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RICARDO BRESSANI and LUIZ G. ELIAS
Institute of Nutrition of Central America and Panama (INCAP), Guatemala, C. A.

All-Vegetable Protein Mixtures for Human Feeding. The Development of INCAP Vegetable Mixture 14 Based on Soybean Flour

SUMMARY

Study of the complementary value of soybean and corn proteins in young growing rats resulted in the formulation of INCAP Vegetable Mixture 14: 58% corn flour, 38% soybean flour, 3% torula yeast, 1% calcium phosphate, and 4,500 I.U. vitamin A per 100 g. The mixture contains approximately 27% protein. Formula 14, evaluated in young growing dogs, was slightly lower in protein quality than casein. Its main limiting amino acid is methionine, but only when fed at a low level of protein. The addition of lysine and threonine in the presence of methionine caused a highly significant increase in protein efficiency ratio. Only a small response was obtained when milk protein replaced Formula 14 protein in isoproteic diets.

INTRODUCTION

The widespread prevalence and great public-health significance of protein-calorie malnutrition among young children in protein-poor areas of the world is now well recognized. During the last few years, several laboratories throughout the world have developed vegetable-protein mixtures for supplementary feeding of children and adults as a means of controlling protein-calorie malnutrition in underdeveloped areas (National Academy of Sciences, 1961). These mixtures usually consist of various combinations of a cereal grain with a vegetable protein concentrate. The vegetable protein concentrates that have received the greatest attention are soybean, cottonseed, peanut, and sesame flour (Altschul, 1958).

In our laboratories, a great deal of attention has been given to vegetable-protein mixtures based on cottonseed flour and corn (Bressani *et al.*, 1961). One of the mixtures developed was tested extensively in experimental animals (Bressani *et al.*, 1962) and children (Scrimshaw *et al.*, 1961) and is now being produced on an industrial scale under the commercial name of Incaparina (Béhar, 1963). Emphasis was placed on using cottonseed flour because of its availability in the Central American countries (Bressani, 1965) and other parts of the world (USDA, 1963) where protein-rich foods are needed.

Vegetable-protein mixtures containing other vegetable-protein concentrates are highly desirable because the availability of such concentrates varies from one area to another. Therefore, soybean flour was tested as the main vegetable-protein concentrate to develop mixtures with cereal grain protein.

The literature on the nutritive value of soybean protein has been reviewed in an excellent manner by several workers (e.g. Cicle and Johnson, 1958). Also, a considerable amount of work has been reported on the supplementary effect of soy flour on the protein of wheat flour (Jarquín *et al.*, 1966) and other cereal grains (Dean, 1958). In this report, soybean flour protein was tested for its complementary value to corn proteins, resulting in the development and testing of INCAP Vegetable Mixture 14.

MATERIALS AND METHODS

The experiments were done with a toasted soybean flour (kindly supplied by General Mills) containing 50% protein and local whole corn flour. On the basis of the nitrogen content of both ingredients, the complementary and supplementary value of soybean protein to corn protein was studied. For the experiments on the complementary value of soybean flour protein, a series of diets were prepared in which protein level was kept at approximately 9%. In one of the diets, all the protein was derived from corn flour, while in remaining diets, corn protein was replaced progressively by soybean flour protein, so that the protein of the last diet of the series was all derived from soybean flour and all contained equal amounts of total protein. The partial composition of the diets fed is shown in Table 1.

The supplementary value of soybean flour to corn protein was studied by adding increasing levels of soybean flour, from 0 to 20%, to a fixed level of corn in the diet. In this case, the protein content of the diet increased with increasing levels of soybean protein. As before, the partial composition of the diets used is shown in Table 2.

Table 1. Optimum combination between the proteins of corn and of soybean flour.

