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9. ABSTRACT
This report includes analyses of the corn fortification project in Guatemala, the wheat fortification project in Tunisia, and the rice fortificarion project in Thailand. In the area of plant breeding, there are examinations of high lysine corn, wheat, high lysine sorghum, millets, barley, rice, and food legumes. Other discussions include the relative importance of protein and calories, the possibility of developing an adequate diet by modification of existing food patterns, and the question of whether yield can be maintained when protein levels in grains are increased through genetic manipulation. General rccommendations were: 1) Qualitative or quantitative improvements in cereal grains should be directed toward specific population groups. 2) Adequacy or availabilit of proteins should be evaluated only in the presence of adequate calories. 3) Communica tion and cooperation on all improvements are essential. 4) Acceptance of a decrease in yield must be considered on a cost-benefit basis. 5) The question of bulk of foods must be considered and evaluated. 6) The possibility of providing special foods for special purposes must be considered. 7) Attempts to increase vitamin or mineral content of cereals by genetic means generally offer only limited success. 8) Food nutrients should be evaluated with consideration of nutrient losses in processing and cooking. 9) The U.S. Government should consider using grains with greater amounts of utilizable protein where they could help to reduce malnutrition. 10) Preliminary research should be conducted, as it may obviate the necessity for field trials.

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Report of Second Workshop on Breeding and Fortification

September 1976

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IMPROVING THE NUTRIENT QUALITY OF CEREALS

Report of Second Workshop on

Breeding and Fortification

University of Colorado Conference Center

Boulder, Colorado

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Edited by

H. L. Wilcke

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TABLE OF CONTENTS

Page

Opening Remarks
Irwin Hornstein and Earl Leng. 1

Summary
Harold L. Wilcke 7

General Recommendations 12

Working Group Recommendations
Maize. 15
Wheat. 18
Rice 22
Sorghum, Pearl Millet, and Barley. 24
Legumes 26

Background Papers

--Maize
-Fortification
Report of the Maize Fortification Project in Guatemala . 28
Juan Jose Urrutia et al
-Breeding
Improvement of Protein Quality in Maize. 69
David V. Glover
Genetic Improvement of Maize 98
Federico Poey
Breeding Maize for Protein Quantity and Quality. . . 100
Marcus S. Zuber
Interactions between Yield, Protein and Lysine in Cereals 107
Edwin T. Mertz

--Wheat
-Fortification
Results of Lysine Fortification of Wheat Products
in Southern Tunisia. 113
Mohamed el Lozy and George R. Kerr
-Breeding
Protein Improvement of Wheat by Breeding 134
Virgil A. Johnson and Paul J. Mattern

--Rice
-Fortification
Rice Fortification in Thailand 145
Stanley Gershoff
-Breeding
Current Status of Breeding High Protein Rice . . . 150
W. R. Coffman and B. O. Juliano
(presented by Charles N. Bollich)

Table of Contents cont.

Page

--Sorghums, Barley, Millet
-Breeding
Gene Sources for High-Lysine Barley Breeding. 154
Walter Newman and R. F. Eslick
Nutritional Quality Research in Sorghum--
Progress Report and Current Status 183
John D. Axtell
Work on Pearl Millet and Grain Sorghum Quality
at Kansas State University 193
Carl Hoseney and Gary Paulsen

--Legumes
-Breeding
Legume Breeding--An Assessment of Problems and Potentials
for Improvement in the Edible Bean 204
M. W. Adams
Potential of Soybeans as a Source of Protein
for Cereal Fortification for the Tropics. 212
D. Keith Whigham

Supplementation with Protein
Supplementation of Cereals with Protein - The State of the Art . 220
Paul R. Crowley
Fortification with Protein Supplements 225
Fred Barrett
A Case Study: Ecuador. 230
William J. Hoover

Economics
Cereal Fortification: Barriers to Implementation 241
James E. Austin and Donald R. Snodgrass

Nutrition
Widespread Malnutrition--a protein problem, a food problem or
a population problem? 255
Alfred E. Harper
Productivity and Improved Nutritional Value in Basic Food Crops . 265
Ricardo Bressani
Nutritive Quality Improvement of Diets Based on Corn 288
Ricardo Bressani et al
The Relative Importance of Protein and Energy in the Diet
of Infants and Children 320
George G. Graham

Closing Remarks
Leon Hesser 328
Irwin Hornstein 329

List of Participants 330

OPENING REMARKS

Irwin Hornstein, TA/N AID
Earl Leng, TA/Ag AID

This is the second workshop on Cereal Breeding and Fortification. Five years have passed since AID brought together individuals working in the fields of breeding and fortification for the purpose of exchanging information and viewpoints concerning their respective approaches to improving the protein quality and quantity of food staples. There was little doubt in the minds of the participants that the protein gap was the most important nutritional deficit to be addressed. A major purpose of that meeting was to make the plant breeder and "fortifier" realize that both had similar objectives and problems and that establishing communication between scientists in these areas would prove mutually beneficial. Some of you were at that meeting and will recall that it was a useful dialogue.

We consider it timely to resume that dialogue. In the intervening five years considerable progress has been made in developing new varieties of improved protein quality and quantity and in developing new processed foods of improved protein content; and as might have been expected, new questions have arisen concerning the merits of these programs.

One should emphasize again the complementarity of the two approaches. The inherent advantages of breeding are that:

1. Coverage may reach 100% both in rural and urban areas if a wholly acceptable variety is developed. It is conceivable that the food supply of an entire region may be improved with no need for changes in processing or food distribution systems and at no increase in cost to the farmer or consumer.

2. A breeding program can reach a cut-off point. Once successful varieties are produced, if they are not hybrids, the program is self-perpetuating.

On the other hand, fortification has an equally legitimate role.

1. Fortification lends itself to the addition of a wide variety of nutrients, i.e., minerals, vitamins, lipids, amino acids, protein concentrates, flavors, etc.

2. Acceptability problems may be minimal if essential nutrients are added at required levels.

The inherent disadvantages of a breeding program are that:

1. The farmer may be reluctant to accept unknown risks for a possibly nebulous benefit.

2. If the new variety is a hybrid, the subsistence farmer may be reluctant to buy seeds each year.

3. New varieties that require large-scale cultivation may exacerbate inequality of income distribution, greater investment requirements for fertilizer, irrigation, etc., and affect overall crop plantings. A shift in crops may be nutritionally detrimental -- witness the increase in wheat production at the expense of legumes in India.

The potential disadvantages of fortification include:

1. Limited coverage of rural areas.

2. High per capita cost of reaching a target group.

3. A fortification program is open-ended. The fortificant must be added as long as the food staple is to be improved.

In any given effort to combat malnutrition, either or both approaches may prove useful.

The purpose of the present workshop, in essence, is to (a) review what has been accomplished during the past five years by the plant breeder and the food technologist, to examine issues that have received increased attention, e.g. the relative importance of protein versus calories and (b) to see if major changes in R&D programs should be recommended.

As a first step, it will be useful to summarize the recommendations that emerged from the Annapolis meeting in June, 1971. At that workshop three task groups were assigned responsibility for preparing recommendations for R&D involving (a) wheat, (b) rice, and (c) corn, sorghum and millet. A fourth general task group considered general policies associated with breeding and fortification programs.

The General Task Group recommendations can be summarized as follows:

A. AID should establish a joint R&D Committee (TA/Ag and TA/N) to provide guidance to both offices in their R&D efforts. This committee was established - it was useful - but was disbanded because of bureaucratic regulations.

B. AID should initiate pilot studies for the development of a good strategy that would examine the entire system of possibilities for intervention in relation to the specific needs of a given country.

   AID has moved in that direction. In fact, the main thrust of the AID nutrition program is to create a capability in LDC's to diagnose the causes of their malnutrition problems by looking at related sectors and, based on the diagnosis, modify existing agriculture, health and education practices and programs so as to include nutrition goals as an integral part of these programs.

C. The third recommendation of the General Task Group was that joint efforts should be made to improve the utilization of legumes. The breeder should look to increased yield and improved quality and the food technologist should develop useful foods from these new varieties.

D. A fourth recommendation was that joint efforts should be increased to make greater use of oilseeds including soy as a protein source for humans.

AID has initiated programs along those lines with particular emphasis on soy. Thus, soy/wheat flours have been developed that can be made into acceptable low cost breads, pastries, pasta, etc., and technologies have been developed or adopted for the preparation of blended foods containing soy.

The Task Group on Wheat recommended that:

A. AID should initiate a project to improve screening methods for mass evaluation of improved nutritional quality in wheat, and, suggested that the screening models should take into account nutrient availability, protein fractions affected by genetic manipulation, identification and determination of second limiting amino acids and of nutritionally important components other than protein.

B. AID should consider the feasibility of establishing, in cooperation with existing crop breeding organizations, an international crop improvement network.

C. A project be developed to study the fundamental biochemical processes that result in seed protein production.

D. A program should be instituted to expedite the utilization of new high protein wheat varieties as they become available for release.

E. AID should consider the establishment of a technological center to serve as a source of expertise in the processing of legume and oilseeds into high protein flours that can be incorporated into bread and bread-like products.

F. AID should initiate a study to determine the feasibility of bringing together a group of LDC's to achieve local production of lysine and other fortification nutrients.

G. And finally, that all PL-480 wheat shipments be fortified with lysine.

Some of these recommendations have been implemented, others should perhaps be implemented and some are probably wide of the mark.

The Task Group on Rice recommended that:

A. Breeding for improved protein content should be stepped up.

B. Studies should be expanded to include improvement of deep water and upland rice varieties.

C. Studies should be initiated to determine the nutritional impact of consuming different varieties of rice and of rice containing different protein levels. Biological utilization and digestibility of protein should be included in the studies.

D. The question of the limiting amino acids in rice should be resolved.

The Task Force on Corn, Sorghum and Millet recommended that:

Corn

A. Primary emphasis be placed on improving yield - the interesting statement was made - that given the objective of producing a corn which is both high in protein quality and highest in yield, greater progress can be expected from efforts to improve yield of normal high protein varieties rather than to attempt to improve protein quality in corn.

B. AID should establish a pilot project to evaluate the potential for Opaque-2 corn.

C. A research program should be established by ecological zones for the growing of Opaque-2 corn.

D. Simple and rapid methods for the determination of lysine and tryptophane should be developed.

For sorghum and millet it was recommended that work be carried out on digestibility, increasing protein percentage, and improving amino acid composition. Again, the task group thought the initial payoffs might be in higher yields. It was suggested that high protein quantity as well as improved quality and digestibility should be looked at in later stages.

Millet research should include (a) attention to the prevention of rancidity -- the need for rapid analytical procedures for evaluation of essential amino acids and biological efficiency; and (b) fortification procedures should be developed suitable for subsistence farmers -- the rationale being since millets and sorghum are normally grown under very arid conditions that are not conducive to production of other crops that could supplement the diet; fortification with appropriate nutrients would be desirable, and finally it was suggested that the reservoir of millet germ plasm be increased and improved.

Again, some of the recommendations have been implemented, others have not.

With the preceding as a background, what do we expect from this workshop? For one thing we would like to review the results of three major fortification pilot projects designed to show the impact of amino acid fortification. These included the fortification of wheat with lysine, the fortification of rice with lysine and threonine and the fortification of corn with soy flour and lysine. We are interested in a candid assessment of the results of these pilot studies.

From the plant breeder, we would like to know the current status of high lysine corn, of improved wheat and rice varieties, high lysine sorghum, barleys, etc.

Perhaps more important, we would like to know if the goals we accepted five years ago to improve protein quality and quantity through breeding and fortification are still worthy goals - under all conditions? Under some conditions? As you all know there is considerable controversy today concerning the relative importance of providing more and better protein or providing additional calories. Some nutritionists believe that if more of the same diet is provided no protein problem will be observed, others say that this may be true for adults but not for children, particularly children suffering from chronic under-nutrition and frequent bouts of illness, or for pregnant and lactating mothers. Still others believe that the protein gap may have been eliminated by a stroke of a pen (FAO/WHO protein requirements have been lowered) but changing the requirement doesn't change the reality - protein may still be all important.

We have asked Drs. Graham and Harper to discuss this issue. We hope they take advocative positions so that the issue can be clearly defined if not resolved. In a similar vein we have asked Dr. Bressani to look at the possibility of developing an adequate diet by modification of existing food patterns through consumption of a better balance of indigenous foods.

We wish to review a second area of controversy - the question of yield versus protein content. There is considerable doubt that yield can be maintained if the plant breeder tries to produce new varieties with increased protein content. Is this true? Does it also hold for improving protein quality?

With the state of the art and discussion of these issues behind us, we will be divided into five task groups along commodity lines -- (1) wheat, (2) corn, (3) rice, (4) legumes, and (5) sorghum, millet and barley. Each group will have a core group of a nutritionist, a plant breeder and an individual interested in fortification. We will look to the workshop participants to spend the last two days in developing recommendations for AID and other interested groups concerning the kind of R&D and applied programs needed to promote the nutritional status of populations dependent on these commodities.

In arriving at recommendations, each group should consider such questions as the following:

1. Should we breed for protein quantity? If yes, is there a point at which we say enough? Is a wheat with 13% protein satisfactory or should we strive for 20% protein? Is a 10% rice desirable but 12% better?

2. Should we breed for improved protein quality? Should the goal be for each food protein to be as close as possible to the "perfect protein," i.e., have an Essential Amino Acid composition close to that of egg protein or casein.

3. Should we be only concerned with yield and not worry about protein quality or quantity and if we are concerned with protein, how much of a loss in yield can be tolerated?

4. Where do minerals and vitamins fit into the picture? Should the breeder pay attention to improving, e.g., vitamin A precursor content or should this best be addressed through fortification?

5. Under what conditions should we look to amino acid or protein fortification?

6. What criteria should be used for selecting fortificants and commodities?

The questions to be considered in arriving at recommendations are not limited to the ones that we have enumerated - other issues developed during the course of the workshop may prove to be of equal or greater importance.* Your recommendations will represent the collective wisdom of those most closely associated with R&D in the effort to improve the quality of food through breeding and fortification. The recommendations that emerge as a result of the workshop will undoubtedly influence AID and other programs in these areas over the next several years. We can all look forward to a challenging and useful week in a setting hopefully conducive to a bit of introspection and soul searching regarding the direction our respective programs should take.

*The conference recognizes that cereals may also be good sources of dietary fiber and that western diets are low in fiber, in part because of current milling practices. However, since this conference is intended to deal primarily with cereals in the diets of the people of developing countries the role of cereals in providing fiber will not be considered.

SUMMARY

Harold L. Wilcke

In December 1970, a Workshop on Improving the Nutrient Quality of Cereals Through Breeding and Fortification was convened by the Office of Nutrition, Technical Assistance Bureau, AID. Out of the conference came recommendations regarding wheat, rice, corn, sorghum, and millet, and some general recommendations on strategy to implement the recommendations. Several individuals who were participants in that conference are here, or have been in attendance in this follow-up conference almost six years later. We are, indeed, appreciative of the conscientious efforts of Irwin Hornstein of TA/N and Earl Leng of TA/Ag, who recognized the need to bring this group together to evaluate the progress that has been made in following up on the recommendations of the earlier conference, to appraise our present position, to re-evaluate those earlier recommendations, and to formulate the objectives.

Dr. Hornstein has stated the objectives for this conference very clearly.

How well have we succeeded in meeting these objectives?

First we have had a very frank and objective analysis of the results obtained in the fortification projects.

A. The Corn Fortification Project in Guatemala.

This was a village project designed to determine the effects of fortifying the basic corn diet of the villagers with soybean flour as a source of protein, plus .1% lysine addition and a mineral/vitamin mix. The supplement was added at the local milling facility when the corn was brought in for grinding. Excellent cooperation was experienced in starting the project, with about 95% of the families participating, but a substantial number withdrew because of differences in consistency of the batter after water was added, with resulting problems with fermentation and bacterial development when the mix was carried over to the next day. As a result, only about 36% of the families were continued throughout the entire experiment. Because of other problems, the effective level of supplementation resulted in only a 5 to 6% level of soybean flour instead of the planned 8%. There was no apparent effect on birth weight or on height at three years, but there was a significant decrease in morbidity during the second and third year of life with fortification, and a significant decrease in infant mortality. The experimental village was destroyed in an earthquake in the fourth and final year of the experiment. However, data collection was essentially complete, and although the village has been rebuilt, no further studies will be carried out at this site.

