Studies on the Use of *Opaque-2* Corn in Vegetable Protein-Rich Foods

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The protein quality of *Opaque-2* corn in comparison to common corn as a component of vegetable protein mixtures was evaluated by growth and PER experiments. The results indicated that mixtures which have a lysine deficiency are improved in quality when prepared with *Opaque-2* corn. Mixtures whose main protein source is soybean flour are not improved in protein quality as a result of preparation with *Opaque-2* corn. Amino acid supplementation of the various mixtures made with common and *Opaque-2* corn gave results which explained the responses observed. The results of additional studies indicated that the proteins of *Opaque-2* corn and cottonseed flour do not complement each other. *Opaque-2* corn and soybean protein complement each other in the range of 80 to 40% of the dietary protein from soybean, and 20 to 60% of the same from *Opaque-2* corn. Similar values were found between *Opaque-2* corn and cooked black beans. These results were interpreted in terms of the lysine concentration in *Opaque-2* corn and the other proteins tested.

The discovery of the improved essential amino acid pattern in the protein of corn due to the presence of the *Opaque-2* mutant gene (Mertz *et al.*, 1964, 1965) has opened a new area of investigation in nutrition. The use of *Opaque-2* corn by corn-consuming populations is now a possibility. Corn proteins are ordinarily deficient in lysine and tryptophan (Bressani *et al.*, 1963; Bressani and Marenco, 1963; Rosenberg *et al.*, 1960), and these amino acids are found in increased concentration in *Opaque-2* corn.

Previous investigations on the nutritive value of the protein of *Opaque-2* corn have demonstrated that its protein quality is higher than that of common corn (Mertz *et al.*, 1965). These studies, carried out in rats, evaluated corn as the sole source of protein in the diet. In the present study, *Opaque-2* corn was evaluated in the presence of other proteins of vegetable origin known to be deficient in lysine and methionine. Specifically, *Opaque-2* corn was tested as a substitute for common corn in vegetable protein mixtures developed to prevent protein malnutrition in the young human population of areas where protein deficiency prevails. Studies on the complementation between the proteins of *Opaque-2* corn and those of soybean, cottonseed, and black beans have also been reported.

MATERIAL AND METHODS

The *Opaque-2* corn used in the present studies was produced in the United States and sent by air mail to Guatemala, while the common corn was local material consumed by the rural population of the area.

The studies consisted of replacing common corn with *Opaque-2* corn in vegetable protein mixtures 9, 14, and 15, described in Table I. These formulations were analyzed for total nitrogen content, which for the biological tests in rats was diluted with corn starch to give 9 or 10% protein in the diet. The other dietary ingredients were 4% mineral mixture, 5% refined cottonseed oil, 1% cod liver oil, and a complete vitamin B supplement (Manna and Hauge, 1953).

Additional biological tests were carried out in which the diets made from common and *Opaque-2* corn, soybean, and cottonseed proteins were supplemented with various amino acids which previous studies (Bressani *et al.*, 1961; Bressani and Elías, 1966; Bressani *et al.*, 1967) had shown to be deficient in the vegetable protein food mixtures. These tests were also carried out using either 9 or 10% protein-containing diets, derived from the vegetable protein mixtures.

Finally, biological studies were performed to determine the points of maximum complementation between the proteins of *Opaque-2* corn and those of soybean, cottonseed, and black beans. For these studies, eight isoproteic diets were prepared for mixtures of *Opaque-2* corn and each protein. The protein in two of the diets was derived exclusively from *Opaque-2* corn or from the protein under study, while the other six consisted of mixtures of the two protein sources. As before, the diets were supplemented with 5% refined cottonseed oil, 1% cod liver oil, and 4% mineral mixture. All diets were also supplemented with a complete vitamin mixture, and corn starch was used to adjust to 100.

