AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
Batch 97

1. SUBJECT CLASSIFICATION

A. PRIMARY: Food production and nutrition — AF30-0120-0000

B. SECONDARY: Plant breeding--Cereals--Maize

2. TITLE AND SUBTITLE

Progress in developing maize with improved protein quality

3. AUTHOR(S)

Bauman,L.F.; Mertz,E.T.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1974	10p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Purdue

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

(In Agr.Experiment Station. Research bul.914)

9. ABSTRACT

10. CONTROL NUMBER

PN-AAG-304

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Maize
Nutrition
Proteins

13. PROJECT NUMBER

931052400

14. CONTRACT NUMBER

CSD-2809 Res.

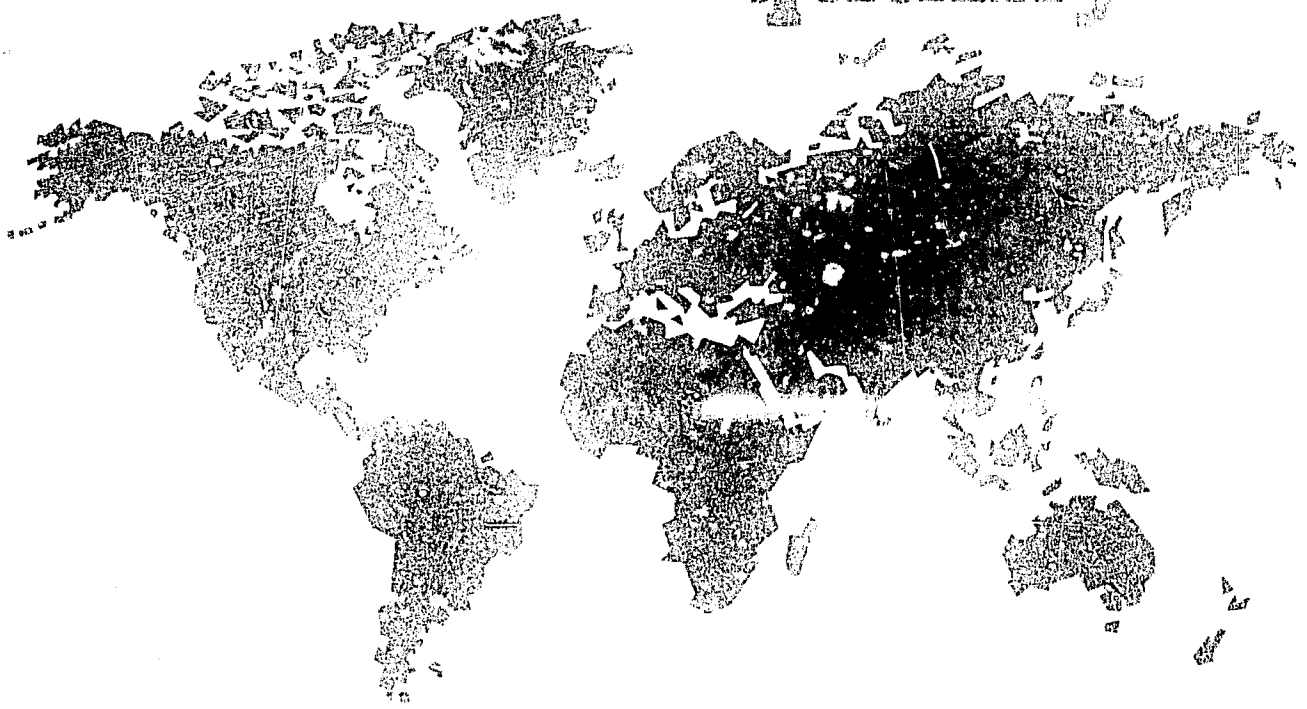
15. TYPE OF DOCUMENT

RESEARCH BULLETIN 914
OCTOBER 1972

progress in developing maize with improved protein quality

PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
WEST LAFAYETTE, INDIANA

progress in developing maize with improved protein quality



L. F. Bauman, Department of Agronomy
E. T. Mertz, Department of Biochemistry
D. V. Glover, Department of Agronomy
P. L. Crane, Department of Agronomy
Purdue University

Supported by the Agency for International Development, U.S. Department of State, under contract AID/csd 2809, "Inheritance and Improvement of Protein Quality and Content in Maize."