B. Wheat Fortification Project in Tunisia.

This was a project to evaluate fortification of wheat with lysine, or with lysine plus vitamins and minerals. Great care was taken to insure the proper addition of the supplements, and a quality control program of anaylsis was set up to insure proper concentrations of the additives. While preliminary results had been encouraging, the final analysis showed no effect of the supplements on birth weight, height, or other parameters measured.

C. The Rice Fortification Project in Thailand.

This project was designed to evaluate the addition of lysine and threonine, with certain minerals and vitamins, riboflavin added later, to essentially rice diets. The supplements were added through metering devices which were quite accurate. However, at the end of four years there was no suggestion of change in any of the parameters measured. The most plausible explanation for the failure of the intervention is interpreted to be a lack of caloric intake of these children in spite of the presence of adequate amounts of foods.

These three projects will be discussed further on the final day of these meetings, with recommendations for follow-up, if any. If this is accomplished satisfactorily, the first objective will have been met.

Second, the plant breeders reported that:

A. High Lysine Corn.

This term is used to designate maize carrying the Opaque-2 gene for higher lysine, and also those varieties of "normal" corn in which the lysine content has been increased by genetic selection.

Progress has been made in developing strains of high lysine corn which approach equality in yield with normal hybrid lines. While some Opaque-2 strains yield as well as normal strains, the general average still appears to be about a 10% depression in yield. Progress has been made in obtaining more density in the endosperm, but this has not been accomplished fully without affecting yield. Minor problems with ear rot are responding to genetic manipulation. There is a general note of optimism that these problems will be resolved satisfactorily.

B. Wheat.

A new, higher protein strain has been developed and released. Wheat breeders are confident of their ability to produce the characteristics, such as higher lysine, desired by nutritionists, but the primary emphasis has been on increasing yield. There is genetic variability for protein levels, higher protein levels are not incompatible with higher yields or good milling characteristics, short stature is not incompatible with high protein or high yield. There is genetic variability for lysine levels, but this is not as great as in corn. In short, the wheat breeder has the necessary genetic material to manipulate total protein level, increase lysine content of the protein, or work with increased yields, all, of course, within limits.

C. High Lysine Sorghum.

High lysine strains of sorghums have been identified and are in the program. Tannins appear to be restricting the biological availability of protein, but these may be largely removed with the testa. High prolamine also results in lower lysine. Some of the higher lysine lines show partially dented kernels, resulting in somewhat decreased yields. Some success has been achieved in increasing lysine content through the use of chemical mutagen treatments. While the PER of sorghum is generally slightly lower than that of corn, higher values are obtained in higher lysine lines.

D. Millets.

Responds both in yield and nitrogen content when nitrogen fertilizer is applied. There is substantial genetic variability. One of the distinctive features is the level of alpha amylase, which may replace the sugar and malt needed for wheat flour when millet flours are incorporated in bread doughs as partial replacements of the wheat.

E. Barley.

The outstanding problem in high lysine barley appears to be yield. The earlier cultivars have been very low in yield, but some improvement has been made. Satisfactorily high levels of lysine have been obtained.

F. Rice.

The primary objective of rice breeding programs has been to increase yields. Much has been accomplished in increasing yield in the past, but only slight increases in protein have been achieved in the period since the first conference of this type held in 1970. The biological value of the protein in rice is quite good compared with other cereals, but the protein levels are low. The objective is to increase the protein level to 7 to 9% for milled rice, and 8 to 10% for brown rice. Experiences of the past several years suggest that to increase yield and protein levels simultaneously in rice will be a very difficult task.

G. Food Legumes.

Food legumes are beset with many problems of diseases, parasites, adaption to climatic variation, etc. However, an international research network has been set up to work on the problems of increasing yields and also increasing levels of protein. While most of this class of food legumes is low in methionine, there seems to be little encouragement to attempt to solve this problem by genetic means.

The soybean is the predominant oilseed food legume, and attempts are being made to increase yields, achieve 100 day maturity, and produce more than 4,000 kg grain per hectare. For direct human consumption, flavor problems are critical in certain parts of the world, but not

objectionable in other areas. Some progress has been made in improving protein levels and in adapting varieties to the more tropical zones.

It appears that this objective, as narrowly stated, has been achieved. However, more discussion on the relative merits of alternate approaches for specific objectives of improvement of value of cereals would seem highly desirable. In other words, are there certain areas where the genetic approach seems the most efficient, certain areas where fortification might be more feasible, and other circumstances where both approaches might seem justified?

Thirdly, are the goals we accepted five years ago to improve protein and fortification still worthy under all conditions? Under some conditions?

The 1970 recommendations were reviewed by each of the working groups. Some of those recommendations are repeated in some form, some have been accomplished, and many required revision. There was no direct discussion of the status of all of the 1970 recommendations, therefore, this objective was met in part, but not completely.

Fourth, to discuss the issue of the relative importance of protein and calories.

A. The discussions developed several very important concepts:

1. Protein and calories are interdependent as nutrients and should not be considered as completely independent variables.

2. Protein is utilized at maximum efficiency only in the presence of adequate calories.

3. Protein-calorie malnutrition is not simply a nutrition problem but may reflect infection, diseases, parasites, etc. There is no single solution solving protein-calorie malnutrition. It may require more extensive treatment than only nutrition intervention.

4. Proteins with optimum amino acid balance for children are also the most efficient for nitrogen repletion in adults.

5. There are probably few instances in which the addition of calories alone to the diet is justified. Such additions may result in nutrient imbalance.

6. If calories are adequate, seven to eight percent of calories as fully utilizable protein is probably an adequate level for two to five year old children.

7. Without reducing the importance of dietary protein, there has been increased recognition of the importance of the total diet, its total energy content, and all its composite nutrients, in determining nutritional and health status.

8. Protein-energy malnutrition is usually a problem of children and less frequently in adults.

9. There are instances when consumption of more of the same type of food will not correct malnutrition even if calories are sufficient. This is particularly true with diets where the major sources of nutrients are starchy foods such as cassava, yams or yucca.

10. Vulnerable groups, that is, the pre-school children and pregnant and nursing mothers, should be targeted when possible to avoid unnecessary supplementation with resultant high costs, of both food and money.

These discussions and recommendations appear to have satisfied this objective, although admittedly, there is much basic information needed to fill in the gaps of knowledge of requirements between the periods from childhood through adolescence to maturity.

Fifth, to discuss the possibility of developing an adequate diet by modification of existing food patterns.

This has been done for cereal-bean and cassava-bean combinations, with data on varying combinations of the two foods. A 70-30 combination of corn and beans appears to be most satisfactory, whereas the levels would be 74-26 for cassava and beans with added methionine, and 67-33 when methionine was not added.

The use of Opaque-2 corn improves the PER significantly in the corn-bean mixtures. An added bonus in this work was the development of recommendations for protein, lysine, tryptophan, and sulfur amino acid levels for food grains, cassava, and beans.

The concept of calculation of recommending foodstuffs on the basis of the efficiency in terms of productivity per unit land was also introduced. This is calculated on the basis of productivity (yield per hectare) corrected by a nutritive value factor and a technological evaluation.

This report satisfied this objective for at least one country.

Sixth, to resolve the question of whether yield can be maintained when protein levels in grains are increased through genetic manipulation.

This can be done in wheat and oats but, in general, when protein levels are increased there is some decrease in yield. This means that trade-offs must be evaluated, and some incentive must be provided to compensate the producer if the lower yielding strain is to be produced on a practical scale. This objective cannot be met fully until some method is developed to measure the costs and benefits of the trade-offs - higher protein or higher yield.

Finally, Dr. Hugh Roberts and his staff are to be commended for the organization and management of this conference.

GENERAL RECOMMENDATIONS

1. All improvements in the quality or quantity of cereal grains, whether by genetic manipulation or by fortification, should be directed toward the use by the intended populations or specific target groups.

2. The adequacy or availability of proteins should be evaluated only in the presence of adequate calories. When calories are inadequate, some of the protein will be utilized to correct the deficiency in caloric intake.

3. Continuous communication and cooperation among all individuals concerned with the improvement of the nutritional value of cereals, and particularly among the scientists, will expedite the success of such attempts in terms of time, the efficient use of scientific man-power and facilities, and in cost.

4. Recommendations to the effect that no decreases in yield be accepted must be considered on a cost-benefit basis. Obviously, if improvements in nutrient level or availability are of sufficient magnitude to increase the total production of nutrients, some decrease in total yield would be accepted.

5. The question of bulk of foods must be considered and evaluated. There is the distinct possibility that certain segments of the population, particularly the infant and young child, might not be able to consume sufficient total food, even though available, to supply nutrient requirements.

6. Consideration must be given to the possibility of providing special foods for special purposes. The use of high-lysine sorghum produced in Ethiopia as a weaning food is a good example, since the conventional foods used for this purpose would probably not be available to much of the population.

7. In general, attempts to increase the vitamin or mineral content of cereals by genetic means offer prospects of only limited success. Fortification with either the individual vitamins or minerals known to be deficient, or with foods known to be a good sources of the nutrients, should receive primary consideration.

8. The nutrient contribution of all foods should be evaluated with due consideration for nutrient losses in processing and cooking.

9. It is recommended that the US government consider the inclusion of grains with greater amounts of utilizable protein (e.g., high protein wheat and Opaque-2 corn) in P.L. 480 programs where these products can help reduce malnutrition due to protein deficiencies.

10. Field trials are expensive, difficult to control adequately, and the results are often difficult to interpret. Therefore, preliminary research, which is less expensive and time-consuming, and may obviate the necessity for field trials, should be conducted.*

*Before a major breeding effort is undertaken to increase the content or quality of protein in a cereal, it would be useful to determine whether the resulting improvement will have a probable health benefit. This can be done by conducting a field trial with the cereal fortified in that protein or those amino acids for which one expects to breed the cereal. After the protein quality or other nutrient quality of a cereal has been improved, field trials are also useful for determining whether their cultivation should be encouraged, either through further breeding for desirable characteristics or through agricultural extension.

The following is one suggestion for research steps to be followed before undertaking large field trials directed towards showing the nutritional and health effects of the nutrition intervention. Many of the steps are already common practice.

The results of Steps 1 and 2 are of universal importance.

Step 1

Animal nutrition studies of the fortified or improved cereal should be conducted to substantiate the theoretically calculated improvements, making certain that these improvements are not accompanied by substances or characteristics deleterious to growth and health.

Step 2

To substantiate the animal results in man, studies should be conducted in metabolic wards of persons, in those age groups or with those physiological conditions, who are expected to benefit from these cereal improvements. Within ethical constraints, these studies should be conducted at the levels of calories and nutrients at which one would expect the largest nitrogen balance difference between the usual cereal and the improved or fortified cereal.

Steps 3, 4, and 5 must take into consideration the local dietary conditions:

Step 3

Studies should be conducted in metabolic wards of the effect of substituting the improved or fortified cereal for the usual cereal in the traditional diet of those age groups, or groups with those physiological conditions, expected to benefit from the substitution. This is to assure that the putative improvement of protein quantity or protein quality in the diet does indeed have a beneficial effect on protein retention or repletion. This would not be the case if other nutrients besides protein are severely limiting in the composition of the diet, if there are toxic or anti-nutritional factors in the diet which prevent the expected effect, or if the

protein concentration of the traditional diet is high enough and its protein quality good enough that improvement of protein is superfluous.

Step 4

Determine whether calories are limiting in the traditional diet of the persons expected to benefit from the improved cereal. This will have to be ascertained from dietary surveys, that are substantiated by metabolic ward studies in which the food intake is reduce from the levels given in Step 3 to the levels traditionally ingeste in the diet.

Step 5

Field-test the improved cereal in the traditional diet, providing at the same time the necessary health care to eliminate epidemics and easily preventable deaths in the persons expected to benefit from the nutritional improvements. **In preschool children the preventable diseases are "immunizable diseases," and the preventable deaths are, above all, those from pneumonia and from dehydration from diarrhea. The reason for the health care is to reduce the variability in those measures of health and growth which are expected to be affected by the improved diet. (The cost of such effective health care is about 10% of an evaluation program for nutritional improvement).

The strongest experimental design so far used in evaluation trials has combined treatment replication over more than one village and has had a gradient of treatment within villages. This design requires a non-protein treatment which duplicates in form and delivery the protein treatment. Results from evaluation studies cannot be interpreted in the absence of controls and replication.

**"Limiting" in this context is used to mean that intake of the nutrients under consideration is so low that no increase in other nutrients will improve growth or function.

MAIZE WORKING GROUP RECOMMENDATIONS

Edwin T. Mertz, Chairman

I. To obtain the goal of productivity, as expressed by yield x nutritional value x technological value, the Maize Task Force recommends that breeding should be continued on the improvement of protein quality and quantity without a material loss in yield for satisfying the essential nutrient requirements of monogastric animals including man. The Task Group specifically recommends that:

A. Breeding research and development be increased on the improvement of protein quality and quantity with particular emphasis on yield, milled and non-milled storage properties, host-plant resistance and grain quality in the following types:

1. Single gene factors.

a) Opaque-2.
b) Modified opaque-2 types and double mutant combinations.
c) Other endosperm mutants (existing and induced), and potential combinations thereof.

2. Multigene factors.

Normal endosperm types in the absence of single gene endosperm mutants.

B. Improved efficiency of nitrogen utilization by the corn plant is necessary if increased grain yields and protein percentages are to be obtained with minimal increases in use of nitrogen fertilizer. Thus, support of research on the mechanisms of nitrogen utilization, genetic variability for nitrogen utilization efficiency, and on methods of incorporating improved efficiency into usable cultivars (particularly of high lysine corn) is needed.

C. Basic research information on variation in digestibility within opaque-2 and normal corn germ plasm is limited. An increase in digestibility of corn would help solve the problem of inadequate caloric intake of young children fed corn diets.

It is recommended that research on the potential for genetic improvement of digestibility of corn be supported.

D. Studies should be conducted to better understand the genetic-physiological relationship of dry matter accumulation and yield by investigating:

1. Interrelationships and compensating effects among carbohydrate, protein, and oil metabolites during endosperm development.

2. Relative rate and length of grain filling period and related maturation moisture loss.

3. The interaction of genotype and environment on the grain-stover relationships.

E. Protein synthesis mechanisms.

Plant breeding procedures have demonstrated the feasibility of genetic modification of maize protein. The opaque-2 and other mutants document that single gene changes can result in useful protein quality alternations. Further advances, including new selection techniques, may be suggested by more basic information on protein synthesis mechanisms in mutant and normal endosperms.

Research should be supported to generate information on the molecular and developmental biology of maize relative to protein synthesis. Specific areas should include the molecular biology of protein synthesis, amino acid bio-synthetic pathways, and storage protein synthetic mechanisms. Biochemical, genetic, and practical selection aspects should be emphasized.

The following examples may help to clarify the type of basic information needed.

1. Molecular biology of protein synthesis involves the elucidation of the DNA$\rightarrow$RNA$\rightarrow$protein mechanisms.

2. Understanding of the lysine biosynthetic pathway may lead to feedback resistant mutants to deregulate the synthesis of lysine.

3. Structural genes coding for storage proteins (none of which have been identified) and regulatory genes other than opaque-2, floury-2, etc., may be modified to give protein alterations not yet discovered.

4. Developmental biology information on maize endosperm may lead to greater understanding of what triggers various storage proteins to be synthesized, and DNA levels in parts of the endosperm and how this relates to protein types and levels.

5. Screening systems for useful mutants should be considered for the above areas.

II. The Maize Task Group recommends that:

A. Target levels of nutritional components in corn grain be established as guidelines for corn breeding.

B. A nutritional research evaluation of practical protein quantity and quality levels in corn be established to determine adequacy of target levels for application to the widest types of possible corn based diets.

C. Target levels be modified as necessary, based on current and future field-scale nutritional research projects.

D. The current target levels be as follows (at 10% grain moisture):

1.	Protein (%N x 6.25)	13 - 14%
2.	Lysine	3.5 - 3.8 g/100 g protein
3.	Tryptophan	0.8 - 0.9 g/100 g protein
4.	Total sulfur amino acids	3.2 - 3.4 g/100 g protein
5.	Oil	At least maintain present levels.