Table I. Composition of INCAP Vegetable Protein Mixtures

Ingredients	Vegetable Protein Mixtures 9, %	14, %	15, %
Common corn	58	58	58
Cottonseed flour	38	—	19
Soybean flour	—	38	19
Torula yeast	3	3	3
Calcium carbonate	1	1	1
Vitamin A, I.U./100 g.	4500	4500	4500

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Table II. Effect of Replacing Common Corn by *Opaque-2* Corn on the Protein Quality of INCAP Vegetable Protein Mixtures

Vegetable Protein Mixture	Corn	Protein in Diet, %	Av. Wt. Gain, G.	PER
Vegetable mixture 9	Common	9.9	55[a] ± 10.5	1.72 ± 0.21
	Opaque-2	10.3	72[a] ± 15.5	2.00 ± 0.26
Vegetable mixture 14	Common	10.0	82[a] ± 15.9	2.14 ± 0.24
	Opaque-2	10.8	86[a] ± 18.4	2.07 ± 0.23
Vegetable mixture 15	Common	10.6	71[a] ± 17.2	1.92 ± 0.22
	Opaque-2	9.3	81[a] ± 13.1	2.39 ± 0.16
Casein	—	11.4	119[a] ± 14.0	2.67 ± 0.16
Vegetable mixture 9	Common	8.7	36[b] ± 17.1	1.61 ± 0.61
	Opaque-2	9.3	54[b] ± 23.2	2.03 ± 0.62
Vegetable mixture 14	Common	7.3	52[b] ± 8.5	2.40 ± 0.13
	Opaque-2	9.0	60[b] ± 13.8	2.01 ± 0.29
Vegetable mixture 15	Common	8.7	38[b] ± 18.0	1.76 ± 0.55
	Opaque-2	10.1	61[b] ± 14.0	1.93 ± 0.27

[a] Average initial weight, 49 grams.
[b] Average initial weight, 44 grams.

Table III. Effect of Amino Acid Supplementation of Vegetable Protein Mixtures Made with Common and *Opaque-2* Corn

Corn	Amino Acid Supplement, %	Protein in Diet, %	Av. Wt. Gain, G.[a]	PER
	Mixture No. 9			
Common	None	11.2	67 ± 12.1	1.77 ± 0.22
	0.25 L-lysine · HCl	10.5	94 ± 21.8	2.28 ± 0.33
	0.25 L-lysine · HCl 0.10 DL-methionine	10.5	90 ± 16.7	2.33 ± 0.19
	0.25 L-lysine · HCl 0.10 DL-methionine	11.6	132 ± 21.5	2.53 ± 0.22
Opaque-2	None	10.5	100 ± 13.4	2.20 ± 0.17
	0.25 L-lysine · HCl	11.2	112 ± 16.0	2.24 ± 0.24
	0.10 DL-methionine	10.3	101 ± 12.8	2.32 ± 0.15
	0.25 L-lysine · HCl 0.10 DL-methionine	9.4	101 ± 24.9	2.79 ± 0.25
	0.25 L-lysine · HCl 0.10 DL-methionine 0.20 DL-threonine	11.2	131 ± 23.7	2.72 ± 0.24
	Mixture No. 14			
Common	None	11.0	86 ± 18.9	2.23 ± 0.26
	0.20 DL-methionine	10.1	113 ± 32.1	2.81 ± 0.26
	0.20 DL-methionine 0.20 DL-threonine	10.1	113 ± 16.7	2.85 ± 0.15
	0.20 DL-methionine 0.20 DL-threonine 0.20 L-lysine · HCl	10.1	128 ± 31.9	3.06 ± 0.33
Opaque-2	None	11.1	107 ± 14.5	2.30 ± 0.24
	0.20 DL-methionine	10.9	136 ± 26.1	2.65 ± 0.34
	0.20 DL-methionine 0.20 DL-threonine	10.5	137 ± 30.5	2.95 ± 0.28
	0.20 DL-methionine 0.20 DL-threonine 0.10 L-lysine · HCl	11.3	146 ± 21.3	2.96 ± 0.26
	0.20 DL-methionine 0.20 DL-threonine 0.20 L-lysine · HCl	12.2	138 ± 23.3	2.66 ± 0.31
Casein	—	10.9	108 ± 10.7	2.65 ± 0.14
	Mixture No. 15			
Common	None	9.4	109 ± 14.6	2.75 ± 0.16
	0.2 DL-methionine 0.2 DL-threonine	10.2	100 ± 11.6	2.61 ± 0.20
	0.2 DL-methionine 0.1 L-lysine · HCl	9.9	99 ± 10.7	2.77 ± 0.12
	0.2 DL-methionine 0.2 DL-threonine 0.2 L-lysine · HCl	9.8	135 ± 17.4	3.20 ± 0.27
Opaque-2	None	9.9	112 ± 10.8	2.70 ± 0.12
	0.20 DL-methionine 0.20 DL-threonine	9.8	124 ± 17.9	2.84 ± 0.13
	0.20 DL-methionine 0.10 L-lysine · HCL	10.4	128 ± 15.8	2.78 ± 0.16
	0.20 DL-methionine 0.20 DL-threonine 0.20 L-lysine · HCl	9.9	142 ± 20.9	3.43 ± 0.15
Casein	—	11.0	88 ± 16.7	2.46 ± 0.21

