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Protein Quality of Opaque-2 Corn Evaluation in Rats [1,2]

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ABSTRACT A study was made on the protein quality of opaque-2 corn as tested in rats. Opaque-2 corn showed a protein efficiency ratio (PER) of 2.79 as compared with 2.88 for casein at comparable levels of protein in the diet. The results also indicate that processing the corn into corn-masa or tortilla does not alter its high protein quality, although slightly lower PER values were found. Amino acid-supplementation studies indicated that lysine is the first limiting amino acid in opaque-2 corn when it provides less than 10% protein in the diet. Results are presented on the significant improvement obtained when common corn is supplemented with lysine and tryptophan, added together. Evidence that niacin from opaque-2 corn is available to the niacin-depleted rat, as opposed to its low availability for rats in the same condition in common corn, is also provided. Furthermore, the data show that opaque-2 corn has more niacin than common corn. Because of the high quality of opaque-2 corn proteins, efforts should be made to introduce the gene causing high lysine content into local varieties, especially in areas where corn is the most important staple food.

It has been known since 1914 that the quality of corn proteins is poor because they are deficient in the essential amino acids, lysine, and tryptophan (1). The reason behind these deficiencies is that zein, the corn protein which is soluble in alcohol, contributes up to 50% of the total protein in the corn kernel (2–5) in most corn varieties. Zein has been studied extensively, and results have indicated that it contains very low levels of lysine and tryptophan (6–8). Numerous studies (3, 4, 9–12) on the factors affecting the protein quality of corn indicated that both environment and variety had, in several cases, a significant effect on lysine content. It has also been shown that fertilization increases protein content and decreases protein quality. This is due to a total increase in the zein fraction which causes protein quality to decrease (2, 10, 13, 14). None of these studies had indicated the possibilities of increasing the nutritive quality of corn proteins, until Mertz et al. (15) showed that the opaque-2 gene of corn caused a genetic increase in lysine concentration. The studies by Mertz and co-workers (15) indicated also that the lysine increase in opaque-2 corn was the result of change in the distribution of endosperm proteins; opaque-2 corn contains only approximately 22% zein. Chemical analysis of corn protein for amino acids showed that opaque-2 corn contains 4.2 g lysine/100 g of protein, as compared with 2.8 g in regular corn. These results were corroborated by several workers (16–20), and by biological trials carried out with rats by Mertz et al. (17). Because corn constitutes the main staple of many population groups in which protein deficiencies are prevalent, it was of interest to study the nutritional quality of opaque-2 corn more extensively. Furthermore, in several countries in Latin America, corn is consumed in the form of tortillas. Therefore, it is important to learn whether or not the process of tortilla preparation affects the quality of opaque-2 corn protein. This paper presents the results of these studies.

MATERIAL AND METHODS

The opaque-2 corn [3] used in all the experiments was obtained from the United States. It was shipped by air and stored at INCAP laboratories until used.

Representative samples were analyzed for proximate chemical composition by AOAC official methods (21), and for ly-

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sine and tryptophan by microbiological assay (22). Niacin content was determined also, by microbiological assay for which *Leuconostoc mesenteroides* with Difco media was used (23).

Tortilla preparation. The method used was previously described by Bressani and Scrimshaw (22) and Bressani et al. (24). The raw corn was first washed with water to remove foreign matter. To 12.5 kg of corn, 24 liters of water and 200 g of lime were added. The mixture was cooked for 90 minutes. It was allowed to cool, the cooking liquor was discarded and the cooked kernels were washed four or five times with water, or until the washings were clear. During this process, the kernels were scrubbed to remove the seed coat which breaks down during the alkaline cooking. The cooked and peeled corn was then ground to a fine masa. Part of the masa was dried with hot air at 80° and part was used for tortilla preparation. The tortillas were dried also by hot air at 80°. The procedure described was applied to both the opaque-2 corn and to a common Guatemalan highland corn. Both the masa and the tortillas were analyzed for lysine and tryptophan content as indicated before, and for niacin content by microbiological methods (22).

Tests with rats. Several studies were carried out, using groups of 12 weanling white rats of the Wistar strain from the INCAP colony. Each group was made up of six male and six female rats, and care was taken that the initial weight was the same between groups within each experiment. The animals were placed in individual all-wire screen cages with raised-screen bottoms. The diets, as well as water, were provided ad libitum for a 28-day period. Changes in weight and amount of food consumed were measured every 7 days.

