Value of Opaque-2 Corn Protein for Chicks

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The protein quality of opaque-2 corn was compared to that of common corn with chicks. In some experiments the corn samples were tested with other protein sources and in others as the sole source of dietary protein. Opaque-2 corn was shown to be superior in protein quality to common corn, primarily because of its higher lysine content. In the studies carried out, the difference in protein quality, however, could be detected only when opaque-2 corn was fed as the sole source of protein, or when tested with lysine deficient protein sources. In a cottonseed flour–corn mixture, lysine is the first limiting amino acid whether the corn is opaque-2 or common corn. Methionine was the second limiting amino acid only for the cottonseed flour–common corn diet. When fed as the sole source of protein, opaque-2 corn had a superior quality when compared to common corn with or without lysine and tryptophan added, cottonseed flour, soybean flour, sorghum or wheat middlings, and opaque-2 corn supplemented with methionine.

Corn is a high energy grain commonly used as a source of calories in animal nutrition. The proportion in feed formulations for monogastric animals usually surpasses 50% of the diet, rendering its protein nutritionally significant. It is also well documented that corn proteins are deficient in lysine and tryptophan (Bressani *et al.*, 1968; Sauberlich *et al.*, 1953) and that they must be supplemented with sources of these amino acids, which at present are either expensive or difficult to find in developing countries.

The opaque-2 gene changes the amino acid composition of corn protein, causing a substantial increase in the lysine content, through a decrease in the alcohol soluble proteins of the cereal grain (Mertz *et al.*, 1964; Mertz *et al.*, 1965).

The fact that opaque-2 corn is nutritionally superior to common corn is supported by biological evidence, for the rat (Bressani *et al.*, 1969b; Mertz *et al.*, 1965), the pig (Cromwell *et al.*, 1967a), and humans (Bressani *et al.* 1969a; Clark, 1966). In recent studies, carried out with baby chicks (Cromwell *et al.*, 1967b), a corn–soybean diet was used and results favored opaque-2 corn only after the deficiency of the first limiting amino acid in the diet was corrected by methionine supplementation, which is also the first limiting amino acid in soybean protein (Bressani and Elías, 1968a). Under the dietary conditions used by Cromwell *et al.* (1967b), it was concluded that the beneficial effects of opaque-2 corn for the chick in comparison with normal corn were caused by its increased lysine content.

It is also known that soybean protein is a good source of lysine. Therefore, it is difficult to demonstrate the superior quality of opaque-2 corn by using a corn–soybean diet, because of its high content of lysine in comparison with common corn, unless lysine becomes limiting by addition of methionine to the diet. If the protein source in the diet, however, is sufficiently lysine deficient, the higher quality of opaque-2 corn should be easily detected.

The purpose of the present study was to evaluate the nutritional value of the protein of opaque-2 corn from the United States and Colombia, in comparison with the protein of common corn. Protein sources, deficient in lysine, were used in the basal diets in one series of experiments, supplemented with various levels of opaque-2 and common corn. In other studies common and opaque-2 corn were fed as the sole source of protein in the diets of chicks.

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MATERIALS AND METHODS

The protein sources used in the experiments were two cottonseed flours prepared by the pre-press solvent extraction method (Bressani and Elías, 1968b), sesame flour prepared in the laboratory by extracting the oil with a hydraulic press followed by hexane extraction, soybean meal, corn gluten meal, two sources of opaque-2 corn, and common highland Guatemalan corn. All of the material was analyzed for total crude protein by the Kjeldahl method (A.O.A.C., 1960) and for available lysine by the method of Conkerton and Frampton (1959).

The protein concentrates were used in the basal diet, described in Table I, in the first series of studies. The amounts used provided 16% protein in the diets.

The corn samples under study were added to the basal diet in the amounts needed to provide 2.0, 3.1, and 4.2% protein. Their weight replaced an equal weight of glucose or starch. These diets were then supplemented with additional calories, vitamins, and minerals, to satisfy the needs of the growing chick.

The diets were fed *ad libitum* to groups of 2-day old chicks of the Vantress breed, for 4 to 5 weeks, and water was available at all times. Each group consisted of 12 to 20 birds, depending on the availability of materials, which were placed in battery brooders with temperature control. In four out of seven experiments each dietary treatment was tested in two replicates with the same number of birds, which were distributed by weight so that initial average weight was the same in all groups. Details of the number of birds per group are given in the Tables under Results. Weekly records of weight change and feed consumption were kept.