III. The Maize Task Force recommends that corn-based diets be improved by the addition of other foods or that they be fortified to meet the requirements of the weanling infant, pre-school child, and pregnant and lactating mothers. The diets should contain adequate energy, adequate protein content and quality, and adequate minerals and vitamins for each target group. The practical value of "normal" corn supplemented with other sources of protein and/or amino acids should be compared with better quality protein corns such as opaque-2 which contain higher levels of protein and a better balance of limiting essential amino acids. Assessment of the biological effect of improved corn diets should be made by changes in anthropometry and morbidity, and also by evaluation of host defense mechanisms.

WHEAT WORKING GROUP RECOMMENDATIONS

G. Richard Jansen, Chairman

I. Improving the nutritional quality of wheat.

A. Premises.

1. Wheat does not have sufficiently high levels of vitamins, minerals and utilizable protein to meet completely the nutritional needs of the most vulnerable population groups under conditions where wheat furnishes two-thirds or more of the food energy consumed.

2. The vulnerable groups consist primarily of infants, pre-school children, and pregnant and lactating women.

3. Situations in fact exist where wheat does provide at least two-thirds of the food energy to significant numbers of vulnerable population groups.

4. Vitamins and minerals can best be added by fortification. However, it is recognized that this mechanism will have great difficulty in reaching rural subsistence farmers and their families.

5. Utilizable protein can be added to wheat by breeding, protein fortification, amino acid fortification and combinations of all of these methods. An increase of up to 50% in the utilizable protein of wheat consumed in less developed countries is a reasonable goal.

6. The exact form and need for a protein improvement program in a country or region will depend on the composition of the wheat, extraction rate, form of the food as finally consumed, cultural factors, availability of resources, and economic constraints.

B. Recommendation for plant breeding programs.

The following are listed in descending order of priority.

1. First priority should be placed on achieving maximum yields, with no sacrifice in nutritional quality, or in desirable functional properties.

2. The utilizable protein in wheats as commonly consumed in less developed countries when wheat is the cereal staple, should be increased. This should be accomplished with no sacrifice in yield, nutritional quality or functional quality.

3. In view of current knowledge concerning protein and lysine variability in wheat, primary emphasis should be placed on increasing the percentage of protein in the cereal grain with no sacrifice in protein quality.

4. Wheat germplasm in which high grain protein has been combined with desirable agronomic traits is available from the ARS-Nebraska program. Development of procedures by which this germplasm can be most rapidly and efficiently utilized by breeders and farmers in LDCs already has been implemented by the Nebraska group in collaboration with CIMMYT and Oregon State University. This activity needs to be expanded and accelerated.

5. The implications of recent observations in which wide crosses of existing cereal species have resulted in new inter-specific and inter-genetic hybrid cereals should be evaluated in terms of their possible utility in increasing the productivity and quality of cereals in LDC's.

6. At the present state of knowledge, the breeder should give little priority to improvement in the vitamin and mineral content of wheat.

C. Recomendations for fortification programs.

The following are listed in descending order of priority.

1. Vitamin and mineral fortification of wheat should be implemented as widely as possible within economic and technological constraints.

2. Amino acid and protein fortification are capable of improving the amount of utilizable protein in wheat. Because of difficulty in demonstrating that this in fact results in a health benefit under real-life conditions, it is suggested that such fortification only be carried out if the cost is minimal. When the actual limiting amino acid in the diet is not certain, protein fortification has a higher likelihood of achieving a nutritional impact.

II. Special Foods for Women, Infants and Children

A. Premises

1. Infants, pre-school children, pregnant and lactating women have higher nutritional requirements than do the general population.

2. Special foods developed for these population groups have a high likelihood of achieving a nutritional impact. For this to occur these foods need to be consumed in significant amounts by the target groups.

B. Recommendations.

1. Further develop special foods based on wheat, as discussed above, for specific countries with full consideration given to such factors as acceptability, cost, availability of local food resources and processing capacity, and sanitation.

2. In the development of such special foods, full utilization should be made of existing technologies available from plant breeding, protein and amino acid fortification, and vitamin and mineral fortification programs.

III. General Recommendations

A. Protein and amino acid requirements for pre-school and school age children need to be determined more accurately. Research should be carried out under metabolic ward conditions that will define the following for children ages 2-18:

1) Amino acid and protein requirements
2) Protein quality-quantity relationships, and
3) Protein-energy relationsips.

B. Evaluation of Breeding and Fortification Programs

Taking full advantage of everything learned from all current fortification studies, a field study should be carried out in which the protein improvement of wheat by several alternative mechanisms is evaluated, with specific emphasis on the following interventions:

1) Vitamins and minerals only.
2) High protein variety of wheat.
3) High protein variety, vitamins, minerals.
4) Soy, vitamins and minerals.
5) Lysine, vitamins and minerals.
6) Additional unfortified wheat.
7) No treatment.

Parameters measured should include food consumption of vulnerable groups, birth weights, mortality, morbidity, school attendance and growth of children.

C. Methodology Improvement

1) Specific attention should be directed to development of techniques for field evaluation of nutritional status and health benefits resulting from nutritional intervention programs.

2) Reliable, but simple and more rapid screening methods to assist the plant breeder should be developed.

D. Transfer of Production and Utilization Technology to LDC's

1) Transfer wheat improvement and production technology which is appropriate and which would maximize wheat yields for subsistence farmers.

2) Transfer food technology skills in areas of processing, storage and nutrient retention.

3) Transfer nutrition education skills in area of consumer education and "mother craft" type activities.

RICE WORKING GROUP RECOMMENDATIONS

Charles N. Bollich, Chairman

In order to consider the role of breeding and fortification for rice eating populations, our Task Force considered it important to develop a hypothesis about the consequences of high levels of rice consumption in terms of nutrition. This hypothesis is based on the research of the last five or more years and is considered to be applicable to most if not all rice eating cultures. Rice eating people are hypothesized to suffer growth retardation in young children where little other food is a part of the diet. A number of other nutrition and health factors are believed to be contributing to the observed retardation, including insufficient trace nutrients and poor health (high levels of parasites and infections). It also appears that caloric deficits in some areas are due to insufficient food supplies, but in other areas the deficits exist even though foods are available. It appears that if rice is the only source of protein during the most rapid periods of growth, the present levels of protein in the grain are 10-20 percent low. Recommendations for the alleviation of malnutrition among rice eaters are being made in the context of the above, recognizing that the cause of the growth retardation may vary among rice eating countries, the specific cause being yet to be determined for each area.

Recommendations for Research

I. Breeding

Primary emphasis in breeding should continue on improving yield, without diminishing consumer acceptability. The committee feels strongly that increased yields in new varieties should not compromise current protein levels in milled grain.

In view of the nutritional status of peoples receiving 10 percent or more of their calories from rice, any improvement in the protein content of rice through breeding would be valuable. It is recommended that efforts to develop higher protein rices be strengthened, with the goal of developing varieties with a protein content of 9 percent in the milled grain. Any achievements in developing higher protein varieties should not negate improvements in grain yield. Although all rice breeders should be encouraged to explore, on a limited scale, the development of higher protein varieties and be alert for experimental selections with higher protein content, the committee recommends that at least one location be selected for a strengthened breeding effort for higher protein. Since rice protein has high biological value relative to other cereals, lower priority should be directed to improving protein quality than to increasing levels of protein.

The committee believes that high priority should be given to genetic, physiologic, and biochemical studies of protein synthesis in the rice grain. Such information is urgently needed by the breeder to design an efficient program to develop higher protein varieties with satisfactory yielding ability.

II. Nutrition

1. The rice food systems may be subject to a number of nutrient loss factors that field research needs to take into consideration.

   a) Lysine loss potential during the heat developed in milling.

   b) Vitamin B_1 loss during storage.

   c) Protein losses from over-milling (perhaps a reduction from 7 percent to 5 percent).

   d) Losses from cooking procedures.

   A different type of loss is occurring in these countries from early weaning with the consequent loss of breast milk as a food resource.

2. Rice fortification research in Thailand has resulted in a number of unexpected conclusions.

   a) Rice fortified with lysine and threonine plus Vitamins B_1, B_2, and A, and iron did not result in any measurable health benefit in a population of children retarded in growth but with an adequate quantity of food.

   b) Failure to observe beneficial effects was not associated with morbidity.

   It is from these conclusions that the hypothesis "the causes of growth retardation..." should be the subject of any future research in rice eating cultures. It is believed that such research will show that the caloric and protein density of rice as consumed is too low to meet the needs of this age group.

3. a) Dietary fortification research should have the objective of adding foods rich in protein and calories together to the diets of children. Variations among countries make it imperative also to give careful attention to the design of research regarding supplementation with vitamins and minerals. Broadly based studies on soy food introduction are recommended.

   b) Research on rice grain fortification (as one component of the diet) with nutrients is considered of lesser priority than studies of the fortification of the complete dietary regimen.

4. Inadequate consumption in the presence of an adequate supply of rice should be investigated.

SORGHUM, PEARL MILLET, AND BARLEY WORKING GROUP RECOMMENDATIONS

Lloyd Rooney, Chairman

Improvement in food and nutritional quality of sorghum, millet and barley can best be achieved through genetic improvement to increase and maintain yield, improve protein quality and maintain food quality. These crops are consumed by the very poor subsistence farmers where central processing facilities and distribution systems are lacking. Therefore, in the opinion of the committee, the likelihood of effective fortification programs is questionable. However, some attention should be given to finding methods to fortify diets of subsistence farmers.

In general, the committee feels strongly that current knowledge of the nutritional properties of these cereals is inadequate. There has been drastically less research on these cereals and much of the research that has been done is related to feed use and may not apply directly to food use. It is essential that research be done to provide basic and applied information on these crops. For example, there is little or no experimental information on the adequacy of these cereals for human nutrition. Currently, predictions of their value are extrapolated from data obtained for corn, wheat and rice.

More data on the digestibility of these grains as used in the diets are required. It has been well documented that sorghum has lower digestibility than other grains when used in animal feeds, and very rigorous processing is required to improve the biological availability of the nutrients in this grain.

The total amount of research accomplished on sorghum and millets in the world is much less than that of other major food commodities. Therefore it is imperative that existing and new research on sorghum and millet be carefully coordinated and integrated into an effective vehicle to achieve maximum efficiency in the improvement of these crops.

Specific recommendations, listed in order of decreasing priority, are:

1. The major thrust should be to produce high, stable yields of grain. Sorghum, millet and barley are usually grown under the most adverse conditions of drought and temperature stress and are subject to several devastating diseases and insects. Therefore, it is very important that varieties have built-in resistance and/or tolerances to these conditions.

2. It is important to improve amino acid balance of sorghum and barley with the goal of increasing lysine content 50% while maintaining acceptable yield and end use food characteristics. The genetic variability of the amino acid balance of pearl millet should be determined to see if it is feasible to increase the amino acid balance.

3. Define and provide, for sorghum, millet, and barley, information of importance in determining their functional properties and acceptance for traditional village food use. Use this information to develop reliable methods to screen for the important food grain functional properties.

4. Determine the content and components of the tannins and other polyphenols of sorghum, barley and other food grains, and their interactions with proteins and other nutrients. This should include research to understand the chemistry of the tannins and their role in reducing biological value.

5. There is a need to determine the level and variability of vitamins and minerals in millet. Niacin content and availability is an important variable in the nutritive value of sorghum for some areas and should be considered. However, the problem can be solved through improvement of the amino acid balance.

6. Determine if special varieties of these cereals could have an important place in the feeding of weanling children. If so, then varieties with high protein content and improved amino acid balance should be developed while accepting some reduction in grain yield.

7. Evaluate the composition of the diets of people consuming these cereals to provide information required:

   a) To intelligently devise strategy for improvement of their nutritional status and,

   b) To provide information to establish guidelines for crop improvement programs.

8. Nutritional studies should be designed to determine the minimum level of protein quality and quality in sorghum, millet and barley required to meet human requirements when those cereals constitute the principal source of protein in the diet.

9. A better understanding of kernel development, morphology, and the genetic control of the biosysthesis of the major kernel components of these grains is needed. This should include information on the composition and structure of those constituents.

10. Protein content should be maintained at levels of 10-11% while yields are increased. Increases in protein content are desirable provided no losses in yield occur.

11. Carotenoid pigments exist in these grains at low levels but there is little hope of improvement in their level through breeding.

Finally, the committee is concerned that because sorghum and millet are considered coarse grains and because they are grown in very poor areas, too often they do not receive an equitable share of research attention and funds. Yet the consumers of these crops are certainly among the most in need of assistance.

LEGUMES WORKING GROUP RECOMMENDATIONS

Robert A. Luse, Chairman

The food legumes are concentrated sources of protein with particular value for direct utilization in the diet and as fortificants of foods with lower protein. Their high protein content and amino acid composition make them good nutritional supplements to cereal grains and the chief protein source in diets based on sturdy roots and tubers.

The nutritional value of food legumes, and hence their impact on improving the nutritional status of vulnerable groups are limited by generally low seed yield; poor protein digestibility, and deficiency in sulfur amino acids; and poor functional (technological) characteristics, such as long cooking times and hard seed. Progress towards the solution of these problems can be made by plant breeding and selection from existing germplasm, but these require further cooperative efforts by plant breeders, nutritionists and food technologists.

It is recommended that the legume breeder concentrate on high, stable seed yield and the primary factors of yield including pest, disease, and virus resistance; response to management and resistance to stress. Protein content and quality should be maintained in improved cultivars, especially the sulfur amino acids and lysine, though to absolutely increase these amino acids has limitations and has lower priority. Functional factors that determine consumer acceptance of the legume as a food must also be considered and optimized.

It is proposed that the following improvements can be made by the plant breeder, with little or no loss of seed yield:

A. Beans (*Phaseolus vulgaris*)

1. Increase protein content to 26-28%.
2. Raise protein digestibility.
3. Reduce cooking time, seed hardness, unsatisfactory taste.

B. Soybeans (*Glycine max*)

1. Increase protein content to 44-46%
2. Reduce heat stable anti-nutritional factors.
3. Reduce cooking time of dry seeds when used as human food.
4. Develop vegetable types for consumption as green vegetables.

C. Cow pea (*Vigna anguicalata*)

1. Increase protein content to 26-28%.
2. Improve sulfur amino acid content.

D. Pigeon pea (Cajanus cajan)

1. Raise protein content to 26-28%.

2. Improve amino acid balance in tryptophan and sulfur amino acids.

E. Other legumes and oil seeds with production potential in the tropics.

1. Improve protein content or protein to oil content ratio.

2. Improve amino acid composition when deemed desirable in actual food use situations (e.g., with sturdy staples)

3. Raise digestibility when low.

4. Improve functional (technological) properties.

It further is recommended that the nutritionist and food scientist should cooperate with the plant breeder in the following research:

1. Revise the above nutritional targets as more information is obtained from field trials and laboratory experiments where legumes are eaten together with cereals and/or sturdy staples.

2. Define functional factors more carefully, so that they may be screened more easily in breeding programs.

3. Determine the reasons for hardening of the bean seed upon storage and ways to reduce or eliminate this problem in utilization.

4. Determine the effect of tannins, phenolics, and carbohydrates on proteins, dry matter and digestibility.

5. Develop food processing techniques for less developed countries that permit increase in consumption of legumes by all social and age groups, but especially by post weaning infants, young school children, pregnant women and nursing mothers.

6. Introduce into food systems legumes with high oil content (e.g. soybean, peanut) so that their high caloric density is fully utilized.

To meet the needs of a fortification program based on cereal-legume blends, it has been found that addition of whole soybeans or soy flour to the extent of 15-20% by weight of cereals provides a high protein, high energy product. It is recommended that other legumes be considered as fortificants, using the criteria: availability, functional properties of the product and its acceptance, protein content and protein quality. The possibility to grow legume fortificants in the area of nutritional need should be considered. The feasibility of any fortification project should be based on clearly defined nutritional needs of the target group, economic feasibility and level of incentives to use (and pay for) the fortified product.