Percentage protein distribution in diet		Protein distribution in diet		Weight distribution in diet[a]		Weight gain[b] (g)	PER
Corn	SBF	Corn	SBF	Corn	SBF		
100	0	8.0	0	90.0	0	33	1.43
80	20	6.4	1.6	72.0	3.2	57	2.15
60	40	4.8	3.2	54.0	6.4	73	2.53
40	60	3.2	4.8	36.0	9.6	83	2.71
20	80	1.6	6.4	18.0	12.8	83	2.61
0	100	0	8.0	0	16.0	68	2.33

[a] The other ingredients of the diets were: 4.0% mineral mixture (Hegsted *et al.*. 1941), 5% refined cottonseed oil, 1% cod liver oil, and cornstarch to adjust to 100%. All diets were supplemented with 4 ml of a complete vitamin solution (Manna and Hauge, 1953) per 100 g.
[b] Average initial weight: 48 g (12 animals per group).

A procedure similar to that described above was followed in studies to find the complementary effect between skim milk and the best corn-soybean flour mixture. These experiments were done at the 10 and 20% protein levels in the diet. The partial composition of the diets used is described in Table 3.

Finally, the limiting amino acids in Mixture 14 were determined in young growing rats with diets containing 10 and 20% protein. The amino acids added, as well as the amounts, are shown under "Results." The other components of the diets are also shown in Table 4.

In all these studies, assays were carried out with young growing rats of the Wistar strain of the INCAP colony. Six to 12 rats were assigned at random to each experimental diet. The rats were placed in individual all-wire cages, with raised screen bottoms. Both feed and water were provided *ad libitum*, and the experimental period lasted 28 days. Weight of the rats and consumption of food was recorded every 7 days. Protein efficiency ratios (PER) were used to assess the effect of the different treatments. Partial description of the diets used is given in the results section, together with the performance of the experimental animals.

The best protein combination between corn and soybean flour served as the basis for the formulation of Mixture 14, which was then fed to 4 young growing dogs to determine its nitrogen balance index (NBI).

The animals were fed decreasing levels of protein from a diet made of: 87% Formula 14; 10% hydrogenated vegetable fat, 2% mineral mixture (Hegsted *et al.*, 1941), 1% cod liver oil, and 3 ml of a complete vitamin solution (Manna and Hauge, 1953) per 100 g. The intake of calories was maintained at 135 Kcal/kg/day throughout the study. When needed, the intake of calories was adjusted with an N-free diet made of: 20% cornstarch, 40% dextrose, 24% sugar, 3% cellulose, 10% hydrogenated vegetable fat, 2% mineral mixture (Heg-

Table 2. Effect of complementing and supplementing corn protein with soybean flour protein in rats.

Percentage protein distribution in diet		Protein distribution in diet		Protein in diet (%)	Weight distribution in diet		Weight gain[a]	PER
Corn	SBF	Corn	SBF		Corn	SBF		
100	0	7.82	0	7.82	90.0	0	36	1.60
80	20	6.41	1.60	8.01	72.0	3.2	58	2.29
60	40	4.70	3.13	7.83	54.0	6.3	74	2.72
40	60	3.20	4.79	7.99	36.0	9.5	88	2.91
20	80	1.63	6.54	8.17	18.0	12.7	79	2.67
0	100	0.00	8.07	8.07	0.0	16.0	77	2.56
100	0	6.99	0.00	6.99	70.0	0.0	22	1.31
76.0	24.0	6.32	2.00	8.32	70.0	4.0	62	2.30
59.8	40.2	5.96	4.00	9.96	70.0	8.0	101	2.61
48.4	51.6	5.63	6.00	11.63	70.0	12.0	129	2.59
39.6	60.4	5.24	8.00	13.24	70.0	16.0	158	2.69
33.2	66.8	4.96	10.00	14.96	70.0	20.0	154	2.55
57.9	42.1	5.64	4.10	9.74	70.2	8.2	100	2.63
15.8	84.2	2.14	11.39	13.53	32.4	22.9	141	2.40
15.1	84.9	2.26	12.70	14.96	36.0	25.4	139	2.21

[a] Average initial weight: 47 g (6 animals per group).

Table 3. Complementation between the proteins of Formula 14 and skim milk at two levels of protein in the diet.