To test the protein quality of opaque-2 corn, a different

Table I. Composition of Basal Diet

Ingredients	%
Protein source[a]	38–45
Corn under test[b]	. . .
Refined cottonseed oil	5.00
Bone meal	2.10
Calcium carbonate	1.50
Iodized salt	0.45
Vitamin and minor elements	0.55
Glucose	52.40–45.40

[a] For cottonseed and sesame flour 38.0% and for corn gluten 45.0%.
[b] Added in amounts equivalent to 2.0, 3.1, and 4.2% protein. Their weight replaced part of the glucose.

Table II. Protein and Available Lysine Content of Materials Used in Feeding Trials

Ingredients	Protein Content, g., %	Lysine Content[a] g., %	Lysine Content[a] g./16 g.N
Cottonseed flour[b]	49.3	1.92	3.9
Cottonseed flour[c]	47.6	1.53	3.2
Soybean meal	44.7	...	...
Sesame meal	43.8	1.06	2.4
Corn gluten	34.9	0.18	0.5
Opaque-2 corn (U.S.)	10.5	0.42	4.0
Opaque-2 corn (Colombia)	9.5	0.37	3.9
Common corn	8.0	0.30	3.7

[a] Epsilon amino lysine. [b] Cottonseed flour B, pre-press solvent. [c] Cottonseed flour DS, pre-press solvent.

approach was used in a series of studies. In these, baby chicks, 1-day old, were fed for 15 days an 18.5% protein commercial ration. At this time the chicks were distributed by weight among various feeding treatments. These treatments consisted in feeding the chicks each of the materials as the sole source of protein in the diet at a level sufficient to provide the same amount of protein as that coming from 90.4% of corn. The rest of the ration contained the same sources and amounts of calories, minerals, and vitamins as indicated in Table I. These diets were fed during 4 or 5 weeks. Weekly records of weight changes and feed intake were kept.

RESULTS

The protein sources used as well as their protein and the available lysine content are shown in Table II. The protein concentrate sources ranged in protein content from 34 to 44%. All of them except soybean meal have lysine as the first limiting amino acid, which in the materials used ranged from 0.18 to 1.92%, or 0.5 to 4.0 g. lysine/100 g. protein of available lysine.

The corn samples used ranged from 8.0 to 10.5% protein content, and from 0.30% (3.7 g./100 g. protein) for the common corn to 0.42% (4.0 g./100 g. protein) for the U.S. opaque-2 corn of available lysine. The Colombian sample was lower in available lysine. It should be indicated also that lysine content of the common corn used was uncommonly high.

Table III shows the results of the first experiment in which cottonseed flour with 1.92% available lysine was used as the main protein source. The average final weight of the animals, fed the three levels of opaque-2 corn or the three levels of common corn, were higher than the final weight of the control groups. The effect was probably caused by the higher protein content in the diets with opaque-2 or common corn. Final weight, however, was higher for the diets containing common corn when compared to the diets prepared with opaque-2 corn. Since lysine content was slightly higher in the rations with opaque-2 corn, the response observed was unexpected.

As indicated previously, common corn was added to the diet in amounts calculated to provide equal levels of protein as opaque-2 corn; however, because common corn had a lower content of protein, higher amounts of it were used in the diet, providing higher levels of starch. This decreased the levels of added glucose. Therefore, a second experiment was carried out in which the carbohydrate added to the basal diet was either glucose or starch. The cottonseed flour used contained 1.53% available lysine. In this experiment an additional group was tested using common corn supplemented with lysine and tryptophan. The results are shown in Table IV. As before, the addition of either common or opaque-2 corn to the basal diet produced better growth and feed efficiency. The opaque-2 corn from the United States induced higher weight gains than both opaque-2 corn from Colombia and common corn. The differences in weight and feed efficiency of the latter two corns were smaller. Except for the animals fed on cottonseed alone or with opaque-2 corn from the United States, starch was not as effective as glucose that tended to produce better weight gains and feed efficiency.

The best performance was obtained from the chicks fed common corn supplemented with lysine and tryptophan, which was significantly higher than that observed with the better of the opaque-2 corns tested.

Table V shows the results when sesame flour is used as the main protein source. Sesame protein is known to be a good source of methionine with, however, a relatively high lysine deficiency. The results show that the U.S. opaque-2 corn sample gave the higher final weight, followed by the Colombian sample.