REPORT OF THE MAIZE FORTIFICATION PROJECT IN GUATEMALA*

J. J. Urrutia,** B. Garcia,** R. Bressani,** and L. J. Mata***

Introduction

Many studies have been carried out by INCAP to determine the content of protein and amino acid of local maizes. These studies indicate that the protein content of Guatemalan maizes varies between 6.5 and 10.0% with highland corn usually lower than lowland varieties (2,4). The results of biological tests carried out with maize have shown that the limiting amino acids definitely improves the quality of maize protein (1,3,6).

The protein efficiency ratio (PER) of lime-treated maize is about 1.2 and increases to 1.5 by adding 0.31% of lysine. The addition of 0.31% lysine and a very small amount of DL-tryptophan (0.05%) dramatically increases the PER tO 2.76 (6). This is equivalent to adding 8% soybean flour (50% protein) and 0.125% L-lysine HCl.

The nutritional surveys carried out in the population of the Central American area, have shown a deficit in the intake of high biological quality protein and calories. This deficit is still more marked in preschool children and pregnant and lactating women (9,12,18,21). The problem stems from the fact that the main source of available dietary protein is maize or other cereals. It has been determined that in Guatemala, maize provides more or less 60% of the protein and calories in the diet of rural areas.

Based on this knowledge, it was decided to test the possibility of implementing and conducting a maize fortification project at the level of a community, in order to determine the biological effect of the improvement of the quality of maize protein. One important aspect of this project was that the implementation of this field trial did not require any change in the dietary habits, and more important that the whole family could be reached (17). The most appropriate method to fortify the maize was by adding a soy mixed fortifying flour plus lysine, vitamins, and minerals, as explained in Table 1. The procedure was to be carried out at the level of the village wet corn mills, where maize is customarily milled in Guatemala.

This study was aimed at determining the biological effect on the health of the members of a community. It was planned to measure changes induced by the intervention mainly on the health status of preschool children and pregnant women. The variables selected to assess the biological effect were: fetal and postnatal growth, morbidity in children from birth to 3 years of age, as well as infant and 1 to 4 years mortality.

The place of the research project was the Mayan Cakchiquel Indian village of Santa María Cauqué, located in the Guatemalan highlands at an altitude of 1,600 meters, 35 kilometers from Guatemala City. The number of inhabitants at the beginning of the study was 1,410.

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**INCAP
***University of Costa Rica

Table 1

Fortifying mixture for maize
Santa María Cauqué

Ingredient	Composition of supplement %	Content in 8% added to corn, g
Soybean flour	97.5000	7.800000
L-lysine HCl	1.5000	0.120000
Thiamin	0.0268	0.002144
Riboflavin	0.0162	0.001296
Niacinamide	0.1930	0.015440
Ferric orthophosphate**	0.6000	0.048000
Vit. A 250-SD***	0.0313	0.002504
Corn starch	0.1327	0.010616
Total	100.0000	8.000000

* Developed by R. Bressani, unpublished data.

** Contains 28.1% Fe.

*** 75,000 mcg Retinol/g.

Previous research conducted from 1964 to 1972 in this village by the same team which was in charge of the maize fortification project, provided basal information on intrauterine and postnatal growth, dietary intake, morbidity and intestinal infection in preschool children, and mortality for the whole village. In addition, information on the socioeconomic characteristics of the village was obtained by means of the three censuses conducted in 1963, 1967, and 1971. During the time these data were collected, no interventions were imposed.

The main findings of the basal data have been published (13,15,16,17). These data showed that the health problem in Santa María Cauqué was very complex. It can be summarized by stating that not only the poor diets but the high incidence of infections are the main responsible factors for malnutrition and growth retardation in children, as well as for the lack of adequate fetal growth.

The information in this report has been divided into two sections, dealing first with the implementation of the field methodology and acceptability of the intervention, and second with the biological effect of the fortified maize.

I. Implementation of the Field Methodology and Acceptability of the Fortification

1. Description of the Research Project

Santa María Cauqué was chosen as the experimental village. Another Indian village, Santo Domingo Xenacoj, located at 4 kilometers from Santa María Cauqué, was selected as a control (17). The variables to be studied in both villages were: fetal growth and postnatal growth from birth to 5 years, as well as preschool mortality. In the experimental village, the effect of maize fortification on preschool morbidity and on their dietary pattern was also to be assessed.

a. Social Work Previous to the Implementation of the Fortification Project

The fortification project started on the 29th of May, 1972 and ended unexpectedly on the 3rd of February, 1976. Santa María Cauqué suffered the loss of 5% of its population and the destruction of all but one of its houses during the earthquake on the 4th of February.

The purposes and characteristics of the maize fortification project were explained to the villagers prior to the initiation of the field work. During a three month period several meetings were held with the village authorities and other village principals, such as teachers and community leaders, to explain the project in detail. In addition, the field personnel carried out many home visits to explain to the housewives the purposes of the project, and especially the fortification methodology.

As maize is eaten in the form of tortillas, several trials of taste differentiation with fortified and unfortified tortillas were practiced with male and female adults, as well as with adolescents. The observations were carried out in 154 subjects with a total of 274 observations. The comparisons between fortified and unfortified tortillas were done utilizing the triangle method. The trials were conducted either at the health center, or at the Municipality Building. The percent of correct answers was 32.5, which was

not statistically significant. It was concluded that villagers could not differentiate between fortified and unfortified tortillas (Urrutia, Garcia, and Mata, unpublished observations).

b. Installations and Equipment for Fortification

First of all, it was necessary to obtain the collaboration of the mill owners. At the beginning of the project, there were two mills in the village. During the course of the project, two more mills came into operation. The same methodology that was applied to fortify the maize in the two existing mills, at the beginning of the project, was applied later on in the other two mills.

Considering that there was not any previous experience to fortify maize on a daily basis at the village level, special simple equipment to be utilized by the millers had to be designed. The equipment consisted of a balance with a dial to read the number of pounds of cooked corn (nixtamal), and measuring cups of various sizes to deliver the corresponding amounts of fortifying mixture to the nixtamal at the level of 8%. For example: Measure number 10 delivered the amount of flour needed to fortify 10 pounds of nixtamal (see Figure 1).

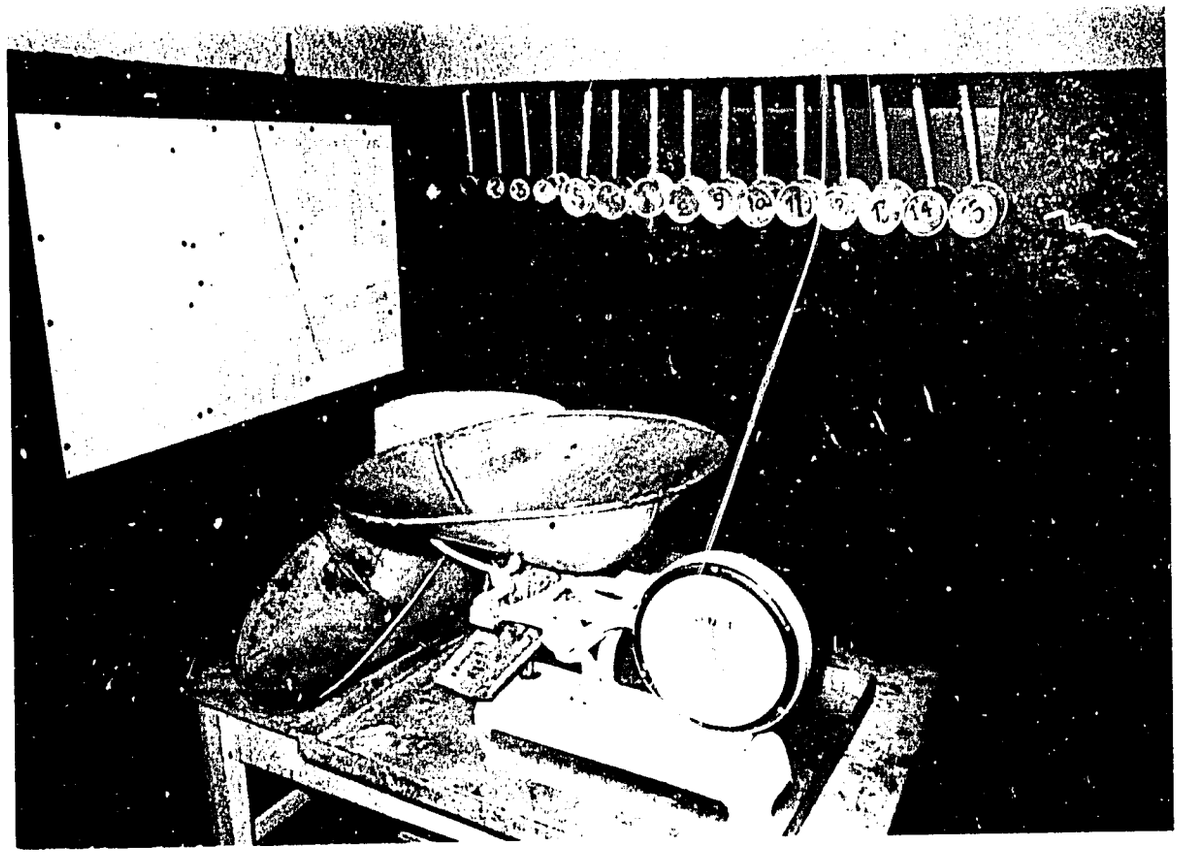

FIGURE 1

Equipment employed for the fortification operation. It consists of (a) an adjusted balance with blocked dial to show the approximate number of pounds of "nixtamal" (lime-cooked and pealed kernels); (b) measuring devices (hanging on the wall) to add the corresponding amount of fortifying mixture; (c) bulletin board with forms for recording visits of each family.

Printed forms with the number and names of the housewives of the village families were used to keep a control on the daily fortification to identify the family at the mill when different members of the families carried the nixtamal. This was done by the INCAP staff before the project started.

The procedure for corn fortification was as follows: women or female children carried the nixtamal to either one of the mills. At the mill the nixtamal of all the customers was weighed. Then they were asked if they wanted the fortification. When the answer was affirmative, the proper measuring cup was utilized to deliver the mixture into the nixtamal (Figure 2).

FIGURE 2

The supplement is added to nixtamal. The custom was to cover the powder with kernels to facilitate grinding.

A record of the number of nixtamal pounds and of the number of the measure utilized was registered on the precoded form. Thereafter, the nixtamal plus the fortifying mixture was placed into the hopper of the mill for grinding. During the process of grinding, the nixtamal and the fortifying flour were mixed with a wood spatula (Figure 3).

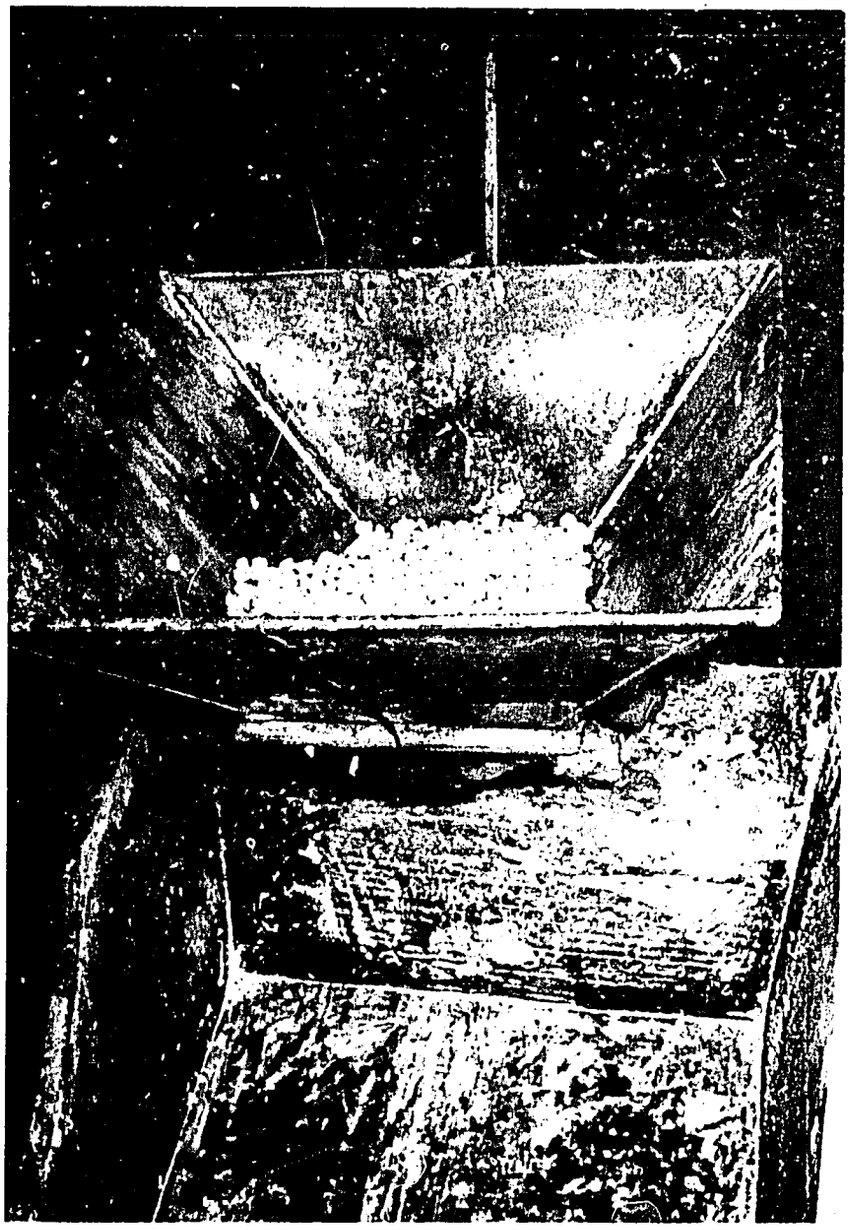

FIGURE 3

Hopper of the mill where the "nixtamal" plus the fortifying mixture is placed before grinding.

Then the women collected the maize dough and carried it to prepare the tortillas at home. In the process the only intervention was the weighing of the corn and the adding of the flour; no other modifications were introduced in the traditional process of cooking maize or preparing tortillas

c. Studies on Pregnant Women and Preschool Children

To assess the biological effect of the improved maize on fetal growth and on the health of preschool children, 90% of the pregnant women and a cohort of 70 children from 0 to 3 years of age were followed up. Intrauterine growth was determined in 189 cases by measuring at birth - weight, height, and head and chest circumference. The maternal variables affecting fetal growth, plus the dietary intake and morbidity during pregnancy, were also determined.

The cohort of children 0 to 3 years of age was formed at the beginning of the study by 63 children distributed as follows: 13 children between 0 to 3 months of age, and 5 groups of 10 children each, representing the age periods of 3 to 6, 9 to 12, 15 to 18, 21 to 24, and 27 to 30 months. The follow-up of children stopped at the third birthday. All babies born after the 29th of May, 1972 were included in the cohort until April 1973, when it was decided to stop the collection of newborn for the cohort; by then, the number of the study children was 70.

The health of the preschool children, 0 to 3 years old, was determined in the cohort children by a weekly surveillance of the morbidity and dietary intake. Growth of these children was determined by measuring weight, height, chest and head circumferences biweekly during the first year of life, and every four weeks during the second and third year of life. Growth records of children other than the cohort group were also available through measurements of the same parameters in children from birth to 10 years of age at 3 month intervals. In the control village, pregnant women were followed only for anthropometrical examination. Newborns were examined at birth, as described for the experimental village. Records of growth from 0 to 3 years of age were obtained at 3 month intervals. A general record of natality and mortality was kept for both villages. The methodology of the field activities has been described previously (13,15, 16,17).