Percentage protein distribution in diet		Protein distribution in diet		Weight distribution in diet[a]				
Formula 14	Skim milk	Formula 14	Skim milk	Formula 14	Skim milk	Protein in diet (%)	Weight gain (g)	PER
100	0	9.65	0.00	36.40	0.0	9.65	123	2.79
80	20	8.00	2.00	29.12	6.06	10.00	135	2.88
60	40	6.12	4.08	21.84	12.12	10.20	122	2.84
40	60	4.20	6.30	14.56	18.18	10.50	137	2.95
20	80	2.23	8.92	7.28	24.24	11.16	131	2.82
0	100	0.00	12.00	0.00	30.30	12.00	129	2.73
100	0	19.10	0.00	72.80	0.00	19.10	143	1.77
80	20	15.92	3.98	58.24	12.12	19.90	151	1.76
60	40	12.00	8.00	43.68	24.24	20.00	154	1.83
40	60	8.12	12.18	29.12	36.36	20.30	157	1.81
20	80	4.00	16.00	14.56	48.48	20.00	146	1.84
0	100	0.00	20.80	0.00	60.60	20.80	138	1.67

[a] The diets were supplemented with: 4.0% mineral mixture (Hegsted *et al.,* 1941), 5% refined cottonseed oil, 1% cod liver oil, and cornstarch to adjust to 100%. All diets were also supplemented with 4 ml of a complete vitamin solution (Manna and Hauge, 1953) per 100 g.

sted *et al.,* 1941), 1% cod liver oil, and 4 ml of a complete vitamin solution (Manna and Hauge, 1953) per 100 g of diet. The Formula 14 diet contained 3.62% nitrogen and 435 calories per 100 g. The N-free diet contained 435 calories per 100 g.

Each level of protein was fed for 8 days, providing two 4-day balance periods. Feces and urine were pooled every 4 days and homogenized before obtaining a subsample for nitrogen analysis. From the nitrogen intake and fecal and urinary nitrogen excretion, nitrogen absorption and balance were calculated.

RESULTS

Complementation between corn and soybean proteins. Table 1 shows the results of the first study designed to determine the best protein combination between corn and soybean protein. From

Table 4. Limiting amino acids in mixture 14 at two levels of protein in the diet.[a]

Amino acid supplement	Protein in diet (%)	Weight gain (g)	PER
None	10.10	110	2.56
+ 0.20% L-lysine HCl	10.30	93	2.35
+ 0.20% L-threonine	10.80	102	2.31
+ 0.20% DL-methionine	10.10	139	2.93
+ 0.20% L-lysine HCl + 0.20% L-threonine	10.10	121	2.69
+ 0.20% L-lysine HCl + 0.20% DL-methionine	10.40	126	2.74
+ 0.20% L-threonine + 0.20% DL-methionine	10.00	142	3.05
+ 0.20% L-lysine HCl + 0.20% L-threonine + 0.20% DL-methionine	10.10	164	3.39
None	18.04	145	2.01
+ 0.20% L-lysine HCl	18.36	156	1.99
+ 0.20% L-threonine	17.80	169	2.11
+ 0.20% DL-methionine	18.00	171	2.20
+ 0.20% L-lysine HCl + 0.20% L-threonine	17.34	162	2.11
+ 0.20% L-lysine HCl + 0.20% DL-methionine	17.00	168	2.25
+ 0.20% L-threonine + 0.20% DL-methionine	17.56	178	2.30
+ 0.20% L-lysine HCl + 0.20% L-threonine + 0.20% DL-methionine	18.05	164	2.19

[a] All diets were supplemented with: 4.0% mineral mixture (Hegsted *et al.,* 1941), 5% refined cottonseed oil, 1% cod liver oil, and cornstarch to adjust to 100%. All diets were also supplemented with 4 ml of a complete vitamin solution (Manna and Hauge, 1953) per 100 g.

both weight gained and PER values, it is evident that the proteins complement each other when corn provides 20–40% of the protein of the diet and soybean flour 60–80%. From these results, and by selecting the higher level of soybean flour and the lower of corn, Mixture 14 was formulated to consist of a mixture of 58 g of corn and 38 g of soybean flour per 100 g of mixture.