The protein in ordinary maize has relatively low nutritional value; and early attempts to improve its quality were both unsuccessful and generally regarded as impractical. In 1964, however, Mertz, Bates and Nelson reported that the *opaque-2* gene improved protein quality markedly by increasing the content of the nutritionally-limiting amino acids lysine and tryptophan. Subsequently, they found that the *floury-2* gene was intermediate between normal and *opaque-2* and had an increased methionine content. More recently, high lysine mutants have also been identified in sorghum and barley.

Nutritional studies have confirmed the potential value of *opaque-2* maize in helping meet the world's human and animal nutritional needs. And, many maize breeding researchers throughout the world have initiated programs to develop *opaque-2* hybrids or varieties.

Today it is apparent that to achieve acceptability and utilization, *opaque-2* types must, to some degree, overcome their generally-observed deficiencies, when compared with normal maize. These deficiencies include: (1) lower yield, although this gap has been narrowed through breeding; (2) greater susceptibility to ear rot; (3) harvest and storage problems, including grain insect damage, and (4) soft endosperm, which is unacceptable as human food for some areas or uses.

In light of increased breeding work over the past several years and the time lag associated with publication in scientific journals, it seems appropriate to present here a brief report of the development of high quality protein maize. This report will discuss first the breeding and genetics, and then the analytical and nutritional aspects. Some of the information is preliminary, and some ideas or conclusions will, no doubt, be modified or changed as more information becomes available.

Current Utilization

A recent worldwide survey indicated some limited production of *opaque-2* maize in Brazil, U.S.S.R. and the United States and very minor use in several other areas (e.g., Colombia, Nigeria and Vietnam). This survey also indicated that grain insects and grain quality, especially in tropical areas, were the most serious problems. Nearly all countries rated the future prospects of *opaque-2* as good to excellent, with only one country indicating poor prospects.

More rapid utilization of high lysine maize has also been limited by economic considerations involving yield and relative price of protein supplement. Economic factors vary greatly in different countries and are too complex to treat in a meaningful way here. However, it can be said that in the developing countries, economic factors are generally not of great significance, and acceptability and grain quality are the more important limiting characteristics of *opaque-2* varieties.

breeding & genetics

Backcross Conversion

The immediate approach has been to backcross the *opaque*-2 gene into inbreds or varieties currently used to produce normal maize. To save time, conversion is generally accomplished by a continuous backcrossing procedure. After the first backcross, the plants are selfed, and pedigreed backcrosses are made with individual plants to the recurrent parent as female. At harvest, the selfed plants are classified as to which are segregating for *opaque*-2. Then, only the backcrosses to the recurrent parent involving these segregating plants are continued into the next generation. Because of the rapid conversion to the recurrent inbred genotype, it is difficult to select effectively for modifiers or interactions.

The *opaque*-2 gene interacts with different genetic backgrounds. Therefore, as opaque hybrids or varieties are reconstituted, some perform poorly with regard to yield and/or grain quality (other than nutrition), while others are more nearly equal to normal hybrids. Yields of the opaque hybrids range from 80 to 90 percent or more of normal hybrids. Therefore, development of varieties with satisfactory performance does appear feasible. In fact, based on limited use in several countries, farmer acceptance of these "first generation" opaque varieties has been somewhat better than expected.

Introduction of *opaque*-2 into varieties or synthetics can follow the same procedure used for inbred conversion, except larger populations are required to insure that the germplasm is adequately sampled. This is particularly true of the final backcross. The genetic variations should enable the breeder to visually select for opaque types with better grain quality.

Improving performance of opaque genotypes in widebase germplasm populations with some sort of cyclic selection procedure, concurrent selfing and selection for modified or vitreous types, and selection for protein quality offers a promising long-range method to develop acceptable types.

Yield Testing Procedures

Small-plot yield comparisons of opaque and normal maize or check hybrids must involve some control to prevent the opaques from outcrossing with pollen from the normal entries. Since the *opaque*-2 gene is recessive, outcrossing to normal results in normal phenotype and amino acid profile. Hand subbing of yield test plots is generally not feasible. Therefore, the most practical method is to detassel the normal entries, being careful not to remove any leaf area which would reduce yield. Outcrossing of normal entries with *opaque*-2 has little or no effect on their yield.