2. Results on the Implementation of the Project and Acceptability of the Fortification

a. Cooperation of the Villagers

During the first two days of fortification, 95% of the families accepted the fortification of their maize. At this time, many villagers began complaining about foul odor and bad taste in the fortified tortillas that were left over from the previous meal. After a few days cooperation dropped to about 30%. The problem was promptly investigated in the field and in the laboratory. It was found that the water content was higher in the fortified tortillas due to the high water solubility of the fortifying mixture. The millers were also adding a greater amount of water in order to facilitate grinding of the maize plus the mixture. Furthermore, the storage of tortillas in closed and not well ventilated baskets, a common practice in the village, favored bacterial proliferation. It was found

that fortified tortillas had greater numbers of Bacillus, coliforms, and cocci. The logical practical solution was to remove part of the water from the nixtamal. For this purpose, holes were drilled in the balance tray (this removed from 5 to 10% of the total weight), and to favor water evaporation housewives were told to leave the tortillas a little longer in the hot dish (comal), where tortillas are cooked as well as to store them in a well ventilated basket.

These recommendations plus intensive field work resulted in better cooperation, but the percentage of regularly fortifying families dropped to 40%. The problem of high water retention was never solved satisfactorily, as will be seen later.

b. Reasons for Accepting or Not Accepting the Fortifying Flour

A survey intended to determine the concepts villagers had about the fortifying flour was carried out at the end of 1974 (more than two years after the project had started). Thirty-nine collaborator and 46 non-collaborator families were investigated.

In Table 2 are presented the reasons for accepting the fortifying flour in 39 families (Santa María Cauqué, June 1975-January 1976).

Table 2

Reasons	Number of cases and percent
Advice of the clinic personnel	23(59)
Own decision (to increase body strength to resist illnesses)	8(21)
Were convinced by other persons from the community, because it gives strength	4(10)
Volume of dough is increased (produces more tortillas)	4(10)

The majority of them (59%) responded that they had accepted the fortification because it was advised by the clinic personnel. Thirty-one percent mentioned that they were accepting it because the mixture gave them strength and it was good to prevent illness. Ten percent said that they were obtaining more tortillas because the flour increased the volume of the dough.

The concepts about the fortifying flour in 39 collaborator families are presented in Table 3 (Santa María Cauqué, June 1972-January 1976).

Table 3

Concepts	Number of cases and percent
It is good and avoid illnesses	22(56)
It is good, avoid illnesses and has good flavor	10(26)
They did not have a concept	7(18)

Everyone expressed the belief that the fortified tortillas had good flavor, but were not accepted by pregnant women because fortified tortillas caused nausea.

It can be seen that 82% of the families believe that the flour was good to avoid illnesses and was acceptable for everybody, except pregnant women, because fortified tortillas caused nausea.

The reasons for not accepting the fortification in the same 46 non-collaborator families are presented in Table 4 (Santa María Cauqué, June 1972-January 1976).

Table 4

Reasons	Number of cases and percent
Tortillas cannot be stored for next day	20 (43)
Change in flavor	17 (37)
Stomachache and/or diarrhea	9 (20)

The main reason was that tortillas could not be stored for the next day. A noticeable change in flavor was reported by 37% of the families, mentioning that this change became stronger when tortillas were mixed with coffee. Twenty percent said that fortified tortillas produced stomachache and/or diarrhea.

Finally, the reasons given by the collaborator families for not fortifying maize on a daily basis are shown in Table 5.

Table 5

Prepare tortillas for two or more days*
Feed other persons**
Preparation of corn tamales or tamalitos
Pregnancy (early months)**
Maize destroyed by insects
Maize not completely ripe

*Related to: Tortillas cannot be stored.
**Related to: Change in flavor.

The main reasons were related to either change in flavor or to problems related to storage of tortillas. The preparation of corn tamales or tamalitos was also mentioned. This is related to the problem of the high water solubility of the soy flour. They also mentioned that flour increased the sticky consistency of dough made from unripened maize or maize destroyed by insects and consequently tortillas could not be easily prepared.

c. Level of Fortification (Control of Fortification)

The level of fortification of the families was determined on a weekly basis in accordance with the information collected in the daily records of attendance at the mills. A weekly index of fortification of the family (family weekly fortification index: FWFI) was determined as presented in Table 6.

Table 6

Calculation of fortification index (F.I.)

$$\text{Family weekly FI} = \frac{\text{Days of attendance to the mill}}{\text{Days of the week (6)}} \times \frac{\text{Amount of fortifying mixture added to cooked maize}}{\text{Pounds of cooked maize}}$$

$$\text{Average family FI} = \frac{\text{Sum of family weekly F.I.}}{\text{Sum of weeks of observation}}$$

$$\text{Average child FI} = \frac{\text{Sum of family weekly F.I.}}{\text{Age of child in weeks} + \text{39 weeks of gestation}}$$

From this index, the average family fortification index (FI) or the average child FI could be calculated for any specific time. As the indexes were cumulate, they permitted the classification of the families in accordance with their level of fortification. The FI for one specific week was equal to the sum of the weekly indexes since the beginning of the fortification project, divided by the number of added weeks. A special index was prepared for each child born since February 1964 when the previous study in Santa Mariá Cauqué started. To be able to compare children born at different periods the time of gestation was considered. In this manner the child fortification index at birth was equal to the family fortification index corresponding to the 39 weeks previous to delivery (average duration of pregnancy).

For the children who were born before the onset of the corn fortification project, the index corresponding to the date of any of the anthropometrical, dietary, or morbidity surveys, was calculated taking the sum of the weekly family indexes at the moment of the survey, divided by the child's age in weeks, plus the 39 weeks of gestation.

An analysis of the frequency distribution of the weekly family indexes permitted the classification of the families in accordance with the levels of cooperation. The lower fortified group had a FI less than 20 and it constituted 51% of the families. The FI for the medium fortified group oscillated between 20 to 39 and corresponded to 13% of the families. The highly fortified group had a FI of 40 and more 36% of the families belonged to this group. The consistency in the collaboration of the highly and medium fortified group is shown in Tables 7 and 8.

Table 7

Variability in the Fortification Frequency
in the Highly Fortified Group* (FI: 40-100)
Santa María Cauqué, June 1972-May 1975

Trimesters of observation	Fortification index	Trimesters of observation	Fortification index
1	64±22**	7	71±17
2	69±20	8	75±16
3	71±21	9	78±22
4	75±19	10	55±26
5	79±17	11	51±31
6	55±13	12	60±34

*94 families
**Mean ± one standard deviation.

Table 8

Variability in the fortification frequency
in the medium fortified group* (FI: 20-39)
Santa María Cauqué, June 1972-May 1975

Trimesters of observation	Fortification index	Trimesters of observation	Fortification index
1	42±27**	7	32±27
2	47±27	8	24±24
3	42±27	9	27±31
4	37±23	10	14±19
5	27±33	11	17±25
6	26±20	12	23±31

*23 families
**Mean ± one standard deviation.

It can be observed that the highly fortified group was very consistent in the fortification. However, there were two periods when the collaboration dropped. The first period corresponded to the sixth trimester of observation, when their corn stocks became attacked by insects. The second period corresponded to the 10th and 11th trimesters, when two new mills came into operation in the village. About one third of the families decided to mill their maize in these two mills. It took about six months to convince the mill owners to participate in the program, after this period the collaboration began to increase.

On the other hand, the medium fortified group started with a FI between 42 to 47 during the first three trimesters. However, during the fourth trimester their FI began to drop steadily. During the twelfth trimester half of the families of this group did not fortify their maize at all.

The intense campaign to convince the families to fortify their maize was done only during the first three months of the study. Later on, it was found that the families were fortifying with a definite pattern, and it was decided to stop the enrollment campaign. However, the fortifying mixture was available free of charge in the four mills. Any family could decide whether they wanted to fortify their maize or not.

d. Socioeconomic Characteristics of the Collaborator and Non-collaborator Families

With the data collected through the census of 1971, the socioeconomic characteristics of the collaborator and non-collaborator families were determined. The following variables were considered: land tenure, cattle and poultry possessions, material utilized for house construction (floor, walls, roofs), water availability, fecal and waste disposal, literacy, scholarity, and shoe wear. Each variable was given a maximum value of 3 points and a minimum of 1. The highest value was assigned to the families who had better conditions. The socioeconomic index was formed by the sum of these variables. The lower and maximum values were 12 and 36 respectively.

The analysis of this information did not show differences among the three groups of families classified in accordance to the fortification index. The frequency distribution of the family socioeconomic index of the families with children less than 10 years old by fortification index is presented in Table 9.

Table 9

Fortification index	Number of cases	Socioeconomic Index				
		≤19	20-23	24-27	28-31	≥32-36
0-19	108	9(8)*	24(22)	38(35)	32(30)	5(5)
20-39	22	0	8(36)	5(23)	9(41)	0
40-100	93	7(8)	17(18)	38(41)	28(30)	3(3)
		X^2 = 8073		P = Not significant		

*Number of cases (rounded percentage).

Table 10

Family socioeconomic index of three variables in the families with children (<10 years old) in relation to the family fortification index
Santa María Cauqué, June 1971

Fortification index	Number of cases	Socioeconomic index		
		Land tenure	Scholarity	Shoe wear
0-19	108	1.98±0.83*	1.94±0.67	2.24±0.98
20-39	22	2.05±0.84	1.73±0.63	2.09±1.02
40-100	93	2.06±0.76	1.88±0.64	2.17±0.98

*Mean ± one standard deviation.

Three main variables (land tenure, scholarity, and shoe wear) were analyzed independently in the three groups of families. Table 10 shows that there were no differences.

Besides, a study of attitudes toward care of children and utilization of health facilities among two groups of mothers did not show any significant differences. Finally, a survey of the village clinic record of attendance did not show differences in the number of consultations by preschool children between the families with or without children (see Table 11).

Table 11

Medical consultation of preschool children in relation to maize fortification
Santa María Cauqué, June 1972-December 1975

Groups of fortification*	Child-months of observation (number of children)	Number of consultations	Mean number of consultation per 100 child-months
0-19	4339(203)	1243	28.6
40-100	3930(165)	1171	29.8

*Classified by Fortification Index.

These data show that the tendency to fortify the corn was not related to social or economic class.

e. Monitor of the Fortification Project

Monitoring was done at two levels. First, as explained previously, the level of fortification per family was determined daily. Second, tortillas were examined in the chemistry laboratory to determine total free lysine and protein. Random sampling of tortillas was obtained for 100 families every month. The results of these determinations may be observed in Table 12.

Table 12

Protein and total free lysine in a sample of 918 tortillas

Santa María Cauqué, June 1972-May 1975

Variables	498 fortified tortillas	420 unfortified tortillas	t value	P
Protein (g%)	10.7±1.2	8.7±1.1	26.45	<.001
Total free lysine (mg%)	55.9±23.2	19.7±13.1	28.31	<.001

It was found that although fortified tortillas had a larger content of protein and total free lysine and the differences were highly significant, the amount of protein in the enriched tortillas was only half of the expected value. The PER of fortified tortillas was between 1.90 and 2.19 and did not reach the expected value of 2.76. In this manner, it can be concluded that the fortification was done at a level of 4.6% and not at 8%, as planned.

Several explanations have been found for this partial failure in the fortification procedure. First of all, it was found that it was difficult to compress the flour into the measuring cups due to the fluffy consistency of the fortifying flour (mesh 150). Besides, due to the large volume of flour plus the amount of water added during the milling process, the volume of the dough was increased, reducing the final concentration of the fortifying mixture in the cooked tortillas.

3. Discussion of the Implementation of the Field Methodology and Acceptability of the Fortification

The field project here presented was the first attempt to fortify maize at a village level, utilizing a technology devised to be applied as an industria process. Although the fortification started successfully, several problems were immediately identified during the first two days of fortification.

The majority of the reasons for not accepting the maize fortification was related to the quality of the fortifying flour, especially because of its high water solubility. Housewives claimed that because of the big amount of the flour to be added, fortified tortillas could not be sotred for more than 12 hours. Furthermore, people began detecting changes in flavor, which became more noticeable when tortillas were mixed with coffee. An important aspect of the flavor change was that the fortified tortillas were a cuase of nausea in pregnant women. Besides, due to the finess of the fortifying flour it was not possible to fortify the miaze at the desired concentration of 8%. In conclusion, it can be said that the main causes for failure in the study were related to the quality of the flour that was utilized and not to the fortification per se.

The fortification technology devised for industrial purposes requires research at the field level, previous to the introduction of any food fortification program. For instance, the acceptability tests of fortified tortillas showed that villagers were unable to identify the differences in flavor. However, when fortified tortillas were consumed daily, flavor differences were easily found.

It can be said that the implementation of the maize fortification methodology was a success, considering that people allowed an intervention in their staple food. A positive aspect of the study is the fact that, in spite of the problems identified with the fortification procedure, 36% of the village families accepted regularly the fortification of their maize. The achievements and failures of the maize fortification methodology are summarized as follows:

1. It was possible to develop methodology to fortify maize.

2. It was possible to keep on the operation at mill level.

3. It was possible to maintain a surveillance on the frequency of fortification.

4. It was possible to obtain the initial collaboration of 95% of the families.

5. It was possible to maintain the collaboration of 36% of the families.

The identified problems can be solved by changing the characteristics of the fortifying flour. Recent experience at INCAP has shown that when a different flour is used, it is possible to add the required volume of the mixture and less water is retained (5).

II. Biological Effect of the Maize Fortification

In accordance with the design of the project, the fetal and postnatal growth rates, the morbidity in children 0 to 3 years old, and the preschool

mortality observed during the period of fortification were to be compared with the basal data collected in the same village from 1964 to May 1972. The data collected in the control village on fetal and postnatal growth as well as on preschool mortality was to be compared with changes observed in the experimental village. It was also to be utilized to determine secular changes occurring in the area during the period of the project. The methodology for the data collection as well as the plan of analysis has been published previously (17).

The data presented here correspond to the observations on fetal and postnatal growth, dietary intake, morbidity during the first three years of life, and preschool mortality.

The information collected in the control village showed that the patterns of growth during the fetal and 0 to 3 years of age periods, as well as the preschool mortality, were similar to the patterns of the experimental village before fortification (Urrutia, Reynoso, Rizzo, Mata, unpublished observations). However, it was decided that the most valuable comparison to detect changes induced by the maize fortification should be by analyzing the differences between the pregnant women and preschool children of the experimental village with different levels of fortification. This was decided after it was found that the tendency to fortify maize was not related to socioeconomic class.

The effect of maize fortification on fetal growth was assessed in the children who were born during the duration of the project. Changes induced by the improvement of the maize diet on growth from birth to 10 years of age were determined in the village children of these ages. The studies on dietary intake and morbidity during the first three years of life were conducted in the cohort of 70 children, as described in the first section of this report.

The records of natality and mortality, kept by the INCAP personnel, were utilized to determine the preschool mortality rate.

1. Results of the Biological Effect of Maize Fortification

a. Effect of Maize Fortification on the Dietary Pattern of Preschool Children

Dietary intake was measured utilizing the weekly recall method. Tables 13 and 14 show that there were no differences in the intake of calories and protein of preschool children in relation to their fortification index during the first three years of life.

This finding points out that the fortification did not change the amount of food consumed by the children, but it is important to consider that the group of fortified children received a better protein during a critical period of their lives.

Table 13

Relationship between the fortification index and the mean daily intake of calories in children from 18 to 36 months of age, Santa María Cauqué, June 1972-May 1975

Age (months)	Fortification index	Number of children	Calories
18th	0-19	23	405±194*
	20-39	7	488±229
	40-100	29	459±272
	Total	59	453±250
24th	0-19	28	712±289
	20-39	5	485±113
	40-100	32	733±360
	Total	65	705±319
30th	0-19	22	966±283
	20-39	6	1004±318
	40-100	33	903±258
	Total	61	936±271
36th	0-19	15	1121±349
	20-39	6	1131±494
	40-100	24	1019±273
	Total	45	1068±329

* Mean ± one standard deviation.

