Studies on protein quality of flint phenotypes of opaque-2 modified maize[1]

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SUMMARY

The development of a maize which combines a flint (crystalline) endosperm with enhanced protein quality appears to be possible. The quality of a flint selection has been confirmed in laboratory analyses, rat feeding trials, and studies of nitrogen balance in children. Priority must be given to this work in breeding and nutrition programs in the lowland tropics where quality protein is so badly needed.

The introduction of the opaque-2 gene into highly productive maize hybrids has provided one of the most significant changes in protein quality in recent years. This potential for production of a large quantity of high quality protein can provide a solution to many human nutrition problems in the developing tropics, as well as assure a quality substitute for expensive concentrates in the production of poultry and swine. Incorporation of this gene into adapted commercial hybrids is a realty in Colombia and several other countries, and the performance of these hybrids has been documented (1, 2, 3).

Wide acceptability of this new maize has been limited to date in the middle altitudes and lowlands of Colombia by the floury endosperm which is virtually unknown outside the highlands (4).

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There is an inmediate and critical need for the development of maize with improved protein quality, combined with a crystaline (flint-type) endosperm which would be readily accepted by the consumer and would pass easily through market channels. Commercial harvests of the yellow endosperm, double cross opaque-2 hybrid H. 208 and white H. 255 were observed to include a range in endosperm type from the predominant class of opaque kernels through intermediates to almost completely flint types (5). About 30% of the kernels of the H. 208 and 10% of H. 255 have a hard appearance; however H. 255 protein quality decreases when hard patches appear whereas the 208 remains fairly constant. Semi-flint and flint kernels were selected from harvests of H. 208 and tested in the laboratory and in weanling rats in the present study. In addition, standard nitrogen balance studies were performed in children in the Metabolic Unit of the University of Valle Hospital. Results of these several tests are summarized in this report.

MATERIALS AND METHODS

Kernels from commercial harvests of the yellow opaque-2 hybrid ICA-H.208 were separated on an illuminated table into two samples: completely opaque grains and essentially crystalline (flint) grains. These samples were tested in the laboratory for protein, lysine and tryptophane contents, and compared to normal maize. For the purpose of this study only kernels more than 75% crystalline endosperm and pure flouries were selected by hand. Germ was eliminated manually and only the endosperm used for the studies. Aliquots were taken for proximal analysis. The endosperm flour was properly mixed for diet preparation.

The method of Campbell (6) was used in the feeding of weanling rats with experimental diets based on normal, (H. 207), H. 208 opaque, and H. 208 flint maize.

Eight rats were assigned to each treatment, with the exception of the crystalline (flint) H.208 selection which had 16 rats. Data from three rats were not included in the analysis, since they showed gains enterely non-representative of their respective groups. The three maize types were incorporated

into diets, and a control diet of casein and corn starch included in the experiment.

A vitamin and salt fortification mixtures (NBC), and oil, were included in all diets (7). The percentage composition of these diets is presented in Table 1. These diets were formulated and balanced to contain 10 percent protein, however actual laboratory analysis of the diets reveled a substantially lower level, as shown in the table. These actual protein levels were used to calculate corrected PER values.

TABLE 1
PERCENTAGE COMPOSITION OF FOUR EXPERIMENTAL DIETS FED TO WEANLING RATS

	H. 208 floury	H. 208 flint	H. 207 flint	Casein
H. 208 floury	88	--	--	--;
H. 208 flint	--	88	--	--
H. 207 flint	--	--	88	--
Casein	--	--	--	11
Corn starch	--	--	--	73
Corn oil	6	6	6	10
Vitamin fortification mixture (NBC)	2	2	2	2
Salt fortification mixture USP XIV (NBC)	4	4	4	4
Protein content	7.9	7.6	8.4	6.0

Feeding was administered as a formulated diet with 1.0 g protein and 100 calories per kg body weight per day. Carbohy-

Standard nitrogen balance studies were performed in three repleted children (36 months of age) in the Metabolic Ward. drate and fat contents of the diets were 55 and 35% respectively, of the total calorie intake.

The daily diet was divided into five equal meals. The patients received first a protein free diet for 3 days in order to establish the level of endogenous nitrogen. Test protein diets were administered for 10 days. After three days of diet adaptation, five two day periods were used for calculations. An oral marker was given at 8 a.m. at days 1, 3, 5, 7, 9, 11 and stools were separated accordingly. Diet, refusals and urine were separated in the same days. Duplicates of the two diets refusals, urine and stools were analized for nitrogen (Kjeldahl) and

fat (Van de Kramer). Each day urine was analized for creatinine. Based on nitrogen intake, urinary, fecal, and endogenous losses, the following parameters of protein quality evaluation were calculated: Digestibility (Digest.), Net Protein Utilization (NPU), Biological Value (BV), Nitrogen Retention per Day (NRet/Day).