Table VI presents the results obtained when corn gluten meal was used as the source of protein. There was no difference between opaque-2 and common corn when these materials were added in amounts equivalent to 2% protein. When the corns, however, were added to provide 4.2% protein, better performance was obtained with opaque-2 corn. All animals on the basal diet died, which is an indication of the poor quality of corn gluten meal.

It has been suggested that methionine is the first limiting amino acid in opaque-2 corn (Clark, 1966; Cromwell *et al.*, 1967b). Studies were therefore conducted to learn whether such a deficiency could be detected by the use of a cottonseed flour–corn diet. The results are shown in Table VII. The cottonseed flour used contained 1.53% available lysine. The addition of opaque-2 corn increased final weight significantly, when compared to the basal diet and to the basal diet with

Table III. Response to Opaque-2 and Common Corn Added at Three Levels to a Cottonseed Flour Basal Diet

Treatment	Protein in Diet, %	Available Lysine in Diet, %	Glucose in Diet, %	Average Final Weight, g.[b]	Feed/Gain[a]
Cottonseed flour[c]	18.5	0.73	52.4	527 ± 25.1[d]	2.4
Opaque-2 corn[e]	21.4	0.81	32.4	573 ± 17.3	2.1
	21.9	0.86	22.4	597 ± 26.4	2.1
	22.5	0.90	12.4	642 ± 14.8	2.0
Common corn[e]	21.0	0.80	27.4	627 ± 24.8	2.2
	21.3	0.84	14.4	634 ± 29.7	2.1
	22.8	0.88	1.4	686 ± 13.5	2.0

[a] Calculated from the feed consumed/group. [b] Average of 14 chicks/group with an average initial weight of 49 g. Each group consisted of two replications with seven chicks per replication. [c] Human grade flour, level used 38.0%. Available lysine, 1.92%. [d] Standard deviations. [e] Added in amounts equivalent to 2.0, 3.1, and 4.2 g. of protein.

Table IV. Response of Baby Chicks to Opaque-2 and Common Corn in Diets Containing Starch or Glucose

Treatment	Carbohydrate Used in Diet	Protein % Diet	Average Final Weight, g.[a]	Feed/Gain[b]
Basal	Glucose[c]	18.6	212 ± 50.4	3.1
	Starch[c]	19.3	258 ± 42.6	3.0
Basal + 30.0% Opaque-2 corn (U.S.)	Glucose[d]	21.6	411 ± 52.2	2.4
	Starch[d]	21.5	413 ± 68.8	2.6
Basal + 30.0% Opaque-2 corn (Colombia)	Glucose[d]	20.9	368 ± 86.8	2.4
	Starch[d]	20.3	357 ± 77.4	2.5
Basal + 30.0% Common corn (Guatemala)	Glucose[d]	20.6	331 ± 70.2	2.8
	Starch[d]	20.6	307 ± 60.3	2.9
Basal + 30.0% Common corn + 0.25% lysine + 0.05% tryptophan	Glucose[d]	20.7	634 ± 63.2	2.1
	Starch[d]	21.0	597 ± 67.7	2.2

[a] Average initial weight: 47 g. 20 chicks/dietary treatment, experimental period: 5 weeks. [b] Calculated from the feed consumed/group. [c] 52.4% glucose or starch. [d] 22.4% glucose or starch.

Table V. Response to Opaque-2 and Common Corn Added at Two Levels to a Sesame Flour Basal Diet

Treatment	Protein in Diet, %	Available Lysine in Diet, %	Glucose in Diet, %	Average Final Weight, g.[b]	Feed/Gain[a]
Sesame flour[c]	15.5	0.40	52.4	216 ± 24.4[d]	4.6
Opaque-2 (U.S.)[e]	17.9	0.48	32.4	325 ± 19.9	3.1
	20.5	0.57	12.4	418 ± 26.4	2.8
Opaque-2 (Colombia)[e]	17.7	0.47	32.4	315 ± 15.3	3.1
	19.7	0.55	12.4	365 ± 30.3	2.8
Common corn[e]	18.7	0.46	32.4	263 ± 22.5	3.5
	20.4	0.52	12.4	326 ± 21.6	2.8

[a] Calculated from the feed consumed/group. [b] Average of 12 chicks/group; average initial weight: 65 g. Experimental period: 5 weeks. [c] Level used in diet: 38.0%. [d] Standard deviations. [e] Added in amounts equivalent to 2.0 and 4.2 g. of protein.