Table 14

Relationship between the fortification index and the mean daily intake of protein in children from 18 to 36 months of age, Santa María Cauqué, June 1972-May 1975

Age (months)	Fortification index	Number of children	Animal protein (g)	Vegetable protein (g)	Total protein (g)
18th	0-19	23	1.2±1.8*	7.5±3.1	8.7±4.5
	20-39	7	4.5±4.0	7.8±3.6	12.2±6.7
	40-100	29	3.6±3.6	8.8±5.0	10.4±7.1
	Total	59	1.8±3.2	8.2±4.3	10.2±6.3
24th	0-19	28	2.7±2.5	13.8±6.2	16.5±7.3
	20-39	5	1.5±1.0	10.1±2.4	11.6±1.9
	40-100	32	3.0±5.1	14.8±7.3	17.8±10.8
	Total	65	2.7±4.0	14.0±6.7	16.8±9.0
30th	0-19	22	2.4±2.4	19.5±5.0	21.9±6.2
	20-39	6	2.6±0.8	22.5±7.5	25.0±7.3
	40-100	33	2.3±3.1	20.6±6.8	22.9±7.3
	Total	61	2.4±2.6	20.4±6.2	22.7±6.9
36th	0-19	15	3.7±2.3	23.1±7.9	26.7±7.9
	20-39	6	3.1±1.3	24.5±10.5	27.6±10.3
	40-100	24	2.2±1.4	24.1±7.4	26.3±7.3
	Total	45	2.8±1.8	23.8±7.8	23.8±7.8

* Mean ± one standard deviation.

b. Effect of Maize Fortification on Fetal Growth

Fetal growth was determined in accordance to the anthropometric characteristics of newborns. Data on weight, height, head and chest circumferences at birth were available for 189 cases which represented 95% of the babies born in the village during the fortification project. Weight was recorded in the first hour of life by a nurse. Height and head and chest circumferences were measured by the physician during the first 24 hours of life. Several maternal variables such as birth order and mother's height were also obtained. For the analyses of the effect of maize fortification on fetal growth, the fortification index for each newborn was calculated as explained previously.

The relationship between birth weight, height, head circumference, birth order and mother's height with the fortification index for all full term babies born during the length of the project was analyzed. Table 15 shows these results for Santa María Cauqué, June 1972-May 1975).

Table 15

Variables	Number of cases	Correlation coefficient (r)
Birth order	189	.153
Mother's height	189	.094
Newborn weight	189	-.115
Newborn height	189	.030
Newborn head circumference	189	-.051

There was no apparent impact of the intervention on birth weight. Table 16 also shows that this finding was not due to differences in birth order of the child or maternal height. Moreover, similar analyses with height and head circumference of the newborn did also fail to show an effect. Lastly it was found through regression analyses that the fortification index was not related to growth in the newborn, either before or after controlling for the influence of the potentially confounding factors already mentioned (Santa María Cauqué, June 1972-June 1975). (Table 16)

Table 16

Variables	Fortification Index 0-19 $\bar{x}$ + S.D.	20-39 $\bar{x}$ + S.D.	40-100 $\bar{x}$ + S.D.
Birth weight* (grams)	2643±324	2702±285	2571±314
Fortification index	4±6	29±5	65±13
Birth order	4.1±3.1	4.2±2.9	4.8±3.6
Mother's height (centimeters)	143±4.2	145±5.3	144±4.7
Gestational age (weeks)	39.3±1.2	39.3±1.1	39.1±1.5
Number of cases	124	27	71

*Premature babies (< 37 weeks of gestational age) excluded.

Therefore, it was concluded from these analyses that the maize fortification program had no impact on fetal growth.

c. Effect of Maize Fortification on Postnatal Growth

Growth records for all village children born since the year 1964 were available. Measurement of weight, height, head and chest circumferences were obtained at 3 month intervals for children under 6 years of age, and at 6 month intervals for children older than 6 years. The measurements were collected by an anthropometrist, following procedures already established for the long-term interventions being carried out in Santa María Cauqué since 1964 (13).

The analyses on the relationship between the fortification index and attained height from birth to 8 years of age is shown in Table 17 (Santa María Cauqué, June 1972-May 1975).

Table 17

Age (years)	Number of cases	Correlation coefficient (r)
0	214	.045
1	168	-.118
2	167	-.045
3	153	.000
4	139	.010
5	126	.057
6	109	.021
7	108	.091
8	109	.021

None of the correlation coefficients were significant. A similar analysis with weight gave similar results suggesting that there was no apparent relationship between fortification and postnatal growth. In order to control for the influence of factors constant to the family, such as socioeconomic status and level of collaboration with the program, the following analysis was carried out. Sibling pairs were selected in families where growth information was available for both siblings at any of the following ages: birth - 6, 15, 24, 36, and 60 months, and where the older sibling had reached the age in question before the fortification project, while the younger sibling reached the same age during the course of the project. Thus, for every age the older sibling had no opportunity to benefit from the maize fortification. The analyses consisted of comparing the height and weight of both siblings, the expectation being that the younger sibling would be taller and heavier on account of fortification. The results of these analyses are shown in Table 18.

Table 18

Mean and standard deviations ($\bar{x} \pm$ S.D.) of heights and weights in sibling pairs, Santa María Cauqué, June 1972-May 1975

Age (months)	Number of sibling-pairs	Height (centimeters) Sibling 1*	Height (centimeters) Sibling 2**	Weight (grams) Sibling 1	Weight (grams) Sibling 2
0	76	45.7±1.8	46.3±1.6	2569(307)	2634(380)
6	76	59.6±2.3	58.9±0.9	6242(687)	6351(857)
15	76	68.0±2.7	67.5±2.6	7320(893)	7210(878)
24	64	74.4±3.8	74.1±3.6	8586(1004)	8641(998)
36	64	79.9±3.9	80.2±4.3	10304(1276)	10389(1253)
60	66	92.2±4.4	92.7±4.1	13509(1498)	13615(1580)

*Sibling 1 born before corn fortification.
**Sibling 2 born after corn fortification.

None of the comparisons between sibling pairs either for height or weight at the various ages revealed differences. Therefore, it was concluded that even after controlling for constant factors in the family, maize fortification did not seem to have had an impact on physical growth.

The analyses shown in Table 19 relate the increment in physical growth with the increment in fortification index, from 3 to 5 years of age. Although the correlation coefficients were not statistically significant, they do indicate a strong tendency for growth increments in both weight and height to be related to changes in the fortification index (Santa María Cauqué, June 1972-May 1975).

Table 19

Variables	Number of children	Correlation coefficient (r)
Weight	56	.169
Height	55	.100

Finally, an analysis was made of the effects of the fortification of maize on maturation utilizing bone development as an indicator of maturation. Table 20 shows that no significant correlation was found between the number of hand and wrist ossification centers and the fortification index from 6 to 96 months of age. An analysis of variance of the number of the carpal ossification centers in relation to the fortification index was also done. No differences were found in the three groups of children as can be seen in Table 21.

Table 20

Correlation between the number of hand and wrist ossification centers and the fortification index, from 6 months to 8 years of age
Santa María Cauqué, June 1972-December 1975

Age (months)	Number of cases	Number of ossification centers	Correlation coefficient (r)
6	76	2.4±1.8*	.060
12	114	3.3±2.6	-.051
18	139	4.9±3.9	.018
24	158	7.0±4.9	-.019
30	137	9.9±5.8	-.001
36	135	13.9±5.7	.000
42	124	16.5±4.9	-.095
48	121	18.0±4.6	.013
54	121	20.5±3.3	.045
60	109	22.1±3.5	.079
72	86	23.9±2.2	.106
84	42	25.4±2.3	-.139
96	19	26.6±2.0	-.250

*Mean ± one standard deviation.

Table 21

Analysis of variance of the number of the carpal ossification centers of children in relation to the fortification index
Santa María Cauqué, June 1972-December 1975

Age (years)	Fortification index 0-19	20-39	40-100	P
1	2.00 (67)	2.00 (23)	2.14 (23)	N.S.**
2	2.08 (102	2.05 (19)	2.03 (37)	N.S
3	2.17 (72)	2.22 (23)	2.20 (40)	N.S.
4	2.47 (75)	2.50 (16)	2.50 (30)	N.S.
5	3.09 (58)	3.33 (12)	3.41 (39)	N.S.
6	4.03 (38)	4.26 (19)	4.50 (30)	N.S.
7	5.64 (22)	5.30 (8)	5.05 (19)	N.S.

* Mean (number of cases).

** N.S. = Not significant.

Table 22

Correlation between the cortical thickness of the second metacarpal and the fortification index from 6 months to 8 years of age, Santa María Cauqué, June 1972-December 1975

Age (months)	Number of cases	Cortical thickness (mm)	Correlation coefficient (r)
6	76	0.92±0.19*	-.066
12	114	0.95±0.24	.176
18	139	0.99±0.26	-.055
24	158	1.12±0.25	-.156
30	137	1.18±0.28	-.018
36	135	1.18±0.30	-.082
42	124	1.21±0.28	-.067
48	121	1.25±0.29	.099
54	121	1.38±0.31	-.081
60	109	1.41±0.33	.140
72	86	1.54±0.32	.041
84	42	1.68±0.34	.008
96	19	1.82±0.35	-.271

* Mean ± one standard deviation.

A correlation analysis between the cortical thickness of the second metacarpal and the fortification index also did not show any relationship as presented in Table 22.

In summary, the analyses carried out suggest that maize fortification had had no significant effect upon physical growth and bone development.

d. Effect of Maize Fortification on the Morbidity by Infectious Illnesses

In order to test the effect of maize fortification on the morbidity of infectious illnesses, a subsample of 70 village children collected at random as explained in Section I were carefully monitored through weekly home visits by the field physician. Their health status was determined every week on each visit. The date of onset and duration of every illness were determined. The severity of the symptomatology was assessed by the number of days of fever and in the diarrhea cases, by the number of days with 4 to 6, 7 to 9, and 10 or more stools per day. The presence of vomiting and of mucus and/or blood in the feces was also recorded.

The results of the morbidity analyses are presented for disease incidence, duration, and severity, in relation to the fortification index. No differences in morbidity in the three groups of children were found during the first years of life. However, a significant reduction in morbidity was found in the children with high fortification during the second and third year of life. For this reason the results of the morbidity analyses are presented for this period of life only.

The size of the cohort of children for the morbidity study, as well as the number of episodes of infectious illnesses observed in the children per category of the fortification index, are presented in Table 23.

Table 23

Morbidity analysis, size of the sample
(number of children and episodes of illness)
Santa María Cauqué, June 1972-January 1976

Groups of Fortification*	Age intervals, months							
	12-17		18-23		24-29		30-35	
0-19	35**	189***	40	216	42	218	33	129
20-39	11	49	11	47	14	67	12	54
40-100	14	73	16	85	19	93	22	75
Total	60	311	67	348	75	378	67	258

*Classified by Fortification Index.
**Number of children.
***Number of episodes of illness.

A total of 1295 episodes of illnesses were observed; these were utilized for the analysis of the effect of maize fortification.

No differences in disease or incidence number of episodes of illness were found among the children with low, middle, and high fortification index, as shown in Table 24.

Table 24

Episodes of infectious illnesses per categories of the fortification index
Santa María Cauqué, June 1972-January 1976

Groups of fortification*	Age intervals, months			
	12-17	18-23	24-29	30-35
0-19	5.4**	5.4	5.2	3.9
20-39	4.5	4.3	4.8	4.5
40-100	5.2	5.3	4.9	3.4
P***	NS	NS	NS	NS

*Classified by Fortification Index.
**Average number of episodes of infectious illnesses per child.
***Analysis of variance.

This indicates that children of the three categories were subjected to the same force of infection. The relationship between the fortification index and various morbidity items are presented in Tables 25 to 29. In these analyses, the morbidity information is expressed in terms of percentage of the time ill per semester of life. For example, 10% of the time ill with diarrhea is equivalent to 18 days with diarrhea in a six month period. The results of the analyses of the morbidity data are expressed as the percent of time ill in the three groups of children classified in accordance to the fortification index.

Table 25

Percent of the Time Ill with Upper Respiratory Tract Infection

Santa María Cauqué, June 1972 - January 1976

Groups of Fortification*	Age intervals, months 12-17	18-23	24-29	30-35
0-19	10.9**	9.1	9.5	9.8
20-39	10.1	7.6	8.3	6.7
40-100	6.8	5.3	6.5	5.3
p*** 0-19/40-100	$<.005$	$<.005$	$<.005$	$<.005$

Table 26

Percent of the Time Ill with Lower Respiratory Tract Infection

Santa María Cauqué, June 1972 - January 1976

Groups of Fortification*	Age intervals, months 12-17	18-23	24-29	30-35
0-19	8.3**	7.4	8.8	4.0
20-39	6.8	5.4	2.9	3.7
40-100	5.7	3.1	4.6	4.6
p*** 0-19/40-100	$<.005$	$<.010$	$<.005$	N.S.

*Classified by Fortification Index
**Rate per 100 child-days
***Chi-square test.

Table 27

Percent of the Time Ill with Diarrhea

Santa María Cauqué, June 1972 - January 1976

Groups of Fortification*	Age intervals, months 12-17	18-23	24-29	30-35
0-19	16.8***	13.6	15.8	9.5
20-39	12.1	7.2	9.8	8.6
40-100	7.6	9.9	6.9	8.4
p**** 0-19/40-100	.005	.005	.005	N.S.

Table 28

Percent of the Time Ill with Associated Illnesses*

Santa María Cauqué, June 1972 - January 1976

Groups of ortification**	Age intervals, months 12-17	18-23	24-29	30-35
0-19	7.3***	6.5	6.7	3.5
20-39	6.0	5.7	4.9	2.6
40-100	4.6	2.7	2.9	2.4
p**** 0-19/40-100	.005	.005	.005	.025

*Respiratory plus diarrhea; respiratory plus conjunctivitis and/or stomatitis; conjunctivitis plus stomatitis; respiratory plus diarrhea plus conjunctivitis and/or stomatitis.
**Classified by Fortification Index
***Rate per 100 child-days
****Chi-square test.

Table 29

Percent of the Time Ill with Infectious Illnesses

Santa María Cauqué, June 1972 - January 1976

Groups of Fortification*	Age intervals, months			
	12-17	18-23	24-29	30-35
0-19	33.3**	29.6	32.8	20.7
20-39	32.8	24.5	21.1	20.2
40-100	22.8	19.5	17.8	19.8
p*** 0-19/40-100	.005	.005	.005	N.S.

Table 30

Percent of the Time Ill with Fever

Santa María Cauqué, June 1972 - January 1976

Groups of Fortification*	Age intervals, months			
	12-17	18-23	24-29	30-35
0-19	9.2**	7.8	10.4	5.1
20-39	7.4	4.8	5.8	5.5
40-100	4.0	4.3	3.7	5.6
p*** 0-19/40-100	.005	.005	.005	N.S.

*Classified by Fortification Index
**Rate per 100 child-days
***Chi-square test

Table 31

Percent of the Time Ill with Diarrhea of
7 or More Stools Per Day

Santa María Cauqué, June 1972 - January 1976

Groups of Fortification*	Age intervals, months 12-17	18-23	24-29	30-35
0-19	4.8**	4.6	4.6	1.9
20-39	5.0	2.2	2.7	2.7
40-100	1.4	1.8	1.3	2.1
p*** 0-19/40-100	.005	.005	.005	N.S.

*Classified by Fortification Index
**Rate per 100 child-days
***Chi-square test.

Tables 25 and 26 show that the group with low fortification index was nearly twice as sick with upper and lower respiratory tract infection as the high fortification group during the second and third year of life. The differences in duration of diarrhea, between the two groups of children were even larger (Table 27). Table 28 suggests that maize fortification also leads to fewer days ill with associated illnesses, that is to say, association of respiratory and diarrhea illness, or also respiratory or diarrhea illness associated to conjunctivitis and/or stomatitis. These differences were noticeable during the second and third year of life. This trend was not found for other disease, such as skin infections, conjunctivitis, and stomatitis alone. Table 29 shows that there is a negative correlation between the corn fortification index and the percentage of time ill with any infectious illness.

There was also a significant reduction in the severity of disease manifestation. Tables 30 and 31 show that the children consuming the highly fortified diet suffered less days of fever and of diarrhea with 7 ormore stools per day (severe diarrhea).

As a way of summary, Tables 32 and 33 present the differences between the high and low fortification groups (high-low) and the total reduction in number of illness days and of days with less severe manifestation of illness respectively.