[a] Average initial weight, 47 grams.

For all studies, weanling white rats of the Wistar strain from the INCAP colony were used. Each dietary treatment was tested with a total of eight animals, four males and four females, which were distributed by weight so that the average initial weight was the same for all groups in each study. The animals were placed in individual all-wire screen cages with raised screen bottoms, and fed *ad libitum* for a total of 28 days. Water was made available at all times; weekly records of changes in weight and food intake were kept.

RESULTS

Table II summarizes the results of studies in which *Opaque-2* corn replaced common corn in three of the INCAP vegetable protein mixtures. At both levels of protein in the diet, replacing common corn by *Opaque-2* corn increased both weight gain and protein efficiency ratio (PER) for mixtures 9 and 15, but not for mixture 14, in which a small decrease in PER was obtained. Mixture 9 contains cottonseed flour as the main protein source, while 15 contains equal amounts of protein from cottonseed and soybean flours. On the other hand, mixture 14 contains soybean flour as the main source of protein.

To identify the deficient amino acids in the mixtures when these are made with common corn, in comparison with those made with *Opaque-2* corn, the three mixtures were supplemented with the amino acids which previous studies (Bressani *et al.*, 1961; Bressani and Elías, 1966; Bressani *et al.*, 1967) had indicated were deficient. Table III presents the results. The addition of lysine to mixture 9, made with common corn, improved growth and PER. However, this was not true when the mixture was made with *Opaque-2* corn. The addition of both lysine and methionine together increased PER in mixture 9, made with common or *Opaque-2* corn, but growth was increased only when the animals were on the mixture made from common corn. The addition of methionine, lysine, and threonine increased growth, but not PER, for both corn mixtures, over the value obtained from the addition of lysine and methionine.

Table III also shows the results obtained with mixture 14. The addition of methionine improved both weight gain and PER when the mixture was made from either common or *Opaque-2* corn. The addition of threonine with methionine, on the other hand, improved only FER of the mixture made from *Opaque-2* corn. Finally, the addition of methionine, threonine, and lysine increased weight gain and PER of the mixture made from common corn, but not when made from *Opaque-2* corn, even when the level of lysine added was one half as high.

The results for mixture 15 are also presented in Table III. With this mixture, the addition of three amino acids, methionine, threonine, and lysine, resulted in improved growth and PER when the mixture was made with either common or *Opaque-2* corn.

Since the mixtures were formulated from results of biological tests with the protein concentrate and common corn, it became interesting to learn if *Opaque-2* corn would complement the proteins of cottonseed and soybean as common corn does. The results of the nutritive value of combinations of the proteins of *Opaque-2* corn and cottonseed are shown in Table IV. The values obtained indicate that there is no complementation, as there is a linear response in both growth and PER as *Opaque-2* protein replaces cottonseed protein in the diet. *Opaque-2* protein gave a higher PER and weight gain than cottonseed protein when they were the only source of protein in the diet.