In the first study, all animals were killed, and blood and liver were collected for total serum protein analysis (25) and for determination of the chemical composition of the liver.

Table 1 describes the composition of the basal diet used in the study for the evaluation of the protein quality of corn made into tortillas, as well as the composition of the basal diet used in all amino acid-supplementation experiments. Total nitrogen was determined in the diets by the macro-Kjeldahl method, for the calculation of protein efficiency, defined as weight gain per gram of protein consumed. The amounts of amino acids added to the basal diet are given in each section, under Results. Their weight replaced an equal weight of corn starch although no corrections were made for the small amounts of nitrogen contributed by their addition.

Physiological availability of niacin. For these studies, 50 rats were given the niacin-depletion diet described by Kodicek and Silson (26) for 35 days, as shown in table 1. After this period, the surviving animals were distributed by weight into eight groups; they were fed the basal diet in which the corn was replaced by different corn preparations, as described under Results. The diets made with raw or cooked common corn contained an average of 8.2% protein, those from raw and cooked opaque-2 corn contained 8.9% protein.

TABLE 1

Composition of basal diets used in feeding tests

	Diet			
	1[1]	2[1]	3[2]	4[3]
Corn	90.0	—	72.0	40.0
Casein (vitamin free)	—	11.0	—	3.5
Mineral mixture[4]	4.0	4.0	4.0	3.0
Cottonseed oil	5.0	5.0	5.0	2.0
Cod liver oil	1.0	1.0	1.0	—
L-Cystine	—	—	—	0.1
Sucrose	—	—	—	51.4
Corn starch	—	79.0	18.0	—
Total	100.0	100.0	100.0	100.0
Vitamin solution, ml[5]	4	4	4	4[6]

[1] Diets 1 and 2 were used for PER assay of corn preparations. Three diets were prepared from opaque-2 corn and three from common corn, in which the amount of raw corn was replaced by an equal quantity of corn-masa and tortilla flour.

[2] Diet 3 was used for amino acid supplementation studies. Opaque-2 corn was always tested in the amount shown. The level was raised to 82% for the common corn because of its lower protein content. The amino acids added replaced an equal weight of corn starch. The amounts are indicated under Results.

[3] Diet 4 was fed for 35 days to cause niacin depletion in the rats. For the availability study, the common corn was replaced by different preparations as indicated under Results.

[4] Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart 1941 Choline in the nutrition of chicks. J. Biol. Chem., *138:* 459.

[5] Manna, L., and S. M. Hauge 1953 A possible relationship of vitamin B_{13} to orotic acid. J. Biol. Chem., *202:* 91.

[6] Same vitamin solution (Manna and Hauge), but without added niacin.

The second part of the experiment was carried out for a 28-day period, and the rats were allowed to feed and drink water ad libitum. As before, the animals were placed in individual all-wire screen cages with raised-screen bottoms, and weight change and food consumed were recorded every 7 days.

RESULTS

The nitrogen, lysine, tryptophan and niacin content of opaque-2 corn and the common corn used in the study, as well as the content of the above nutrients in the respective corn-masa and tortilla, are shown in table 2. As indicated, opaque-2 corn contained higher amounts of all these nutrients. Table 3 shows the results obtained when common and opaque-2 corn, raw or processed into masa and tortillas, were tested in rats. Weight gain was essentially the same for the three preparations from the common corn. Slightly lower values were obtained from opaque-2 corn made into masa and tortilla than those obtained with raw corn. In all cases, growth of the rats fed raw opaque-2 corn and the two preparations was four to five times greater than the corresponding response from rats fed common corn. Food intake was similar within the same corn preparations, but it was about twice as high for the animals fed opaque-2 corn as compared with common corn. The PER values for the masa and tortilla made from common corn were slightly higher than the value obtained for the raw corn. The PER value of the tortilla made from opaque-2 corn, however, was slightly lower than the value observed for the raw corn and the masa. The differences

TABLE 2

Lysine, tryptophan and niacin content of opaque-2 and common corn and preparations

Corn	Nitrogen	Lysine	Tryptophan	Niacin
	%	*g/16 g N*	*g/16 g N*	*mg/100 g corn*
Opaque-2				
Raw	1.64	4.5	1.5	2.20
Masa	1.67	4.6	1.5	2.47
Tortilla	1.64	4.2	1.5	2.01
Common				
Raw	1.16	3.6	0.62	1.62
Masa	1.12	3.1	0.57	1.63
Tortilla	1.06	3.1	0.49	1.87