Table 32

Reduction in number of illness days in the highly fortified compared with the low fortified children, during the second and third year of life, Santa María Cauqué, June 1972-May 1975

Diseases	Age intervals, months				Total
	12-17	18-23	24-29	30-35	
Total diarrhea	16.7*	6.7	16.2	2.0	41.6**
Upper respiratory tract infections	7.5	6.9	5.5	8.2	28.1
Lower respiratory tract infections	4.7	5.3	7.6	0.0	17.6
Total days of infectious illnesses	28.9	18.9	29.3	10.2	87.3

* The figures given show the reduction of illness days per semester of life between the highly fortified and low fortified groups.

** Reduction of illness days during the second and the third year of life.

Table 33

Reduction in number of days with fever and diarrhea with 7 and more stools per day in the highly fortified compared with the low fortified children during the second and third year of life Santa María Cauqué, June 1972-May 1975

Symptoms	Age intervals, months				Total
	12-17	18-23	24-29	30-35	
Total days of fever	9.4*	6.4	12.2	0.0	28.0**
Days of diarrhea with 7 and more intestinal movements a day	6.1	5.1	6.0	0.0	17.2

* The figures given show the reduction of illness days per semester of life between the highly fortified and low fortified group.

** Reduction of illness days during the second and the third year of life.

Clearly children in the high fortification group suffered fewer days of illness. The total reduction of disease duration during the two year period amounts to 87.3 days (Table 32), 28.0 days less of fever and 17.2 days less of diarrhea, with 7 and more stools per day (Table 33). This corresponds to a reduction of 31% in relation to disease duration during the second and third years of life in the groups of children with low fortification.

e. Effect of Maize Fortification on Infant and 1 to 4 Years Mortality

Since the beginning of the field operations in Santa María Cauqué in 1974 preschool mortality data have been collected continuously. The causes of death were determined either by premortem diagnosis done during the course of the disease, or by interrogations of the child's parents on the characteristics of the final episode of the fatal disease. In more than 90% of the cases the physician or the nurses had the opportunity to examine the child during the course of the fatal disease.

All births and deaths were recorded; in this manner it was possible to determine the number of newborns and inhabitants for each specific age from 1 to 4 years of age.

The mortality rate during the maize fortification project of 3 sets of families grouped according to the family fortification index were compared. Because of the long term data available it was possible to calculate in the same families, the infant mortality rates during the period before corn fortification (January 1964 to May 1972). These baseline mortality data are presented in Table 34. Though the low fortified group appears to have the highest mortality rates, the differences are not statistically significant.

Table 34

Infant mortality before corn fortification

Santa María Cauqué, January 1964-May 1972

Groups of fortification*	Number of deliveries	Number of deaths			Rate**
		Stillbirths	0-11 months	Total	
0-19	255	8	26	34	133
20-39	43	0	5	5	116
40-100	206	8	14	22	107
Total	504	16	45	61	121

* Classified by Fortification Index.

** Rate per 1000 deliveries.

The infant mortality rate for the period of corn fortification is presented in Table 35. The rates are expressed per 1000 deliveries, in each of the three fortification groups. The highest infant mortality rate corresponds to the low and medium fortified group. These figures are not significantly different from the baseline rates shown in the same groups of families before the fortification period (P .05). On the other hand, the infant mortality rate for the group of children with high fortification was reduced to nearly 3 times lower than that of the other two groups. In comparison to the baseline values, the high fortified group experienced a 50% reduction in mortality.

Table 35

Infant mortality during corn fortification

Santa María Cauqué, June 1972-January 1976

Groups of fortification	Number of deliveries	Deaths			Rate*
		Stillbirths	0-11 months	Total	
0-19	166	6	15	21	127
20-39	23	0	4	4	174
40-100	115	2	3	5	43
Total	304	8	22	30	99

*Rate per 1000 deliveries.

Table 36 shows that this reduction in infant mortality was not due to a decrease in the proportion of low birth weight babies. First of all, the proportion of low birth weight babies was similar across all three groups. Secondly, the relationship between corn fortification and infant mortality was still evident within the range of low birth weight babies. The mechanism involved, therefore, appears to be independent of birth weight.

Table 36

Infant mortality in low birth weight babies*

Santa María Cauqué, June 1972-January 1976

Groups of fortification	Number of deliveries	Number of LBW babies	Percent of all deliveries	Number of deaths	Rate
0-19	166	64	38.6	11	172**
20-39	23	9	39.1	3	333
40-100	115	43	37.4	2	47

<*2500 g birth weight.
**Rate per 1000 deliveries.

TABLE 37

Second to fifth year mortality during corn fortification*

Santa María Cauqué, June 1972-January 1976

Mortality (years)	Groups of families by Fortification Index											
	0-19			20-39			40-100			Total		
	No. of children	No. of deaths	Rate	No. of children	No. of deaths	Rate	No. of children	No. of deaths	Rate	No. of children	No. of deaths	Rate
Second	163	5	31	24	4	167	126	3	24	313	12	38
Third	143	3	21	24	0	0	118	1	8	285	4	14
Fourth	103	2	19	18	3	167	88	0	0	209	5	24
Fifth	89	1	11	15	0	0	81	0	0	185	1	5
Total 2nd-5th	498	11	22	81	7	86	413	4	10	992	22	22

* Rate per 1000 children of that age.

The relationship between corn fortification and mortality during the second, third, fourth, and fifth year of life is shown in Table 37.

The rate for the second year of life was similar in the low and the high fortified groups. However, during the third to the fifth year of life only one death was observed for the group of children with a fortification index of 40-100. The total number of deaths were 11, 7, and 4 for the low, medium, and high groups of fortification respectively. The rate of mortality for the whole village corresponding to this period of life here studied for the period before the corn fortification project (see Table 38) was not significantly reduced, but it is important to notice that the mortality for children with high fortification was reduced to a third, and that only one death was observed after the age of two years in this group of children.

Table 38

Second to fifth year mortality
before corn fortification
Santa María Cauqué, January 1964-May 1972

Mortality	Number of children	Number of deaths	Rate*
Second year	454	26	57
Third year	389	13	33
Fourth year	376	5	13
Fifth year	357	1	3
Total	1567	45	29

*Rate per 1000 children of that age.

In summary it could be stated that corn fortification has had an effect on infant mortality and on the third, fourth, and fifth year of life mortality.

Discussion

The significant differences in the morbidity between children with high and low fortification during the second and third year of life, as well as the significant reduction in preschool mortality in the group of children highly fortified, indicates a good effect of the improvement in the quality of the maize protein. No change in the morbidity during the first year of life was observed, but the reduction in the infant mortality is a strong indicator of the effect of maize fortification during that period. As children during their first year of life do not eat tortillas in significant amounts, the effect of the miaze fortification could have been mediated through an improvement in the quality of the mother's milk. Further, the fact that children less than one year old do not become ill as frequently as they do when they are two or three years old (13) must be taken into consideration.

The fact that the intervention had a positive effect during the weaning period (12 to 30 months of age), when the infectious illnesses are more frequent, more severe, and have a longer duration, is of great importance in public health. During this period of life, children are not only growing inadequately, but the infectious illnesses have been identified as an important factor in the causuality of malnutrition (14). A reduction in the duration and severity of the illnesses results in a decrease of the nutritional damage induced by these diseases.

Corn fortification did not produce any change in the dietary pattern of children, and as a consequence the caloric deficit of the diet of children was not corrected; this could be the reason why no effect on growth was observed. However, the positive effect on morbidity and mortality is showing that the nutritional status of children was improved by increasing the host defense mechanisms, which are reduced in malnourished children (16-22). Since highly fortified children received a protein of better quality the cellular immunity could have been improved as it has been shown to occur when acutely malnourished children received an adequate dietary therapy (7,9,22,25).

In dealing with the subject of prevention of malnutrition, the relative importance of improving food intake as opposed to controlling the infectious environment has not been clarified. These results show that improvement in the quality of children's diets during the first three years of life is a good means of enhancing resistance to infections. These facts should be taken into account when deciding which kind of intervention should be done in countries with a high prevalence of protein-calorie malnutrition and a contaminated environment, since changes in the environmental conditions do not occur rapidly.

An aspect to be considered in any nutritional intervention during the weaning period is that the food to be offered should be processed with locally available food, and prepared in a suitable form to be consumed easily by the weanling.

The results of this research project showed that the methodology to evaluate the effect of nutrition improvement programs in children should include, in addition to growth, a very detailed morbidity surveillance, and the determination of the host defense mechanisms by measuring humoral and cellular immunity before and after the intervention.

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IMPROVEMENT OF PROTEIN QUALITY IN MAIZE [1/]

D. V. Glover

There has been a growing awareness of the world food supply situation. We now know of the urgent need for massive efforts to increase agricultural productivity and improve the nutritional value of plant materials for human and animal food, particularly in developing countries and simultaneously to raise the incomes of hundreds of millions of their farmers and other rural people.

Cereals provide a major portion of the calories and protein in human diets in many developing countries. Today the world obtains about 50% of its dietary protein from cereals, about 20% from legumes, and 30% from animal products (13). In developing countries, animal products contribute only 10% of the protein and cereals over 70%. Thus we find in many parts of Asia, the Near East, Africa and Latin America that the people are getting two-thirds or more of their protein from cereals.

As compared with cereals, more land is required to produce each unit of animal protein. Moreover, the cost of animal protein is much higher than protein from legumes and cereals. Scientists are working on better ways to produce animal products, but there is no present prospect for a breakthrough which will use less land, or reduce the price of animal products.

Legumes are much lower in price than meat, and require less land to produce each unit of protein, but yields of legume crops are much lower than those of cereals, and farmers have actually been reducing the land they devote to legumes.

The increased pressure of population upon the world's agricultural land will make it more and more difficult to supply the protein requirements of developing countries from meat and other animal products. Unless scientists can find ways to produce much higher yields in legumes, the farmers are not going to produce enough legumes to satisfy future population growth. Thus cereals are likely to play a larger and larger role in the calorie-protein supply in the human diet.

Maize is the third most important cereal crop grown for human consumption in the world, being surpassed only by rice and wheat in worldwide importance. Maize is a principle source of protein and calories for many millions of people, particularly in the Latin American area, Africa, and other regions of the world. Maize dominates the production statistics of principle grain crops in the U.S. with 143 million tons, exceeding the total production for all of the other grains (Agricultural Statistics, 1974). In the U.S. corn produces an estimated 14 million tons of protein annually, which is nearly 30% of the total produced by all the principle crops (including soybeans).

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If world food production is to keep pace with world population it will be necessary to increase production on our land resources and increase the efficient use of our land resources. Protein production from grain crops can be increased by: 1) increasing total yield, 2) increasing the percentage of protein in the grain without changing yield, 3) improving the nutritional value of the protein, and 4) some combination of the first three factors. Yield, protein content, and protein quality all are subject to the possibility of genetic modification and to increase by suitable cultural practices, especially fertilizer use.

The eventual development and utilization of higher protein quality maize hybrids and varieties which yield competitively is one important way to meet the world's human and animal nutritional needs.

It has been twelve years now since the group at Purdue University (11) demonstrated that the opaque-2 gene almost doubled the lysine content over normal corn. Despite the widespread breeding work with opaque-2 in most countries where maize is of importance (though very small in relationship to the total breeding effort in maize -- perhaps less than 5%), there is today little significant utilization of opaque-2 hybrids and varieties. Undesirable kernel characteristics and relatively low yields associated with earlier developments of opaque-2 endosperm materials have hindered widespread acceptance of high-lysine maize. More rapid utilization of high-lysine maize has also been limited in the U.S. by economic considerations involving yields and relative price of protein supplement.

Research in our laboratory at Purdue has focused upon: 1) a search for and evaluation of new mutants in germplasm sources with improved protein quality and quantity, 2) selection for modified endosperm types with kernel characteristics similar to normal kernel phenotypes and having high nutritive value of the opaque-2 grain, 3) selection of double-mutant endosperm combinations which improve kernel characteristics and protein quality of opaque-2 and 4) developing special varieties and source breeding materials with improved agronomic characteristics for use in the LDC's and temperate zones of the world (9).

The main objective of this paper is to give an update on the state of progress in breeding for improved quality high lysine maize and review a few of the results in the following areas, namely: 1) the selection for high-lysine types in normal maize, 2) improving the kernel characteristics of opaque-2 types through genetic modifiers and the development of double-recessive combinations with opaque-2, 3) the development of high-lysine source germplasm materials, and lastly 4) to explore the yield potentials and variability among high-lysine opaque-2 germplasm developments and the relationship of protein to calorie production.

Selection for High Lysine in Normal Maize. Definitive data are needed on selection for improved lysine content in normal maize. This approach would have the theoretical advantage of improving protein quality without the agronomic disadvantages of lower yield, ear rot, and/or grain insect damage which often is associated with the use of new mutant genes. Normal kernels from 100 segregating families (ears) of the original random-mated versions of Temperate HA and Temperate HB populations have been analyzed for protein and lysine and results are shown in Table 1 (4, 9).

Table 1

Variation in Protein Quality in Endosperms from 100 Normal Families (ears) each in Temp HA and Temp HB.

	Temp HA	Temp HB
Percent Protein		
-Mean	11.1	10.5
-Range	7.3-14.6	7.9-14.3
Lysine/Protein		
-Mean	1.71	1.74
-Range	1.26-2.28	1.43-2.13
Lysine-sample		
-Mean	.187	.181
-Range	.150-.238	.154-.226

Source: Bauman, L. F. (4, 9)

Variations were 7.3 to 14.6 for percent protein, and 1.26 to 2.28 for lysine as percent of protein and .150 to .238 for lysine as per cent of sample. This variation is not as great as we would have liked to have seen but we are continuing the study and perhaps the variation is sufficient to ensure some progress (though limited) in selecting for protein quality in these normal populations.

Modified Opaque-2. A very promising approach to overcoming some of the deficiencies of the opaque-2 types involves visual selection of more specific modifiers of opaque-2. It is fairly easy to develop modified (vitreous) opaque-2 varieties with good ear rot and grain insect resistance. And these varieties have excellent protein quality if selections are made based on analysis. The kernel expression of the modified types might be roughly classified thus: 1) vitreous endosperm that is nearly indistinguishable from normal, 2) 1/2 to 1/2 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 soft starch is distributed irregularly in otherwise vitreous starch (3, 4).

These modifier genes, acting on the expression of opaque-2, are heritable, tend to be recessive, and in most sources 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 (14). There is a negative relationship between vitreousness and protein quality (Table 2), but selection based on analysis can maintain higher levels of protein quality.

Table 2

Mean Values from Four Hybrids of Protein and Lysine in Maize Endosperms

	Modified opaque-2 endosperm			
	Opaque portion	Vitreous portion	Whole endosperm	"Normal" endosperm+
	----------Protein percent----------			
Grand mean	7.8 c*	11.5 a	9.6 b	11.4 a
	----------Lysine as a percent of protein-------			
Grand mean	3.66 a*	2.11 c	2.67 b	1.46 d
	----------Lysine as a percent of sample----------			
Grand mean	0.28 a*	0.24 c	0.26 b	0.16 d

+ Obtained by outcrossing the modified opaque-2 to normal pollen source, giving the endosperm genotype +/o2/o2.

* Means followed by the same letter do not differ significantly at 0.05 level (DMR-test).

Source: Ribeiral, U. C. (14)

An analysis of the amino acid composition of defatted maize endosperms from modified opaque-2 indicate that for the most part the vitreous endosperm portion has intermediate amino acid values between those obtained in the opaque-2 portion versus the normal endosperm types. In Table 3 are illustrated the nitrogen distribution in maize endosperms of modified opaque-2 types. The reduction in the zein fraction is not nearly as extensive in the vitreous portion as is observed in the opaque portion of the endosperm. The modified and opaque portions within the kernel differ greatly in protein with the modified portion being higher in quantity but lower in quality (lysine) than the opaque portion. Amino acid profiles of the modified portion show a shift in the pertinent amino acids towards the "normal" type of profile. The modified expression (thereby probably protein quality also) varies with different environments, with lower temperatures enhancing the modified phenotype.