The results of the complementation between *Opaque-2* corn and soybean protein are also shown in Table IV. The values obtained indicate that the best combinations of these protein sources occur when soybean protein contributes between 80 and 40% of the diet and *Opaque-2* between 20 and 60%. In this range, weight gain and PER are quite similar. Food consumption was slightly lower in this group than in the cottonseed studies, which could explain the lower weight gain and PER of the *Opaque-2* corn group in the soybean study, as compared to the same group in the cottonseed protein experiment.

The results obtained from the protein complementation studies between common black beans and *Opaque-2* corn proteins are shown in Table IV. The nutritive value of the mixture increases as *Opaque-2* corn replaces black bean proteins, reaching the optimum point when 50% of the proteins comes from each one of the components. The highest PER was obtained at this point, and the lower weight gain and PER was found in the diet containing protein only from common black beans. The PER obtained when the *Opaque-2* corn was the sole source of protein is similar to that obtained from the group fed the best combination of the two foods.

Table IV. Protein Complementation

Percentage Protein Distribution from: Cottonseed	*Opaque-2*	Protein in Diet, %	Av. Wt. Gain, G.[a]	PER
Opaque-2 Corn and Cottonseed Proteins				
100	0	11.4	60 ± 23.6	1.59 ± 0.42
80	20	11.3	72 ± 24.4	1.90 ± 0.46
70	30	10.5	79 ± 13.7	2.24 ± 0.31
60	40	11.3	91 ± 14.7	2.08 ± 0.18
50	50	10.2	90 ± 18.3	2.26 ± 0.18
40	60	10.7	86 ± 9.7	2.13 ± 0.10
20	80	11.7	100 ± 12.9	2.44 ± 0.18
0	100	10.7	100 ± 21.6	2.44 ± 0.27
Opaque-2 Corn and Soybean				
Soybean				
100	0	10.7	86 ± 9.7	2.22 ± 0.14
80	20	10.5	112 ± 20.8	2.65 ± 0.28
70	30	10.2	108 ± 23.4	2.67 ± 0.28
60	40	10.3	115 ± 13.4	2.77 ± 0.21
50	50	10.9	111 ± 20.8	2.60 ± 0.27
40	60	10.8	110 ± 15.1	2.63 ± 0.15
20	80	10.9	106 ± 17.9	2.45 ± 0.23
0	100	10.9	90 ± 12.2	2.21 ± 0.16
Opaque-2 Corn and Black Beans				
Black beans				
100	0	10.2	18 ± 8.5	0.78 ± 0.26
80	20	10.2	53 ± 14.6	1.84 ± 0.29
70	30	10.4	64 ± 9.2	1.94 ± 0.07
60	40	10.7	88 ± 19.6	2.16 ± 0.29
50	50	10.2	106 ± 16.4	2.65 ± 0.26
40	60	10.7	113 ± 8.3	2.55 ± 0.17
20	80	10.4	111 ± 17.3	2.58 ± 0.17
0	100	10.2	103 ± 10.7	2.63 ± 0.17

[a] Average initial weight, 47 grams.

DISCUSSION

The results of the present investigation indicate that, in most cases, the replacement of common corn by *Opaque-2* corn causes an improvement in the protein quality of vegetable protein mixtures. The improvement, however, is not as high as was expected, judging from the protein quality of *Opaque-2* corn when tested alone. Apparently, it is dependent on the other sources of protein in the vegetable mixtures. In the present studies, improvements were observed when the other components of the mixture were cottonseed protein alone or an equal mixture of cottonseed and soybean proteins, but not with soybean protein. Cromwell *et al.* (1967) reported recently that only slight differences in growth rate or feed conversion resulted from the substitution of *Opaque-2* corn for normal corn on an equal nitrogen basis in a corn–soybean meal type of diet for chicks. The authors also indicate that the superior protein quality of *Opaque-2* corn in a soybean diet could be detected only when the methionine deficiency of the diet was corrected.