TABLE 3

Average weight gain, food intake and protein efficiency ratio of different preparations from opaque-2 and common Guatemalan corn and their effect on total serum proteins, liver weight, fat and protein contents

Diet fed	Avg wt gain [1]	Food intake	PER	Total serum proteins	Liver		
					Fresh wt	Fat [2]	Protein [3]
		g		*g/100 ml*	*g*	%	%
Common Guatemalan corn							
Raw	25 ± 1.9 [4]	248 ± 11.5 [4]	1.49 ± 0.07 [4]	5.58	3.42	31.7	48.0
Masa	28 ± 2.0	240 ± 10.6	1.66 ± 0.05	5.02	3.18	28.0	49.8
Tortilla	27 ± 1.7	238 ± 8.8	1.55 ± 0.07	5.24	3.42	29.3	49.5
Opaque-2 corn							
Raw	130 ± 6.2	455 ± 12.7	2.79 ± 0.07	5.50	7.36	21.1	47.6
Masa	115 ± 2.9	389 ± 8.3	2.76 ± 0.04	4.89	6.31	18.2	47.9
Tortilla	115 ± 5.2	414 ± 14.3	2.66 ± 0.04	6.47	6.40	20.2	48.2
Casein	132 ± 5.9	408 ± 10.7	2.88 ± 0.06	6.31	7.81	11.6	46.2

[1] Average initial weight: 47 g.
[2] Dry weight basis.
[3] Dry weight basis, fat free.
[4] SE.

were not significant. As with growth, the PER values for the raw opaque-2 corn and preparations were significantly higher (1%) than the respective values for the common corn. The performance of the rats fed the raw opaque-2 corn was only slightly lower than the values observed for the rats fed the casein diet.

Table 3 also summarizes the values for serum proteins and liver composition. Total serum proteins were similar in rats fed either corn, although the rats fed tortilla from opaque-2 corn gave higher values. Fresh liver weight was twice as high in the rats fed opaque-2 corn and its preparations than the values obtained from rats fed common corn and its preparations. Liver fat was lower for the animals fed opaque-2 corn than for those fed common corn, but higher than for the casein control. Liver protein as a percentage was similar for all groups.

Table 4 shows the results of amino acid supplementation of common corn and opaque-2 corn. With respect to opaque-2 corn, a small response was obtained from lysine supplementation, which was not altered when tryptophan or tryptophan and isoleucine were added with lysine. For the common corn, no response was obtained from lysine addition alone. A significant effect was observed, however, when both lysine and tryptophan were added. Isoleucine had only a slight effect which was not significant. Table 5 presents the results of further studies. The addition of tryptophan alone to both opaque-2 corn and to the common corn did not change weight gain

TABLE 4

Effect of amino acid supplementation on opaque-2 and common corn

Amino acid supplement	Avg wt gain [1]	PER
	g	
Opaque-2		
None	90±3.5 [2]	2.72±0.05 [2]
+0.40% L-lysine·HCl	96±5.8	2.94±0.07
+0.40% L-lysine·HCl+0.10% DL-tryptophan	91±4.6	2.82±0.07
+0.40% L-lysine·HCl+0.10% DL-tryptophan +0.20% DL-isoleucine	94±5.2	2.96±0.08
Common corn		
None	38±2.6	1.47±0.08
+0.40% L-lysine·HCl	28±4.0	1.41±0.18
+0.40% L-lysine·HCl+0.10% DL-tryptophan	75±5.5	2.47±0.08
+0.40% L-lysine·HCl+0.10% DL-tryptophan +0.20% DL-isoleucine	77±4.9	2.53±0.09

[1] Average initial weight: 47 g.
[2] SE.