Outcrossing of opaque hybrids to an unrelated opaque pollen source may result in a slight kernel weight increase and an upward yield bias. This, from a practical standpoint, is difficult to control. Therefore, the researcher must keep this possible bias in mind when interpreting his results. Protein and lysine values are not generally greatly affected by outcrossing to unrelated opaque pollen sources.

Comparisons of genetically-identical sibbed *opaque*-2 kernels produced on nonsegregating ears and on ears segregating normal kernels have revealed no effect on kernel weight or protein. Therefore, in breeding programs, characteristics of *opaque*-2 kernels in segregating and homozygous families should be comparable.

Other Effects of Opaque-2

Some additional characteristics of *opaque*-2 phenotypes are:

1. Percent oil is higher than in normal maize. This is because the germ of opaque types comprises a larger percent of the kernel and is actually heavier than in the normal kernel. Apparently, the reduced starch deposition in the endosperm is compensated for, to some extent, by larger germs.

2. Seedling vigor may be poorer than normal counterparts, particularly under cold and wet conditions during germination.

3. Cob weight of opaque maize averages about 10 percent less than normal maize.

4. Percent protein in *opaque*-2 types tends to be slightly lower than that in their normal counterpart.

Genes Modifying Opaque-2 Phenotype

In 1967, Purdue undertook a population approach to improve *opaque*-2 which involved recurrent selection. In the program, the selfed segregating families (ears) from a synthetic showing the least difference in kernel weight (expressed as percent between normal and opaque kernels) were selected and intercrossed for the next cycle. After three cycles, the *opaque*-2 kernel weight (expressed as percent of normal) was 94.9, compared to 88.5 for the control, a significant improvement. However, this was disappointing since the improvement was due primarily to reduce kernel weight of the normal kernels. It should be emphasized that little selection for kernel weight *per se* was practiced, and the effect on total yield was not determined.