Table 2 shows the supplementary effect of soybean flour to corn. Complementation of soybean and corn proteins occurred as before when 60% of the dietary protein was derived from soybean and 40% from corn. It can be seen from the PER values that supplementation was optimum when 8.0% soybean flour was added to 70% corn. Larger amounts of soybean flour did not increase PER, although weight gain increased up to a level of 16% soybean flour in the diet.

Fig. 1A shows the relation between nitrogen intake and nitrogen retained, while Fig. 1B shows the relation between nitrogen absorbed and nitrogen retained in four days as carried out in dogs. From the slope of the lines, the nitrogen balance index was found to be 0.62 for the mixture of 56 g of corn and 38 g of soybean flour. Nitrogen equilibrium is obtained at a nitrogen intake of 230 mg/kg body weight/day. The biological value of Formula 14 was 73.1 calculated from the NBI, and 73.3% from the direct determination. Apparent and true protein digestibilities were respectively 78.6 and 80.2%.

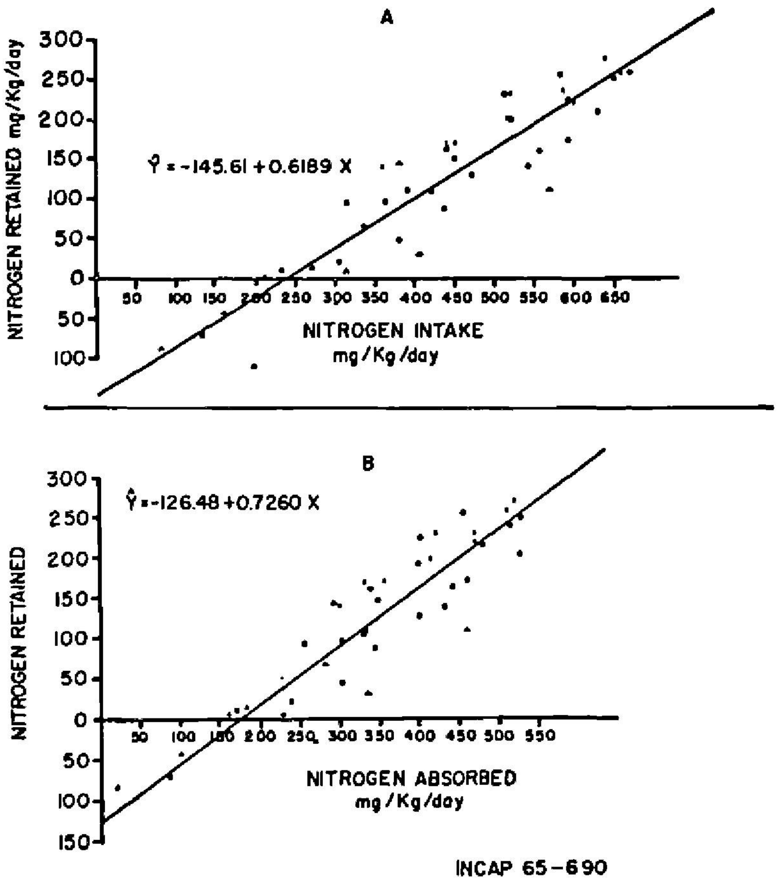

Fig. 1. Relationship between nitrogen intake and nitrogen balance and between nitrogen intake and nitrogen absorbed in dogs fed Mixture 14.

● dog No. 36; × dog No. 37; o dog No. 38; ▲ dog No. 39.
A) $\hat{Y} = -145.61 + 0.6189\,X$
B) $\hat{Y} = 126.48 + 0.7260\,X$

Dogs fed decreasing levels of a casein diet gave a regression equation equal to $\hat{Y} = -90.28 + 0.64\,X$ when nitrogen intake was plotted against nitrogen balance, and of $\hat{Y} = -82.43 + 0.69\,X$ when nitrogen absorbed was plotted against nitrogen balance ($\hat{Y}$ = nitrogen retention; X = nitrogen intake). On this basis, the protein value of Formula 14 was found to be very close to the nutritive value of casein.