TABLE 5

Effect of supplementing opaque-2 and common corn with tryptophan alone and with other amino acids

Amino acid supplement	Avg wt gain [1]	PER
	g	
Opaque-2		
None	67±4.6 [2]	2.60±0.08 [2]
+0.10% DL-tryptophan	67±4.6	2.66±0.09
+0.10% DL-tryptophan+0.40% L-lysine·HCl +0.15% DL-methionine	72±5.5	2.81±0.10
Common corn		
None	27±2.3	1.13±0.11
+0.10% DL-tryptophan	26±2.0	1.16±0.09
+0.10% DL-tryptophan+0.40% L-lysine·HCl +0.15% DL-methionine	60±5.5	2.18±0.16

[1] Average initial weight: 48 g.
[2] SE.

or PER. The simultaneous addition of lysine and of methionine with tryptophan, however, improved both weight gain and PER, particularly in the case of common corn. In all cases, the values obtained from the opaque-2 corn were higher than the values obtained from common corn. In this experiment food intake was lower than in other studies, which could account for the lower weight gains obtained.

Table 6 presents additional results for the amino acid supplementation of opaque-2 corn. Again, it appears that lysine addition, alone or in the presence of tryptophan and of tryptophan and methionine, increased weight gain and PER. Methionine added alone did not have any effect. The lower section of table 6 shows results of further tests, indicating no effect from the addition of threonine or methionine alone. Lysine added in the presence of tryptophan and threonine resulted in a significant increase in PER, but not in weight gain.

Finally, table 7 summarizes the results obtained in the studies carried out to determine the availability of niacin from opaque-2 corn. It can be seen that the animals fed the raw common corn diet lost weight without niacin supplementation, but the addition of the vitamin resulted in a significant increase in weight gain and reduced mortality. On the other hand, feeding the raw opaque-2 corn diet with and without niacin addition resulted in a similar weight gain for both cases, and also for the diet made from common corn supplemented with niacin. Lime cooking of the common corn apparently caused a beneficial effect, since the animals maintained their weight. Lime cooking of the opaque-2 corn reduced weight gain when compared with untreated corn. When both

TABLE 6

Amino acid supplementation of opaque-2 corn

Amino acid supplement	Avg wt gain	PER
	g	
None	86 ± 4.0 [1]	2.45 ± 0.06 [1]
+0.05% L-lysine·HCl	89 ± 4.9	2.65 ± 0.06
+0.10% L-lysine·HCl	92 ± 5.8	2.78 ± 0.06
+0.10% DL-methionine	83 ± 4.3	2.59 ± 0.07
+0.10% lysine·HCl + 0.05% DL-tryptophan	83 ± 3.8	2.70 ± 0.07
+0.10% lysine·HCl + 0.05% DL-tryptophan +0.10% DL-methionine	90 ± 4.3	2.85 ± 0.05
Average initial weight: 50 g		
None	77 ± 3.5 [1]	2.78 ± 0.05 [1]
+0.20% DL-threonine	77 ± 4.6	2.74 ± 0.06
+0.15% DL-methionine	84 ± 4.0	2.51 ± 0.06
+0.40% L-lysine + 0.10% DL-tryptophan +0.20% DL-threonine	69 ± 4.9	2.98 ± 0.11
Average initial weight: 46 g		

[1] SE.

TABLE 7

Niacin and amino acid supplementation of raw and lime-treated opaque-2 and common corn

Treatment	Avg wt gain [1]	Mortality
	g	
Common corn raw	−9	3/4
Common corn raw + niacin [2]	55	1/4
Opaque-2 raw corn	52	1/4
Opaque-2 raw corn + niacin [2]	56	0/4
Lime-treated common corn (masa)	1	3/4
Lime-treated opaque-2 corn (masa)	36	1/4
Common raw corn + lysine + tryptophan + niacin [2]	54	0/4
Opaque-2 corn + lysine + tryptophan + niacin [2]	69	0/4

[1] Average initial weight: 46 g.
[2] Levels added in milligrams per 100 g of diet: L-lysine·HCl, 100; DL-tryptophan, 50; and niacin, 4.

corn samples were supplemented with lysine, tryptophan and niacin, growth response increased for the opaque-2 corn but not for common corn, comparing these results with those of the niacin-supplemented corns. When the comparison is made with the results from unsupplemented corns, however, the amino acids and niacin improved the nutritive value of common corn.