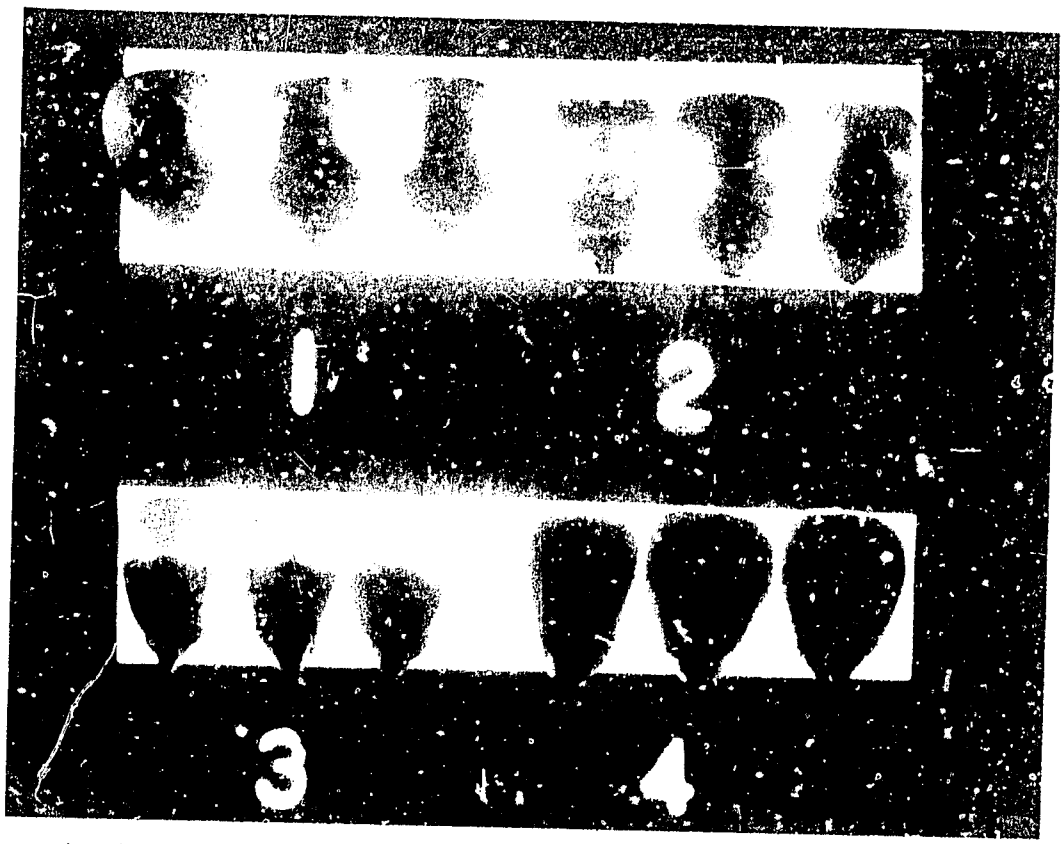


Figure 1. Light transmission with modified opaque types: (1) saddle, (2) diffuse, (3) [illegible], and (4) standard opaque.

Another and perhaps more promising approach to modifying the *opaque*-2 phenotype involves visual selection of more specific modifier genes in segregating families. The kernel expression of the modified types (Figure 1) observed by Purdue, the International Center for Maize and Wheat Improvement (CIMMYT) and others might be roughly classified thusly:

1. Vitreous endosperm that is nearly indistinguishable from normal.

2. A [illegible] phenotype with top or crown part normal or vitreous and the base part opaque. (This is the most frequently occurring type.)

3. A saddle pattern, where the soft or opaque starch is limited to the central portion of the abgerminal side of the kernel.

4. A sectored or diffuse type where soft starch is distributed irregularly in otherwise vitreous starch.

These modifier genes, acting on the expression of *opaque*-2, are heritable, tend to be recessive, probably involve more than one gene, and give variable expression with various environments. The modified types tend to have poorer protein quality than the standard *opaque*-2 phenotype. However, selection can maintain protein quality at a satisfactory level.

These modifiers may be useful in providing more acceptable types for certain traditional human food uses and in improving grain quality, expecially insect and ear rot resistance, for humid tropical areas. CIMMYT has developed some modified *opaque*-2 varieties that offer considerable promise. Selection for modified endosperm does not necessarily improve yield. Some problems may arise, however, in detecting outcrossing in seed production and in maintaining its opaque identity in market channels. The ninhydrin test (discussed later) may provide a simple and quick means of identification in the breeding programs and the market place.

Potential of Floury-2

Tests of *floury*-2 hybrids indicate that yield reduction compared to normal is not so great as it is for *opaque*-2 hybrids. *Floury*-2 grain quality also appears to be somewhat better than *opaque*-2. Repeated observations at Purdue confirm that the *floury*-2 lysine values are less than for *opaque*-2. Selection for specific modifier genes is also difficult with *floury*-2, since this gene shows a semi-dominant or additive gene action in the endosperm dosage series.

New High Lysine Mutant Opaque-7

Dr. K. S. McWhirter (University of Sydney, Australia) has found a new endosperm mutant designated *opaque*-7, which increases lysine and tryptophan in the endosperm. However, potential for *opaque*-7 is not promising because lysine values are intermediate between normal and *opaque*-2 types, and the segregation of the gene is difficult to identify in some genetic backgrounds.

Potential of Sugary-2 Opaque-2 Double Mutant Combination

Purdue has explored the interactions of several endosperm mutant genes in double mutant combination with *opaque*-2 and other genes for possible improvement of the acceptability and nutritional quality of high lysine maize. A promising approach to modifying the *opaque*-2 phenotype involves the mutant combination *sugary*-2 *opaque*-2.

In repeated experiments comparing several near isogenic hybrids, *sugary*-2 *opaque*-2 kernels have more vitreous endosperms and greater kernel density than *opaque*-2 alone. In some backgrounds the double recessive is nearly normal in endosperm texture. We have observed that *sugary*-2 *opaque*-2 ears are less susceptible to ear rot than *opaque*-2 types. And *sugary*-2 *opaque*-2 kernels have slightly higher lysine and protein values than *opaque*-2 counterparts. Evidence indicates that the double recessive has increased oil content and, therefore, possibly greater energy potential (calories). It has also been observed that the kernel volume of the double mutant is less than normal, and as a result, yields are less than in the *opaque*-2 counterpart hybrids.

The starch of the *sugary*-2 *opaque*-2 endosperm is much more readily hydrolyzed by amylases than normal and *opaque*-2 types, suggesting superior digestibility. Nutritional studies at Purdue have shown in isonitrogenous feeding trials that weanling rats fed *sugary*-2 *opaque*-2 and *opaque*-2 diets had, respectively, average daily weight gains that were 2.9 and 2.5 times faster than rats fed the normal counterpart maize diet. Feeding experiments to determine the biological value of this double recessive type are currently being conducted with swine and cattle.