The results presented in this paper can then be interpreted on the basis of the amino acids present in adequate and lower amounts in the proteins of the foods studied. In the case of corn and cottonseed flour, the use of *Opaque-2* corn protein showed an increased protein quality because the deficiency of lysine in cottonseed protein (Fisher, 1965; Howe *et al.*, 1965) was partially met by the relative excess in *Opaque-2* corn. Similarly, in an equal protein mixture of soybean and cottonseed flour, lysine is the amino acid present in deficient amounts, although not as low as in cottonseed protein. Therefore, the use of *Opaque-2* corn resulted in improved growth and PER. Such an effect could not be observed with soybean protein because of its high level of lysine and deficiency of methionine (Howe *et al.*, 1965). The results of the amino acid supplementation studies reported for each mixture corroborate the preceding discussion.

Mixture 9, made with common corn, is deficient in lysine (Bressani *et al.*, 1961), and the addition of the amino acid improves protein quality, but not when the mixture is made with *Opaque-2* corn. As indicated previously, the reason is that cottonseed is lysine-deficient (Fisher, 1965; Howe *et al.*, 1965). A similar interpretation can be advanced in the case of mixture 14, based on soybean, where a definite response was obtained from methionine supplementation, regardless of the corn used.

The amino acid supplementation results also suggest that methionine is present in suboptimal amounts in *Opaque-2* corn, since growth and PER improved in all groups supplemented with this amino acid in the presence of the most limiting protein concentrate used. In these mixtures also, the three amino acids, lysine, methionine, and threonine, are closely related, and the simultaneous addition of the three gave mixtures of high protein quality.

Because of the difference between common and *Opaque-2* corn in essential amino acid pattern, it could be assumed that the combination of highest protein quality between *Opaque-2* corn and vegetable protein concentrate are different from those of the same protein concentrates and common corn. The results presented in this report substantiate the preceding statement. In the case of *Opaque-2* corn and cottonseed, growth data and PER indicate that these two proteins do not complement each other, as reported (Bressani *et al.,* 1961) for common corn and cottonseed protein. In this report it was indicated that the best combination from the quality point of view is when 80% of the protein of the diet is derived from cottonseed and 20% from corn. The nutritional factor controlling this relationship is lysine, which is approximately 2.9 grams per 16 grams of nitrogen in common corn, as compared to 3.6 for cottonseed protein. In the case of *Opaque-2* corn, this value is approximately 4.5 grams per 16 grams of nitrogen. These figures explain the results obtained from different combinations of the two proteins.

Studies on the protein quality of combinations of soybean and common corn proteins indicate that they complement each other when 40% of the protein of the diet is derived from corn and 60% from soybeans (Bressani and Elías, 1966). In the present investigation, optimum mixtures varied from 80 to 40% of the protein from soybean and 20 to 60% of the protein from *Opaque-2* corn. The range in the study with *Opaque-2* corn includes the best combination with common corn. In this situation, however, the amino acid that is playing the role is methionine, which amounts to approximately 3.2 grams per 16 grams of N (methionine + cystine) in *Opaque-2,* common corn, and soybean flour.

The results obtained with both cottonseed and soybean flour and *Opaque-2* corn can be used to formulate vegetable mixtures of high protein quality, particularly with soybean protein concentrate. The results from the studies in which *Opaque-2* corn and black bean protein were combined in different proportions are similar to those reported previously for common corn and black beans (Bressani *et al.,* 1962) and are of practical interest. According to nutrition surveys carried out by INCAP, people living in Central America consume more corn than beans, owing to dietetic habits and economic reasons. The use of *Opaque-2* corn in their diets instead of common corn would be a great advantage to their nutritional status since, with the same amount of beans and *Opaque-2* corn, their diet will be of higher quality than the present one.

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