Table VI. Effect of Opaque-2 and Common Corn on Growth of Baby Chicks Fed Corn Gluten Meal

Treatment	Protein in Diet, %	Average Final Weight,[a] g.	Feed/Gain[b]
Corn gluten basal diet	15.2	...	...
+ Opaque-2 corn[c]	18.8	102 ± 4.5[d]	5.7
+ Common corn[c]	18.3	99 ± 7.3	6.3
+ Opaque-2 corn[e]	21.6	183 ± 15.2	3.3
+ Common corn[e]	20.0	110 ± 5.4	4.5

[a] Average initial weight: 46 g. 10 chicks/group. Experimental period: 6 weeks. [b] Calculated from the feed consumed/group. [c] Added in an amount equivalent to 2.0% protein. U.S. opaque-2 corn. [d] Standard deviations. [e] Added in an amount equivalent to 4.2% protein.

common corn added. Lysine supplementation, however, improved the performance of the chicks fed either the diet with opaque-2 corn or the diet with common corn. Better performance was observed, however, with the opaque-2 corn–cottonseed flour. Methionine addition alone did not alter the final weight of the animals fed either the opaque-2 corn or common corn–cottonseed basal diets. The addition of both lysine and methionine improved the performance of the animals fed the common corn diet but not that of those fed the opaque-2 corn diet, when the results are compared to the diet supplemented with lysine added alone.

Table VIII shows the results of two experiments with 15-day old chicks fed the proteins indicated in the Table as the sole source of protein in the diet. The highest weight gain was obtained with the U.S. opaque-2 corn, followed by the Colombian opaque-2 corn. Addition of methionine once more did not improve the protein quality of opaque-2 corn.

Table VII. Amino Acid Supplementation of a Cottonseed Flour–Common Corn and of Cottonseed Flour–Opaque-2 Corn Diet

Dietary Treatment[a]	Protein in Diet, %	Average Final Weight,[b] g.	Feed/Gain[c]
Basal Diet (B)	16.6	240 ± 19.6[d]	3.0
B + 30% U.S. opaque-2 corn	21.2	403 ± 16.1	2.2
B + 33% common corn	21.0	341 ± 23.0	2.6
B + 30% U.S. opaque-2 corn + L-lysine HCl	21.3	629 ± 29.3	2.0
B + 33% common corn + L-lysine HCl	19.7	550 ± 19.2	2.1
B + 30% U.S. opaque-2 corn + DL-methionine	20.5	429 ± 21.4	2.3
B + 33% common corn + DL-methionine	20.0	326 ± 15.7	2.5
B + 30% U.S. opaque-2 corn + lysine + methionine	20.2	633 ± 28.6	2.0
B + 33% common corn + lysine + methionine	20.4	628 ± 15.4	2.1
B + 30% U.S. opaque-2 corn + lysine + methionine + threonine	20.8	622 ± 15.6	1.9
B + 33% common corn + lysine + methionine + threonine	20.9	608 ± 20.2	2.1

[a] Levels of amino acids used: L-lysine HCl, 0.25%; DL-methionine, 0.20%; DL-threonine, 0.20%. [b] Average initial weight: 42 g. 20 chicks/group. Each group had 2 replications of 10 chicks each. [c] Calculated from the feed consumed/group. [d] Standard deviations.

When common corn was supplemented with lysine and tryptophan, an improvement was obtained over unsupplemented common corn, but final weight did not reach weight levels obtained from opaque-2 corns. Sorghum grain gave the poorest weight gains. These results also show that with this type of test, cottonseed flour protein has a higher quality than

Table VIII. Final Weight of 15-Day Old Chicks Fed Various Feeds as Sole Source of Protein

Protein Source	Experiment No. 1 No. of Live Chicks	Av. Final Wt., g.[a,b]	Feed/Gain[c]	Experiment No. 2 No. of Live Chicks	Av. Final Wt., g.[d,e]	Feed/Gain[c]
Opaque-2 corn	15	580 ± 120.7[f]	3.3	18	446 ± 49.9[f]	3.5
Opaque-2 corn + DL-methionine	15	567 ± 92.8	3.5		...	...
Common corn	11	286 ± 51.9	7.7	17	223 ± 28.3	8.2
Common corn + lysine + tryptophan	18	393 ± 83.3	5.3	17	332 ± 59.2	5.0
Cottonseed flour	18	409[g] ± 97.5	4.2	18	262[h] ± 31.2	6.1
Soybean flour	12	314 ± 102.9	2.9	15	261 ± 140.1	7.1
Wheat middlings	18	491 ± 121.6	4.3		...	...
Opaque-2 corn (Colombia)		...	...	18	402 ± 64.0	3.9
Sorghum		...	...	18	195 ± 27.5	11.3

[a] Average initial weight, 15 days of age: 208 g. 20 chicks/group. Each group had 2 replications of 10 chicks each. [b] 4 weeks. [c] Calculated from the feed consumed/group. [d] Average initial weight, 15 days of age: 147 g. 18 chicks/group. Each group had 2 replications of 9 chicks each. [e] 5 weeks. [f] Standard deviations. [g] Human grade cottonseed flour. [h] Cottonseed meal–42.7% protein.

soybean, although in other studies performance of the two was essentially the same.