Attention must now be directed toward selecting for improved yield, with emphasis on increased kernel volume, weight and vitreousness in the conversion program. Introduction of *sugary*-2 into *opaque*-2 varieties or synthetics can perhaps best be done by maintaining the populations in a heterozygous condition for the *sugary*-2 gene while selecting. Selection for improved performance of *sugary*-2 *opaque*-2 genotypes in widebase germplasm populations may offer a promising long-range method to develop acceptable high lysine maize types.

Waxy and Double Recessive Waxy Opaque-2

There have been several reports that *waxy* maize may give better feed gains in cattle. However, a recent cattle feeding test at Purdue showed no advantage for *waxy* or *opaque*-2 compared to normal maize. The double recessive *waxy opaque*-2 phenotypes have higher lysine values than *opaque*-2 alone, although not as high as the lysine levels found in *sugary*-2 *opaque*-2. The double mutant *waxy opaque*-2 has an opaque phenotype. Our observations have been that *waxy* and and *waxy opaque*-2 are more readily digested by amylases than normal and *opaque*-2, but they are not as readily digested as *sugary*-2 and *sugary*-2 *opaque*-2.

Isonitrogenous feeding trial comparisons conducted at Purdue have shown that weanling rats fed a *waxy* maize diet do not gain any faster than rats fed a normal maize diet. On the other hand, rats fed a *waxy opaque*-2 diet gave faster gains than rats fed *opaque*-2. In the case of *waxy opaque*-2, the response was proportional to its level of lysine (based on protein). Feeding experiments are being conducted with swine and cattle to determine the biological value of the *waxy* and *waxy opaque*-2 types.

Double Recessive Floury-2 Opaque-2

Early reports that this double recessive might give a more normal kernel phenotype than either of the mutants singly have not been confirmed in subsequent observations. Presence of both genes does not result in higher lysine values than *opaque*-2 alone. Since the *floury*-2 gene shows a semi-dominant gene action in the dosage series, and because of the more complicated breeding procedures and greater difficulty in maintaining pure seed stocks and production with this double mutant, this approach is not recommended.

Breeding for Higher Protein Content

The *opaque*-2 gene tends to maintain increased lysine levels at higher protein content. In the long run, this makes it feasible to breed opaque varieties with improved protein content and quality. But the job will not be easy, because protein content is influenced greatly by environment, and heritability is probably not high. There also is a negative relationship between yield and protein concentration.

The correlation between percent protein and percent lysine is positive in *opaque*-2 maize. But the correlation between percent protein and lysine as a percent of protein is negative. The decision to breed for increased protein content may also be influenced by the availability and use of nitrogen fertilizer in the area concerned.

analysis

Figure 2. The darker color in the one test tube and the darker stained kernels (actually deep blue) identify the high lysine kernels.

To make progress in developing high yielding and acceptable *opaque*-2 maize with good nutritional quality, the plant breeder must have rapid, reliable screening procedures for determining both protein quality and quantity. The assay methods found most suitable are described here.

Total Protein

Total protein is usually determined by either the macro- or micro-Kjeldahl method. The amount of protein can also be determined rapidly and fairly accurately by analysis of the reflectance spectrum in the near infrared of a finely ground sample. Instruments using this principle are commercially available.

The rapid biuret method of Craney and Johnson cannot be used to determine total protein in *opaque*-2 maize, and presumably of high lysine mutant cereals.

Total Amino Acids

A complete quantitative amino acid profile on certain promising selections can be obtained by hydrolyzing the protein and separating the amino acids by ion exchange chromatography.

Tryptophan

In most maize samples, colorimetric determination of tryptophan levels in the endosperm is sufficient as an initial screening procedure for protein quality. Endosperm samples containing up to 0.5 grams tryptophan per 100 grams protein are classified as normal quality; samples containing 0.6-1.3 grams may be classified as superior.

Lysine

Usually, lysine value of the endosperm is about four times higher than the tryptophan value. For a quantitative lysine value determination, a simple colorimetric method for lysine can be used.