DISCUSSION

The results of the present investigation corroborate the findings of Mertz et al. (15,17), indicating that the opaque-2 corn has a high protein quality. As shown by the same authors, and likewise by data presented in this paper, the high quality of opaque-2 corn protein is due to its higher content of lysine and tryptophan, in comparison with common corn. Some of the differences in weight gain and PER could be attributed also to the higher levels of total protein in the diets made from opaque-2 corn. These diets contained an average of 10.4% protein whereas those from common corn contained only 6.9%. The difference in protein content, however, probably affected weight gain more than PER, since it has been shown (10) that low protein-containing corn samples have a better protein quality than corns with a high protein content. In other studies (27) common Guatemalan corns with a higher protein content than the one used in the present study gave a PER value of around 1.0.

The results indicate further that lime cooking of opaque-2 corn for the preparation of tortilla causes only a small, not significant, decrease in its protein quality. The small decrease could be due to the inactivation of some of the lysine, since the process of tortilla preparation requires the use of high temperatures (22,24) which are known to make certain amino acids, particularly lysine, less available to the organism (24).

Studies carried out by Rogler (28) have indicated that the high quality of the protein of opaque-2 corn cannot be shown when it is used in combination with protein concentrates, as tested in chicks. Similar results were found by Bressani (29), when testing the use of opaque-2 corn in vegetable protein mixtures with rats. Therefore, it is of interest to learn the reason behind these findings. Because the response is probably related to amino acid balance, studies were carried out to determine the limiting amino acids in opaque-2 corn protein. The results of the present study show that neither methionine, tryptophan nor threonine are first limiting amino acids, since, when they were added alone, no response was evident. Similar results were reported by Clark (30) in man. Rogler (28) reported that opaque-2 corn protein responded to methionine supplementation when used with soybean meal in chick nutrition studies.

Examination of all data obtained when opaque-2 corn was supplemented with lysine, added alone or with other amino acids, showed that the first limiting amino acid is lysine. Statistical analysis of the difference between all PER data from the control groups compared with the PER data of all groups supplemented with lysine, or groups containing lysine together with other amino acids, was highly significant. There was no statistically significant difference in PER between the lysine-supplemented groups and groups fed opaque-2 corn supplemented with lysine plus other amino acids. Apparently, although the content of this amino acid is higher in opaque-2 corn than in common corn, its concentration in the former is not high enough to supply all the lysine needed by the rat at the 9% protein level in the diet. That the corn is deficient can also be deduced from observation of the fat in the liver, which, although lower than liver fat when common corn is fed, was still higher than the fat found in the liver of rats fed casein. Further evidence can be inferred from the studies in which opaque-2 corn was used in three vegetable protein mixtures, one based on cottonseed, one on soybean, and the third on equal parts of both proteins. Opaque-2 corn and cottonseed protein gave a higher index of protein value than common corn and cottonseed protein. Similar results were obtained when opaque-2 corn was tested with cottonseed and soybean proteins, but not when mixed with soybean alone (29). It is well known that cottonseed is deficient in lysine whereas soybean is a rich source of this amino acid (31). It is recognized, however, that the lysine

deficiency is of academic interest. Awareness of this limitation is of value in the use of this corn in diet formulation and in practical applications.

The results on the availability of niacin from opaque-2 corn are of interest. They indicate that the niacin from opaque-2 corn is available to the rat, as opposed to that from common corn, although it is true that opaque-2 corn contained more niacin than common corn. Higher concentration, however, does not necessarily mean more availability, since there are corn samples which contain as much niacin as the opaque-2 corn used in the present study. However, the lime treatment to which corn is subjected when tortilla is made causes a loss of the vitamin, shown previously by Bressani and Scrimshaw (22) and Bressani et al. (24), which could explain the lower weight gain of the animals fed cooked opaque-2 corn. The data also indicate that the theory on the low availability of niacin from corn should be revised (26,32,33). It appears that amino acid balance and tryptophan deficiency are more fitting, and that lime treatment in regular corn causes an increase in tryptophan availability rather than a release of bound niacin. Furthermore, the results showed that common corn supplemented with niacin produced as good a weight gain as the group fed common corn supplemented with niacin, lysine and tryptophan. This would mean that lysine and tryptophan added to common corn in the presence of niacin did not have any additional effect. It must be considered, however, that the basal corn diet contained 3.5% casein, which provided, together with corn, an amino acid pattern which was different from that of corn alone. The loss of animals even after the application of the different treatments, with the exception of the first group, was probably due to the condition of extreme niacin depletion which the animal was in at the beginning of the study.

From the practical point of view, great efforts should be made to introduce corn varieties with the opaque-2 gene, particularly in areas of the world where corn is the main staple food.

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