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Effect of Corn Trypsin Inhibitor on Growth of Rats

Although the opaque-2 gene approximately doubles the lysine and tryptophan contents of corn, it also greatly increases trypsin inhibitor content. To determine the nutritional effect of the inhibitor, it was isolated and added to a 10% casein diet at a level equal to 80% of opaque-2 corn in the diet. The inhibitor did not decrease growth or cause pancreatic hypertrophy. If rats and humans respond similarly, the higher level of inhibitor in opaque-2 corn should not detract from the benefits of the higher lysine and tryptophan contents.

Corn often is the principal component of the diets of people living in rural areas of tropical and subtropical regions (CIMMYT, 1975). However, such diets tend to be of poor nutritional quality because protein of locally grown corn is low in lysine and tryptophan. Following the discovery that the opaque-2 gene approximately doubles lysine and tryptophan levels in the corn endosperm (Mertz et al., 1964), corn strains were developed that contain the gene and are adapted to those regions, thus making available corns which can improve the nutritional status of the people of the regions (Bressani, 1966).

Corn containing the opaque-2 gene also contains about twice as much trypsin inhibitor as does normal corn (Mertz, 1972; Halim et al., 1973). The nutritional effects of the inhibitor are not known. However, soybean trypsin inhibitor depresses rat growth and causes pancreatic hypertrophy (Rackis, 1965; Kakade et al., 1973). Fortunately, the soybean inhibitor is inactivated when soybean products are cooked. Isolated corn trypsin inhibitor in water solution is only slowly inactivated when refluxed for several hours (Chen and Mitchell, 1973), and may survive rapid cooking processes applied to corn products. If corn trypsin inhibitor should possess adverse nutritional properties, the higher level in opaque-2 strains might reduce the nutritional advantages of the higher lysine and tryptophan contents; if so, efforts should be made to reduce inhibitor content genetically without reducing lysine and tryptophan contents. We report a rat growth study designed to assess the nutritional effects of the inhibitor.

METHODS

An opaque-2 strain of corn was finely ground, defatted with acetone, and assayed for trypsin inhibitor content (Erlanger et al., 1961). The inhibitor was removed from 400-g batches of the meal by two 4-h extractions with 0.2 M NaCl. The extract was adjusted to 1 M NaCl, 0.05 M Tris, 0.02 M $CaCl_2$, pH 8.2, and was centrifuged 30 min at 10 000*g*. The extract was percolated through a trypsin affinity column (Loeffler and Pierce, 1973), and the column was washed with a buffer composed of the same salts and of the same pH as the extract. Washing was continued until absorbance of the eluate at 280 nm was below 0.05, thus eliminating noninhibitor proteins. The column then was washed with 300 ml of 0.01 M HCl to elute the inhibitor. The inhibitor was concentrated to about 100 ml in dialysis tubing suspended in front of an electric fan, and the concentrate was freeze-dried. The isolate was highly active, 0.2 mg causing 45% inhibition of trypsin in the Erlanger assay procedure.

Table I. Effect of Trypsin Inhibitor from Corn on Growth of Rats

Control			Inhibitor		
Rat no.	4-week gain, g	PER	Rat no.	4-week gain, g	PER
1	166	3.25	6	134	3.11
2	150	3.04	7	129	3.05
3	95	2.39	8	136	3.29
4	127	2.95	9	156	3.33
5	141	3.08	10	134	3.15
Av	136	2.97		138	3.19

It was established from the initial assay that the opaque-2 corn contained 1325 mg of inhibitor/kg, about twice the amount in normal corn. It was assumed the diets of people in the rural areas of developing regions might consist of 80% corn products. Therefore, the isolated inhibitor was added at a rate of 1060 mg/kg to a purified rat diet containing 10% protein (supplied by casein), vitamins, and minerals calculated to meet the requirements (National Academy of Sciences, 1972), and corn starch–glucose (3:1) to complete the diet. Charles River strain male albino rats were weaned at 21 days and fed commercial laboratory diet until 24 days old. Five randomly selected rats per group (initial weight, 58 to 62 g) were fed the diet with or without the trypsin inhibitor for 4 weeks.

Neither average weight gains nor PER's (Table I) were significantly different ($P = 0.05$). No hypertrophy of the pancreas was observed. Thus, we conclude that the higher levels of trypsin inhibitor in corn containing the opaque-2 gene are not deleterious to rats. If humans and rats respond similarly, the higher levels of inhibitor associated with the opaque-2 gene should not detract from the

benefits of the higher lysine and tryptophan contents.

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Modified Procedure for Purification of Ethoxyquin and Ethoxyquin Nitroxide

The commercial silica gel used originally for the column separation of ethoxyquin nitroxide (Lin, J. S., Olcott, H. S., *J. Agric. Food Chem.* **23**, 798 (1975)) is no longer available. The present procedure depends upon use of a neutralized gel which may have applicability in the chromatography of other stable free radicals.

The procedure used by Lin and Olcott (1975) to separate the free radical, ethoxyquin nitroxide, from other products of the oxidation of ethoxyquin has had to be modified because the silica gel originally used for the column chromatography (Silic AR CC7, 200–325 mesh, Mallinckrodt) is no longer available. The following procedure for preparing a suitable gel yielded satisfactory columns. To 500 g of silica gel (Bio-Sil A, 200–400 mesh, Bio-Rad) in a 2000-ml round-bottomed flask was added, in portions and with stirring, 1500 ml of methanol in which had been dissolved 5 g of sodium bicarbonate and 0.5 g of EDTA disodium salt (Matheson). The methanol was removed by filtration (coarse fritted disk) and the product dried at 100 °C in a draft oven.

Purified ethoxyquin for the synthesis was obtained from technical grade ethoxyquin (Santoquin, Monsanto) by chromatography on a column of the silica described above with chloroform–hexane (1:1). Ethoxyquin eluted as a yellow fraction; yield about 80%. The red material remaining on the column has not been characterized. Ethoxyquin was oxidized as described previously (Lin and Olcott, 1975) for 1.5 h and fractionated on a column of the above silica with chloroform–hexane (2:1) (column size, 450 mm × 25 mm, 65 g dry silica, eluent rate, 2.5 ml/min). A first fraction was colorless, the second was unchanged ethoxyquin, and the third was deep red ethoxyquin nitroxide; yield about 35%. By thin-layer chromatography (13179 silica gel, Eastman) with chloroform–hexane (2:1), ethoxyquin had R_f 0.66 and the nitroxide had R_f 0.33. The column effluent nitroxide fraction gave a single spot but after the solvent was removed by vacuum evaporation, the residue showed two spots only one of which was unchanged nitroxide.

Ethoxyquin nitroxide is unstable in acid media; hence it was necessary to use a silica gel which had been neutralized. The treated gel was in the pH range 6.8–7.2. The same procedure may be useful in the isolation of other free radicals that are unstable in acid media (Forrester et al., 1968).

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