Retained
		g./day	%	%
Basic diet		3.8	54.5	11.2
Basic diet + vegetable mixture	S.M.S.	4.7	67.7	24.1
Basic diet + milk		4.8	64.3	23.8
Basic diet		4.5	57.6	11.3
Basic diet + vegetable mixture	N.C.	4.4	77.1	23.3
Basic diet + milk		4.6	76.9	16.8

[a]See ref. 35.

soybean-corn mixture developed by Dutra de Oliveira (35). As indicated, the supplement increased nitrogen retention over the basal level and in an amount equal to that obtained from milk. Other human tests could be carried out, such as growth studies in children. However, they are usually difficult and costly to conduct. Furthermore, the products should also be tested for their acceptability in the communities where the product is to be utilized. Although such tests do not provide information on the quality of the product, they are of great practical importance in programs of this nature. Otherwise, it is obvious that all experimental evidence, no matter how good it is, will be useless in providing better protein nutrition, particularly in children.

Conclusions

The formulation steps, as well as the testing procedure outlined in this paper, are by no means the best to be followed, since new approaches will be developed. However, it is of great importance to consider the many complex factors of the entire problem. Some of these, particularly those concerned with the organoleptic characteristics and storage properties as related to the nutritive quality of the food, have not been discussed. In any case, it is essential to indicate the need to formulate and test such foods as much as possible in order to obtain the objective, which is the elimination of protein malnutrition, and not to repent at a later date when the world's food situation, and particularly protein nutrition, has deteriorated rather than improved.

Literature Cited

1. World Protein Resources. Advan. Chem. Ser. 57, R. E. Gould (ed.). Am. Chem. Soc.: Washington, D.C. (1966).

2. Bressani, R., and Elías, L. G. Processed vegetable protein mixtures for human consumption. Advan. Food Res. 16: 126-228 (1968).

3. Béhar, M., Viteri, F., Bressani, R., Arroyave, G., Squibb, R. L., and Scrimshaw, N. S. Principles of treatment and prevention of severe protein malnutrition in children (kwashiorkor). Ann. N.Y. Acad. Sci. 69: 954-968 (1957-58).

4. Béhar, M., Bressani, R., Scrimshaw, N. S. Treatment and prevention of kwashiorkor. World Rev. Nutr. Dietet. 1: 75-101 (1959).

5. Scrimshaw, N. S., and Béhar, M. Protein malnutrition in young children. Science 133: 2039-2047 (1961).

6. National Academy of Sciences--National Research Council, Publication No. 843 (1961). Progress in meeting protein needs of infants and preschool children. Proc. International Conference, Washington, D.C. August 1960.

7. Flores, M. Food patterns in Central America and Panama. In Tradition Science and Practice in Dietetics, pp. 23-27. Yorkshire, Great Britain: Wm. Byles and Sons Limited of Bradford (1961).

8. Flores, M. Dietary studies for assessment of the nutritional status of populations in non-modernized societies. Am. J. Clin. Nutr. 11: 344-355 (1962).

9. Braham, J. E., Bressani, R., de Zaghi, S., and Flores, M. Supplementary value of INCAP Vegetable Mixture 9 in the diets of average school children in rural Guatemala. J. Agr. Food Chem. 13: 594-597 (1965).

10. Braham, J. E., Flores, M., Elías, L. G., de Zaghi, S., and Bressani, R. Evaluación dietética, química y biológica de la dieta del niño preescolar en tres comunidades rurales de Guatemala. Submitted for publication to Arch. Latinoamer. Nutr.

11. Braham, J. E., Flores, M., Elías, L. G., de Zaghi, S., and Bressani, R. Suplementación de la mezcla Vegetal INCAP No. 9 a la dieta del niño preescolar de una población rural de Guatemala. Submitted for publication to: Arch. Latinoamer. Nutr.

12. Bressani, R., and Marenco, E. The enrichment of lime-treated corn flour with proteins, lysine and tryptophan, and vitamins. J. Agr. Food Chem. 11: 517-522 (1963).

13. Bressani, R. Supplementazione proteica dei cereali per migliorarne l'equilibrio aminoacidico. Dietologia e Dietoterapia, X (Fasc IV): 338-364 (1966).

14. Bressani, R., Elías, L. G., and Valiente, A. T. Effect of cooking and of amino acid supplementation on the nutritive value of black beans (Phaseolus vulgaris L.). Brit. J. Nutr. 17: 69-78 (1963).

15. Patwardhan, V. N. Pulses and beans in human nutrition. Am. J. Clin. Nutr. 11: 12-30 (1962).

16. Bressani, R., Valiente, A. T., and Tejada, C. All-vegetable protein mixtures for human feeding. VI. The value of combinations of lime-treated corn and cooked black beans. J. Food Sci. 27: 393-400 (1962).