DISCUSSION

The relatively rapid growth of baby chicks requires a high level of dietary protein (Ewing, 1963). This limits its use as a test animal for the evaluation of protein quality of foods of low protein content, such as cereal grains. Thus, the practice has been to provide a basal level of protein to which the protein under test is added. This technique, however, does not measure the protein quality of the food under test, but rather, measures its supplementary effect.

An indication of the nutritive value is obtained, however, since the response is compared to that of an appropriate control. This is particularly true when the protein base has a deficient amino acid pattern which can be corrected to a certain degree by the material tested.

These considerations are apparent in the results presented. In the experiments in which the protein base of the basal diet was derived from a cottonseed flour, with a lysine content of 3.9 g./16 gN, opaque-2 caused weight gains similar to those from common corn. The reason could be that the opaque-2 corn used has a 4.0 g./16 gN of lysine, not increasing significantly the total lysine content of the diet, which was similar to the lysine content of the diets made with common corn. These values were for the opaque-2 and common corn, 0.81 and 0.80; 0.86 and 0.84; and 0.90 and 0.88% total lysine, respectively. That the common corn diets had the above levels of lysine in spite of the content of this amino acid, 3.7 g./16 gN, is explained by the higher amounts added of this corn, because of its lower protein content, to parallel levels of protein as those added from opaque-2 corn. The chicks fed the common corn diets consumed more feed, which could explain the better performance obtained. Since these diets contained more corn flour than those made with opaque-2 corn and less glucose, an experiment was carried out to learn whether the type of carbohydrate could explain the difference. The results, however, were similar. Therefore, factors other than amino acid balance may have been responsible for the results obtained.

That lysine in the protein of the basal diet is of importance is apparent by the results obtained with the cottonseed flour with 1.53% available lysine, the sesame flour with 1.06%, and the corn gluten with 0.18%. All these proteins contained less lysine than opaque-2 corn; therefore addition of opaque-2 corn induced better weight and over-all performance than common corn.

It may be concluded, therefore, that in the studies in which soybean protein was used in the basal diet (Cromwell *et al.*, 1967b), no response was obtained at protein levels above 16% in the diet, in favor of opaque-2 corn because soybean protein has 5.3% lysine, an amount which is higher than that in opaque-2 corn. Better responses were found with opaque-2 corn as compared to normal corn at dietary protein levels below 15–16%.

In a cottonseed flour–corn mixture, the first limiting amino acid is lysine, as indicated by the results in the present studies and by previous results obtained in rats (Bressani and Elías, 1962, 1969).

The present results show that methionine is the second limiting amino acid when the corn in the cottonseed flour–corn diet is common corn, but not when it is opaque-2 corn. These findings suggest that in a soybean–opaque-2 or common corn diet, methionine was limiting (Cromwell *et al.*, 1967b) not because opaque-2 corn is deficient in this amino acid, but because methionine is the first limiting amino acid in soybean protein (Bressani and Elías, 1968a).

It is also of interest to note that if common corn is supplemented with its limiting amino acids, lysine and tryptophan (Bressani *et al.*, 1968), added to cottonseed flour, a better performance than that from opaque-2 corn is obtained, probably because the levels of these two amino acids in the common supplemented corn are higher in the diet than those coming from opaque-2 corn alone.

The results obtained when opaque-2 corn provided the sole source of protein definitely indicate that its overall amino acid pattern is very good, even better at the protein level tested than cottonseed or soybean flour, sorghum protein, common corn with or without amino acid supplements. The mortality of chicks in this type of study was high for some groups, probably because of the relatively low level of protein in the diets used in comparison with the protein needs of chicks of this age. A low level of protein in the diet magnifies amino acid deficiencies from which the chicks died. Because of this, all results per group were pooled together for their analysis. In any case, in both studies the responses to the different proteins were the same.

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Received for review August 1, 1969. Accepted December 3, 1969. INCAP Publication I-506.