Table 3 shows results based on the best protein combination between Formula 14 and skim milk at two levels of protein in the diet. It appears, from both weight gained in 28 days and PER values, that all combinations between Formula 14 and skim milk have a high nutritional value, although slightly higher values for both parameters were found when Formula 14 provided 40% of the dietary protein and skim milk 60%.

Table 4 presents the results on the amino acid supplementation of Formula 14 at two levels of dietary protein. The addition of methionine caused a significant increase in both weight gained and PER at the lower level of protein in the diet, while the addition of lysine and of threonine alone caused a slight decrease in PER and weight gained. The addition of the three amino acids together brought about a highly significant response in both weight gained and PER over the unsupplemented diet and the diet supplemented with methionine. Amino acid supplementation at the higher level of dietary protein brought about only a small increase in weight gained and PER.

DISCUSSION

The findings of the present investigation show that soybean flour protein and corn proteins complement each other, yielding a mixture having a nutritive value higher than that of either component fed alone. The type of response obtained when the protein of one component is replaced by the protein of the second component in isoproteic diets is different from the response obtained from mixtures of cottonseed flour and corn proteins (Bressani, 1965). In this case, replacement of cottonseed protein by corn protein results in a sharp decrease in the nutritive value of the mixture where corn protein contributes more than 60% of the protein in the diet.

Soybean protein supplements corn protein, as has been shown by several workers (Gilbert and Gillman, 1959), but the supplementary effect is not as great as the complementary value between the two proteins, as indicated by the PER values. There is, therefore, a basic difference between the two methods of combining soybean and corn proteins. In one case, that of supplementation, soybean protein is supplying the limiting amino acids in corn protein; however, at the same time other amino acids are added in the form of protein, which may limit the effect of the beneficial amino acids, which in this case are lysine and tryptophan. The situation is similar to that taking place when a fixed level of casein is supplemented with increasing levels of gelatin (Harper, 1959), although the effect is not as marked. In the case of complementation, the amino acid pattern of one protein component adapts with the amino acid pattern of the other component, resulting in a more efficient mixture of amino acids in the form of protein.

A further difference between the two types of protein enrichment is that, with complementation, mixtures ranging in protein content of equal nutritive value from 8 to 30% can be formed, whereas with supplementation the protein content is limited by the amount of the supplement added, to give the highest PER.

From the results on complementation, Mixture 14 was developed. It consists of a mixture of 56% corn and 38% soybean flour. Since these mixtures are being developed for the supplementary feeding of infants and young children whose diets are also deficient in vitamins and minerals, 3% torula yeast is added, as with previous mixtures (Scrimshaw *et al.*, 1962), and 1% calcium phosphate as source of Ca and P. Since vitamin A has also been found deficient in rural diets (Flores *et al.*, 1964), 4,500 I.U. per 100 g are also added.

The NBI of this mixture is superior to that of the Vegetable Mixture 9 (Bressani, 1966) and very similar to that of casein. Furthermore, its true protein digestibility is slightly higher, which may be the reason that its nutritive value is higher than that of Vegetable Mixture 9.

Even though the PER of the mixture between corn and soybean is superior to that of either component fed alone, the mixture is deficient in methionine at low levels of protein in the diet. However, the cost of this amino acid is low, and if its addition is considered practical, it will have only a slight effect on the cost of the final product. Lysine and threonine increased the nutritive value of Mixture 14 only after supplementation with methionine to about the same degree. The addition of three amino acids which appear to be deficient in the mixture of soybean flour and corn, produced a blend of a relatively high protein quality.

From the complementation experiments between milk and Mixture 14, protein mixtures containing some animal protein may be developed. It was of interest to learn that only a small improvement in nutritional value was obtained when skim milk replaced the protein of Formula 14 in isoproteic diets. This may be due to the fact that, at low levels of protein in the diet, methionine would be also the first-limiting amino acid in skim milk, although it contains slightly more of it than in Formula 14. The use of milk has several advantages, and allows the larger distribution of more milk to more people suffering from protein malnutrition.

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