Rapid Ninhydrin Color Test for High Lysine Mutants

High lysine mutants of maize, sorghum and barley contain higher levels of free amino acids than their normal counterparts. Ninhydrin rapidly detects this difference, as shown in Figure 2. To identify segregating ears of maize, the crown on a row of kernels of an ear can be removed and the cut surface stained with ninhydrin. The high lysine kernels show a darker blue color than the normal kernels.

Other Rapid Procedures

The dye-binding method, for example, is being used for screening in some maize breeding programs; but neither it nor the ninhydrin method effectively indicates the precise levels of lysine in *opaque*-2 segregating populations.

On the average, in ion exhange and colorimetric whole kernel analysis, normal maize gives values of 0.4-0.6 grams tryptophan and 2-3 grams lysine per 100 grams protein, whereas *opaque*-2 maize gives 0.8-1.2 grams tryptophan and 4-5 grams lysine. Normal endosperm gives values of 0.3-0.5 grams tryptophan and 1.5-2.5 grams lysine per 100 grams protein, while *opaque*-2 endosperm values measure 0.7-1.2 grams tryptophan and 2-4 grams lysine.

Biological Assay

Final proof of improved protein quality revealed by chemical analyses must depend on biological assay. In a comparative feeding study in 1973 involving four universities (Nebraska, Washington State, Pennsylvania and Purdue) and CIMMYT, the young white rat, the mouse, the chick and the meadow vole were compared as test animals to detect differences in protein quality between several cereals (*opaque-2*, *floury-2* and normal maize, sorghum, wheat and triticale). The Association of Official Analytical Chemists (AOAC) method for determining protein efficiency ratio (PER) was used.

Results of these cooperative studies showed that the male weanling rat gave the best differentiation among the cereals. Only the young rat gave satisfactory PER values. The AOAC method requires 10 male weanling rats on test for 28 days. However, significant differences in PER were demonstrated in 7 days. A 7 day test of *opaque-2* for preliminary evaluations require approximately 600 grams of the cereal grain.

Details on the chemical and biological methods above can be found in a companion brochure entitled "Simple Chemical and Biological Methods Used at Purdue to Screen Cereals for Protein Quality."

nutrition

Studies at Purdue have shown that young white rats fed a 95 percent *opaque-2* maize diet gain weight nearly four times faster than rats fed a 95 percent normal maize diet. Tests with weanling pigs produced similar results (Figure 3). It was also found that pigs weighing 125 pounds or more can be fed opaque maize plus a vitamin and mineral mix without a protein supplement and gain as fast as animals on a balanced maize-soybean meal diet. The ration must contain at least 11 percent total protein.

In Colombia, test on pigs at the International Center for Tropical Agriculture (CIAT) have shown that *opaque-2* maize can be used as the only dietary source of protein during the finishing, pregestation and gestation periods of a pig's life cycle without reducing pig performance. *Opaque-2* maize alone is not an adequate protein source for baby pigs, growing pigs or lactating sows. It must be supplemented with some protein and/or lysine to produce optimum performance. However, less supplemental protein is needed than with normal maize.