17. Elías, L. G., and Bressani, R. Amino acid and protein supplementation of cottonseed flour protein concentrate. (Manuscript in preparation.)

18. Bressani, R. Development of food products. Formulated vegetable mixtures. In: Proc. of the Western Hemisphere Nutrition Congress-1965. Council on Foods and Nutrition-American Medical Assoc., Chicago, Ill.;1966. p. 86-90.

19. Bressani, R., and Béhar, M. The use of plant protein foods in preventing malnutrition. Proc. 6th International Congress of Nutrition, Edinburgh, pp. 181-206 (1963).

20. Bressani, R., Elías, L. G., Aquirre, A., and Scrimshaw, N. S. All-vegetable protein mixtures for human feeding. III. The development of INCAP Vegetable Mixture Nine. J. Nutr. 74: 201-208 (1961).

21. Elías, L. G., Bates, R. P., and Bressani, R. Mezclas vegetales para consumo humano. XVIII. Desarrollo de la mezcla vegetal INCAP 17, a base de semillas leguminosas. Submitted for publication to: Arch. Latinoamer. Nutr.

22. Bressani, R., Elías, L. G., and Braham, E. Cottonseed protein in human foods. Advan. Chem. Ser. 57, pp. 75-100. Am. Chem. Soc.: Washington, D.C. (1966).

23. Bressani, R., Aguirre, A., and Scrimshaw, N. S. All-vegetable protein mixtures for human feeding. II. The nutritive value of corn, sorghum, rice and buckwheat substituted for lime-treated corn in INCAP Vegetable Mixture Eight. J. Nutr. 69: 351-355 (1959).

24. Bressani, R., Elías, L. G., and Scrimshaw, N. S. All-vegetable protein mixtures for human feeding. VIII. Biological testing of INCAP Vegetable Mixture Nine in rats. J. Food Sci. 27: 203-209 (1962).

25. Bressani, R., and Elías, L. G. All-vegetable protein mixtures for human feeding. The development of INCAP Vegetable Mixture 14 based on soybean flour. J. Food Sci. 31: 626-631 (1966).

26. Bressani, R., Elías, L. G., Braham, J. E., and Erales, M. Vegetable protein mixtures for human consumption. The development and nutritive value of INCAP Mixture 15, based on soybean and cottonseed protein concentrates. Arch. Latinoamer. Nutr. 17: 177-195 (1967).

27. Liener, I. E. Effect of heat on plant protein. In Processed Plant Protein Foodstuffs, pp. 79-129. A. M. Altschul (ed.). Academic Press: New York (1958).

28. Bressani, R., Elías, L. G., Braham, E., and Erales, M. Long term rat feeding studies with vegetable protein mixtures containing cottonseed flour produced by different methods. Submitted for publication to: J. Agric. Food Chem.

29. Bressani, R., Aguirre, A., Elías, L. G., Arroyave, R., Jarquin, R., and Scrimshaw, N. S. All-vegetable protein mixtures for human feeding. IV. Biological testing of INCAP Vegetable Mixture Nine in chicks. J. Nutr. 74: 209-216 (1961).

30. Wogan, G. N. Mycotoxin contamination of foodstuffs, pp. 195-215. World Protein Resources, Advan. Chem. Ser. 57, R. E. Gould (ed.). Am. Chem. Soc.: Washington, D. C. (1966).

31. Bressani, R., and Elías, L. G. Mezclas de proteínas vegetales para consumo humano. XI. Aminoácidos limitantes en la mezcla vegetal INCAP 9 y efecto de la adicion de pequeñas cantidades de concentrados proteicos de origen vegetal y animal. Arch. Venezolanos Nutr. 12: 245-257 (1962).

32. Bressani, R., and Gómez-Brenes, R. Amino acid supplementation of cereal grains in mixed diets. Federation Proc. 27: 252, Abstr. 203 (1968).

33. Allison, J. B., and Anderson, J. A. The relation between absorbed nitrogen, nitrogen balance, and biological value of proteins in adult dogs. J. Nutr. 29: 413-420 (1945).

34. Bressani, R., Viteri, F., Wilson, D., and Alvarado, J. The endogenous urinary and fecal nitrogen excretion of children and the nutritive value of various animal and vegetable proteins for human nutrition. (Submitted for publication to J. Nutr.)

35. Dutra de Oliveira, J. E., and de Souza, N. Metabolic studies with a corn and soya mixture for infant feeding. Arch. Latinoamer. Nutr. 17: 197-206 (1967).