RESULTS AND DISCUSSION

The results of laboratory analysis of the three types of maize are shown in Table 2. Protein content as well as lysine and tryptophan concentrations were not significantly different in the two types of grain (floury and flint) selected from commercial opaque hybrid (H. 208). Although the protein content of the normal maize (H. 207) was essentially the same, lysine content was substantially lower. Whole kernel analysis showed higher protein, lysine and tryptophan contents due to presence of the embryo (germ), but the

TABLE 2
LABORATORY ANALYSIS OF OPAQUE-2 SELECTIONS AND NORMAL MAIZE FOR PROTEIN, LYSINE AND TRYPTOPHAN

Grain type	ENDOSPERM					
	Protein gms %	Lysine mgm % of	Tryptophane protein	Protein gms %	Lysine mgm % of	Tryptophan protein
H. 208floury	9.2	3.9	0.89	11.1	4.7	1.04
H. 208 flint	10.0	3.7	0.75	12.0	4.2	0.87
H. 207 flint	9.9	2.5	0.40			

two selections are still similar. These results demonstrate that it is possible to combine a flint type endosperm with the quality characteristics needed in maize.

The average weight gain, food consumption, and efficiency in rats is presented in Table 3. PER values have been calculated using the actual protein contents of each diet (as shown in Table 1). A final calculation was made to relate the results of each diet as percent of the results with the casein control used as 100 (Percent of casein). Although the flint selection from H. 208 showed a lower PER value than the selected opaque sample, the efficiency of this selection was almost twice that of normal maize. Both selections were quite acceptable when compared to the casein control.

TABLE 3

AVERAGE GAIN, PROTEIN CONSUMED AND PER VALUES IN RAT FEEDING STUDY (28 DAY STANDARD TEST)

	H. 208 floury	H. 208 flint	H. 207 flint	Casein
Gain gms	84	62	26	69
Protein consumed	26.1	21.9	18.2	18.7
PER	3.21	2.81	1.43	3.68
PER standard	2.18±0.06	1.91±0.03	0.99±0.02	2.50±0.04
PER as a percent of casein	87.2	76.4	39.4	100

TABLE 4

COMPARATIVE NITROGEN BALANCE IN 3 CHILDREN

	H. 208 floury	H. 208 flint	H. 207 flint	Casein
Offered	175.0	175.0	175.0	175.0
Intake	160.0	159.3	160.0	160.0
Absorbed	145.5	138.0	121.8	156.0
Retained	110.5	109.0	57.2	120.2
Digest.	91	87	78	98
NPU	69	65	36	75
B.V.	76	75	47	77

* Each value is the average in mg of N per Kg per day of fifteen, 2 day periods.

Results from the nitrogen balance studies in children are summarized in Table 4. The intake and balance are shown in the upper half of the table, while calculated digestibility, net protein utilization, biological value, and N retention per day are present in the lower half. Values of the two selections from H. 208 are similar, with the flint type slightly lower in digestibility and net protein utilization than the opaque selection. They are essentially identical in N retention per day and biological value. Although lower than the casein control, these H. 208 selections are definitely superior to the H. 207 normal hybrid maize.

CONCLUSIONS

Results of these studies on selected samples of modified grain from the commercial opaque-2 hybrid H. 208 show that crystalline kernels which contain the opaque-2 gene are almost equal in biological value to the maize normally classifed as "opaque". The results demonstrate that an increased level of lysine and tryptophan in maize is not completely and irrevocable associated with an opaque (floury) endosperm. The improved quality of the crystalline H. 208, when compared to normal H. 207, is due to reduced zein content (8). It will probably be necessary to incorporate the opaque-2 gene into a wide range of genetic backgrounds to search for new and more effective modifying factors.

RESUMEN

Estudios sobre calidad de proteína de los fenotipos duros de maíces modificados por el Gene Opaco-2

El desarrollo de un maíz que combina un endospermo duro (cristalino) con la calidad mejorada de proteína parece ser un hecho factible. La calidad de una selección dura ha sido confirmada por un análisis de laboratorio, ensayos biológicos con ratas y estudios de balance de nitrógeno en niños. Debe dársele prioridad a este trabajo en las tierras bajas de los trópicos, en donde una buena calidad de proteína es tan necesaria.

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