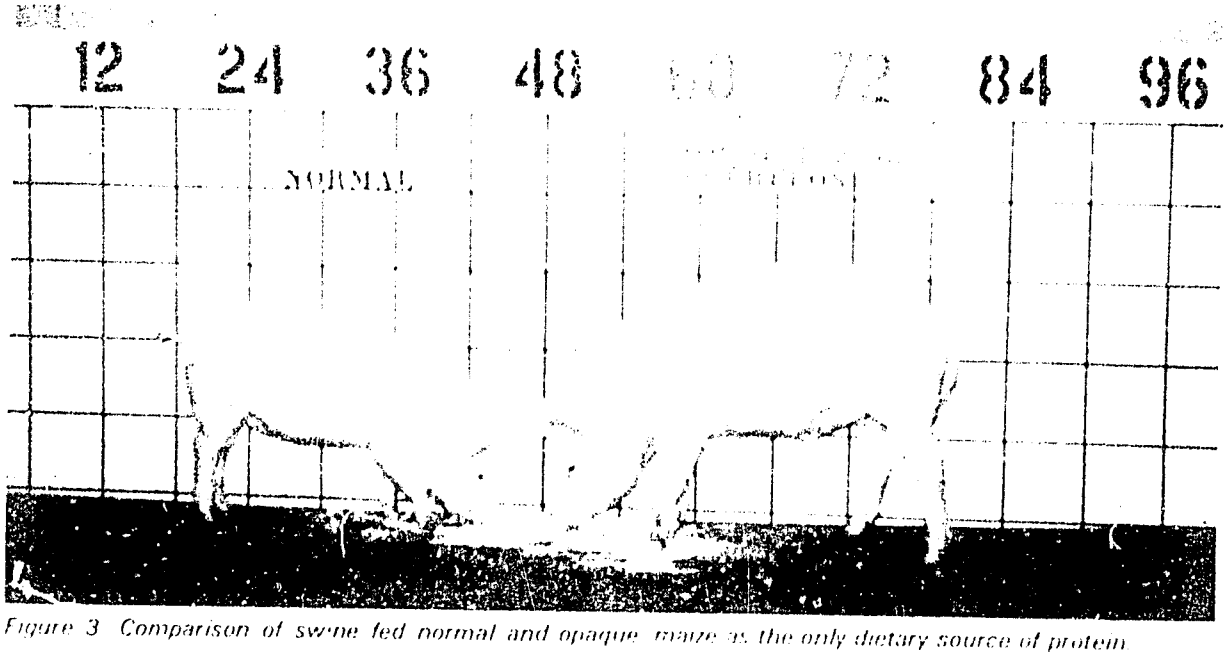


Figure 3. Comparison of swine fed normal and opaque maize as the only dietary source of protein.

Experience with feeding *opaque*-2 maize in the U.S. Corn Belt shows that pigs find *opaque*-2 maize more palatable than normal corn. Farmers are therefore advised to continue to feed *opaque*-2 maize to market weight. Pigs taken off *opaque*-2 maize and placed on normal maize refuse to eat for several days, thus reducing gains and feed efficiency.

Opaque-2 maize has a high nutritive value for humans. Work with young children in Guatemala and Colombia demonstrates that it is equivalent to skim milk when fed at an adequate level in the diet. It can cure kwashiorkor, a severe protein deficiency disease, when it is the only protein source. Although we have found no published feeding tests on the value of the protein in immature *opaque*-2 maize, it is assumed that such maize would be superior to immature normal (roasting ears), because the protein differences in these two types can be demonstrated 15 days after pollination.

It is well known in animal nutrition that acceleration of the growth rate by increasing the first limiting nutrient may accentuate the deficiency symptoms caused by a marginal intake of any other nutrient. Thus, faster growth rates produced by replacing normal maize with *opaque*-2 maize may increase the daily requirements for minerals and vitamins. Therefore, *opaque*-2 maize should always be used in animal and human diets with adequate amounts of minerals and vitamins. If deficiencies exist, supplementation of the diet with the appropriate micronutrient will be required.

Nutritional studies on *floury*-2 maize, although limited, suggest that its response will be in proportion to its level of lysine (based on protein). Usually the lysine level of *floury*-2 maize is about half-way between that of opaque and normal. Nearly all varieties of *floury*-2 maize observed are nutritionally inferior to the opaque varieties.

summary

In the area of breeding for improved quality protein in maize, tentative conclusions reached so far are as follows:

1. Based on lysine levels and feeding trials, *opaque*-2 seems more promising than *floury*-2 or the double recessive *opaque*-2 *floury*-2.

2. Breeders can use genetic background interactions and more specific modifiers to develop varieties that may be satisfactory in terms of their nutritional advantage, yield and a vitreous kernel type where required for consumer acceptability.

3. The double mutant *sugary*-2 *opaque*-2 shows promise for acceptance because of high kernel density and improved nutritive value; however, its yield is somewhat lower than *opaque*-2.

Breeders are cautioned not to rush the release of maize with improved protein quality until they are reasonably certain of acceptable performance for the specific area where production is to be encouraged.

At this time, the preferred analytical techniques for determining protein content and quality of maize are: (1) micro-Kjeldahl (and perhaps the infrared technique) for total nitrogen, (2) colorimetric methods for lysine and tryptophan, and (3) automatic amino acid analyses for complete amino acid profiles. For biological evaluation of a maize variety, the PER method with a 28-day rat feeding trial is recommended. Where amounts of grain are limited, a 7-day trial will give a useful preliminary evaluation as a screening tool for the plant breeder.