Table 3

Nitrogen Distribution in Maize Endosperms

Fraction	Percent of soluble nitrogen+							
	M-3 x M-8				M-3 x M-6			
	Opaque portion	Vitreous portion	Whole endosperm		Opaque portion	Vitreous portion	Whole endosperm	
			Modified	'Normal‡'			Modified	'Normal'
I. (Alb. & Glb.)	17.02	10.11	7.34	4.98	16.95	10.05	13.63	6.08
II. (Zein)	15.82	42.19	24.13	46.36	15.92	38.53	24.15	45.76
III. (Glut.-1)	9.06	20.28	20.80	20.30	10.31	15.75	15.87	20.37
IV. (Glut.-2)	15.46	9.72	16.20	9.45	22.69	12.90	14.22	14.41
V. (Glut.-3)	42.63	17.71	31.54	18.90	34.14	22.78	32.14	13.39

+ Data adjusted for 100 percent recovery.
‡Obtained by outcrossing the modified endosperm to a normal pollen source.

Source: Ribeiral, U. C. (14)

Performance of Modified and Non-Modified Selections in Opaque-2 Populations. Visual selections for kernels with modified and non-modified phenotypes were made in four opaque-2 populations (4). These selected classes were then grown in a yield test of six replicates and data on performance obtained as shown in Table 4.

Table 4

Characteristics of Modified and Nonmodified Selections in Four Populations

	Modified		Nonmodified
Yield (g/plant)	128.5		126.9
Kernel weight (g/50 K)	12.7		12.6
Modification factor[1/]	26.0	*	15.2
Percent modified	36.4	*	23.0
Percent protein	11.6		11.9
g lys/100 g protein	4.0		4.1
Kernel rot grade	1.2		1.3

*Indicates significant differences between modified and nonmodified classes.

1/Based on combination of percent of kernel modified and the degree of modification.

Source: Camerer, W. (4)

The selected versions did differ in modification factor and percent modified, although not by a large margin. The selected versions did not differ significantly for yield, kernel weight, percent lysine, grams lysine per 100 grams protein, nor kernel rot grade.

Skewed selections were made within two of the populations to further evaluate the effect on performance of selection for modified phenotype. The data in Table 5 show a much larger difference in modification factor and percent modified kernels (43.3 versus 8.8%). This skewed selection did not result in any significant difference in yield, kernel weight, or protein characteristics. Kernel rot grade was significantly less in the modified version.

Table 5

Characteristics of Modified and Nonmodified Skewed Selections in Two Populations

	Modified		Nonmodified
Yield (g/plant)	137		139
Kernel weight (g/50 K)	12.0		12.0
Modification factor[1/]	30.6	*	5.0
Percent modified	43.6	*	8.8
Percent protein	11.56		11.8
g lys/100 g protein	4.25		4.27
Kernel rot grade	1.21	*	1.42

*Indicates significant differences between modified and nonmodified classes.

1/Based on combination of percent of kernel modified and the degree of modification.

Source: Camerer, W. (4)

A very critical question involving the practical utilization of selected high lysine "modified" opaque-2 varieties is concerned with the stability of the vitreous expression in high lysine characteristics under random mating and various environmental conditions when used by the farmer. Two groups of materials were developed to evaluate the genetic and environmental stability of "modified" opaque-2.

Genetic and environmental stability were studied in several opaque-2 synthetics (12). High and low lysine versions of HMO synthetic were selected for modification, and Temperate A and Temperate B synthetics were selected for both opacity and modification giving eight different synthetic versions. On each of these synthetics, random mating was simulated three times to give four generations. These synthetics were studied in two different experiments in the summer of 1975. In experiment I, the fourth generation from each synthetic was planted at each of three locations. In experiment II, two different planting dates were studied, with all four generations included of each of the eight synthetics. Plant characteristics measured were ear height, percent lodging, and ear yield per plant. Kernel characteristics measured were percent modified kernels, modification index, weight, and volume of 100 kernels, specific gravity, and percent protein and percent lysine in protein on a whole kernel basis. To summarize some of the conclusions the following points may be cited: 1) selection for higher lysine content in HMO synthetics was effective and this difference was constant throughout the subsequent random mating generations. Lysine values were also constant with these generations (Table 6). Thus random mating modified and non-modified opaque-2 populations for four generations without selection resulted in little or no change in nutritional value and the degree of modification was maintained. This indicates that modified opaque-2 varieties (except for outcrossing to

Table 6

Percent Modified Kernels, Modification Index, Weight and Volume of 100 Kernels, Kernel Specific Gravity, and Percent Protein and Percent Lysine in Protein Whole Kernel Basis for Selections and Generations (Experiment II).*

	Modified		100 Kernel				
	%	Index	Weight (g)	Volume (ml)	Specific Gravity (g/ml)	Protein %	Lysine (g/100 g pro)
SELECTIONS							
N + O	35.50a	0.705a	25.94b	22.30b	1.165a	11.31a	4.12b
M + M	56.55b	1.183b	24.84a	20.75a	1.197b	11.40b	3.93a
LSD	2.21	0.069	0.57	0.44	0.005	0.08	0.04
GENERATIONS							
1	47.63a	1.000b	25.55a	21.55a	1.185a	11.39a	4.02a
2	47.19a	0.954ab	25.39a	21.52a	1.182a	11.32a	4.02a
3	44.94a	0.918ab	25.39a	21.57a	1.180a	11.36a	4.07a
4	44.34a	0.904a	25.24a	21.46a	1.178a	11.37a	4.02a
LSD	3.91	0.084	0.68	0.54	0.010	0.39	0.11

*Values with the same letter were not different with Tukey's procedure at p = 0.05.

Source: Navarro, A. (9, 12).

normal) will tend to retain their desirable characteristics when grown by the farmer for successive generations, 2) selection for higher lysine as a percent of protein resulted in decreased protein percent, frequency of modified kernels, modification index, and specific gravity and increased kernel volume; 3) selection for higher modification resulted in increased specific gravity and decreased kernel volume in all syntehtics. Protein content was increased in the HMO's. Kernel weight was decreased as an effect of selection for high modification only in the HMO synthetics; 4) lysine in protein was decreased as an effect of selection for higher modification.

Feeding Experiments with High and Low Protein with High and Low Lysine in Modified Opaque-2 Germplasm. A feeding experiment with rats to determine the biological value of some available materials falling into a low protein high lysine class (LP-HL) and a high protein low lysine class (HP-LL) was conducted (9). Maize samples used were S_2 families taken out of the high and low (modified selections) lysine intercrosses developed from HMO opaque-2 synthetic. Two S_2 families were selected to represent each class (LP-HL 1, LP-HL 2, HP-LL 1, HP-LL 2). Two checks were also included: A three-way opaque-2 hybrid (OCK) and its normal counterpart (NCK). Casein was used as a standard. Maize was used as the only protein source for each diet, six weanling rats were assigned to each diet.

Table 7 shows values obtained for grams protein per 100 grams sample, grams lysine per 100 grams protein, and grams lysine per 100 grams sample for the six samples. The mean protein efficiency ratio (PER) and the mean feed efficiency ratio (FER) for the seven diets are also included.

Table 7

Protein quality, protein efficiency ratio (PER) and feed efficiency ratio (FER) for modified opaque-2 corns with high and low lysine with high and low protein values.

	Protein %	Lysine/ protein	Lysine/ sample	Mean PER	Mean FER
LP-HL 1	9.08	4.07	.36	2.51	.22
LP-HL 2	9.20	4.08	.38	2.66	.23
HP-LL 1	11.48	3.49	.36	2.20	.24
HP-LL 2	12.35	3.00	.40	2.14	.25
Opaque-2 check	8.70	4.10	.36	2.40	.20
Normal check	7.70	2.85	.22	1.24	.09
Casein control				3.43	.30

Source: Annual Report, 1976 (9)

The mean PER values for two modified samples, LP-HL 1 and LP-HL 2 are comparable to the opaque-2 check. This indicates that with selection, one could breed for modified phenotypes without losing the superior protein quality of opaque-2 phenotype. This study also shows that the PER is influenced more by the quality than by the quantity of the protein that is in the diet.

Feed efficiency ratio (FER) values are almost the same for all the modified samples tested, including the opaque-2 check. This shows that for the two classes, the proportion of lysine relative to the amounts of the other amino acids supplied by the protein resulted in gains in weight which are equal to or even a little bit better than that obtained by the opaque-2 check. It is difficult to predict, however, how far one could shift values for grams protein per 100 grams sample and grams lysine per 100 grams protein and still not produce an amino acid imbalance in the diet.

Single Gene Modifier of Opaque-2. A very promising, apparently simply inherited gene modifier has been found that gives a vitreous kernel in combination with an opaque-2 gene (4, 9). This modifier modifies the soft endosperm of opaque-2 giving more vitreous (normal) appearance. This modifier gene is particularly promising because it does not reduce lysine levels as is true with most modified opaque-2 varieties (Table 8). Results show a slight reduction in the lysine level. This reduction was not statistically significant for the small experiment which was conducted.

Table 8

Kernel weight, protein and lysine of modified and opaque kernels.

Phenotype	Kernel wt. (g)	Percent Protein	Lysine/ Protein
Vitreous*	10.7	11.8	3.7
Opaque*	10.6	11.7	3.6

*Based on analysis of 12 families.

Source: Annual Report, 1975 (4).

This factor has been introduced into different genetic backgrounds. The phenotype expression varies considerably with the different genetic backgrounds. This factor seems to be quite simply inherited with some degree of dominance. This is shown by the fact that the F_1 ear of the modified opaque parent and an opaque parent will usually contain a degree of modification. After two self-pollinations it is possible to recover completely modified ears and completely opaque ears. It is

apparent that this modifier gene would greatly simplify the breeding of more acceptable vitreous opaque-2 varieties with good nutritional qualities, however, this system probably will not result in a yield improvement of opaque-2 varieties.

Studies of the modified (vitreous) opaque-2 types may be summarized as follows: a) modified varieties are more acceptable for human food uses and more resistant to grain insects and ear rots, b) modified varieties can be selected from any heterozygous population, c) there is a negative relationship between vitreousness and protein quality but selection (based on analyses) can maintain higher levels of protein quality, and d) the modified phenotype varies with different environments.

Double-Mutant Combinations. We have been studying the modification of kernel characteristics and the nutritional quality of maize through the genetic interactions which result from double-mutant combinations of endosperm mutants. We have found that the double-mutant sugary-2 opaque-2 has improved kernel vitreousness and kernel density similar to normal (4, 9). In Table 9 are shown means for several hybrids for the kernel characteristics 100 kernel weight, 100 kernel volume and kernel density.

The sugary-2 opaque-2 double-mutant is nearly as dense as the normal kernels (6, 7). However, the kernel size as indicated by 100 kernel volume is considerably reduced. Thus the 100 kernel weight is also reduced. The improved kernel vitreousness and kernel density of the sugary-2 opaque-2 double-mutant kernels should improve the acceptability as well as the ear rot and storage insect resistance and dry milling of the grain.

Table 9

Kernel characteristics (100-kernel weight, 100-kernel volume, and kernel density) from six hybrids of su_2o_2, su_2, o_2 and the normal counterpart hybrids.

Genotype	100 k Weight (g)	100 k Volume (ml)	Kernel Density (g/ml)
Normal	34.52	26.81	1.29
o_2	29.00	25.52	1.14
su_2	29.87	22.44	1.33
su_2o_2	27.07	21.59	1.25

Source: Glover and Tosello (7)

We have demonstrated experimentally with the use of interference-contrast light microscopy that sugary-2 opaque-2 maize has a more extensive protein matrix than observed in opaque-2 (2). The improved kernel vitreousness appears to be associated with a more extensive thick matrix from the subaleurone layer which is continuous into the center of the endosperm with many subcellular enclosures that surround the starch granules, thus there are perhaps fewer air spaces resulting in refractive indices similar to the endosperm of normal maize.

The improved vitreousness of sugary-2 opaque-2 is not necessarily accompanied by a decrease in protein quality as is illustrated in Table 10 (7, 8).

Table 10

Protein and lysine concentration of endosperm fraction samples from six hybrids of su_2o_2, su_2, o_2 and the normal counterpart hybrids.

Genotypes	Protein (%)	Lysine as percent of protein	Lysine as percent of sample
+	10.41	1.75	.18
o_2	8.98	3.25	.29
su_2	11.04	2.20	.24
su_2o_2	9.21	3.72	.34

Source: Glover and Tosello (7).

When sugary-2 is combined with opaque-2 the double-mutant suppresses zein synthesis and shows a synergistic effect. And sugary-2 opaque-2 kernels have slightly higher lysine and protein values than opaque-2 counterparts. The starches of sugary-2 opaque-2 endosperms have a much faster rate of enzyme starch hydrolysis. The sugary-2 opaque-2 kernels also contain a greater oil content. The increase in oil percent and actual content of the grain is associated with approximately a 1% increase in germ size compared to its normal counterpart and also due to some increase in actual density of the oil to the germ fraction. We have demonstrated also that the sugary-2 opaque-2 grain has an apparent biological value equal to or greater than the opaque-2 maize, thus enhancing the potential nutrient value of sugary-2 opaque-2 combinations. Illustrated in Table 11 are the comparisons of the normal, opaque-2, sugary-2, and sugary-2 opaque-2 diets compared to casein control in terms of a rat performance and feeding trial fed at 10% protein levels. The double-mutant has a higher protein efficiency ratio (PER) than does the opaque-2, however, it is not significantly higher.

Table 11

Performance of Oh43 x B37 hybrids in rat feeding trial.+

Genotype	Feed/Gain Ratio	Daily Wt. Gain (g)	P.E.R.
Normal	7.43	1.09	1.37
Opaque-2	4.30	2.68	2.35
Sugary-2	6.81	1.44	1.57
Sugary-2 Opaque-2	3.92	3.17	2.57
Casein	3.63	3.22	2.80

+Ten weanling male rats in each group: Duration 28 days.

We have conducted rather extensive metabolism, growth and digestion trials involving rats and pigs to evaluate the protein quality, energy utilization and digestibility of several different types of corns in near-isogenic backgrounds of the maize three-way hybrid [(Oh 43 x B37) x C103] (15, 16). The six near-isogenic versions of the three-way maize hybrid were normal, opaque-2, waxy, waxy opaque-2, sugary-2, and sugary-opaque-2. These experiments indicated that the double-mutant sugary-2 opaque-2 is similar to opaque-2 in protein quality. Energy utilization in the sugary-2 opaque-2 is as good as for normal corn for growing pigs. The growth results obtained in these experiments indicated that the different starch types had no effect on performance of rats and pigs.

We have studied the nitrogen retention of young men who consumed isonitrogenous diets containing normal, opaque-2, or sugary-2 opaque-2 corn (5). Sugary-2 opaque-2 corn which had a hard or dense endosperm instead of the soft endosperm and opaque-2 maintained nitrogen equilibrium in all men who consumed 5.5 g of nitrogen. Therefore, at this nitrogen level it was equal or superior to opaque-2 corn, but tended to be less effective when whole ground corn furnished only 4.4 g of nitrogen, although the diets were isonitrogenous. Therefore, 5.5 g of nitrogen furnished by approximately 380 g of either opaque-2 or sugary-2 opaque-2 corn, equivalent to 0.5 g of protein per kilogram of body weight, should meet minimal protein requirements of all or most men weighing 70 kg and cover integumental losses of most whereas a similar amount of nitrogen from normal corn would maintain equilibrium in only half of them.

Development of Temperate Germplasm. During the first coordinating conference with CIMMYT the urgent need for varieties adapted to the temperate areas of the world was recognized (4). This project is developing two opaque-2 varieties, Temp HA opaque-2 and Temp HB opaque-2 from diverse germplasm from around the world and the U.S. corn belt (we also

have the "modified" opaque-2 variety HMO). These populations should permit the development of non-modified or modified opaque-2 varieties. Approximately 150 full-sib families are being tested at four worldwide locations. In Table 12 are shown the variation in protein quality of these two populations.

Table 12

Variation in protein quality of three opaque-2 temperate varieties.

	Temp HA C_2	Temp HB C_2	Colus C_1S_1
Percent Protein			
Mean			12.3
Range	10.2-12.9	9.0-12.5	9.4-15.7
Lysine/Protein			
Mean			4.28
Range	3.21-4.76	3.01-4.80	3.63-5.07

Percent protein and lysine as percent of protein at the Indiana location were for Temp HA 10.2 to 12.9% and 3.21 to 4.76% and for the Temp HB 9.0 to 12.5% and 3.01 to 4.80%, respectively. Selections should be quite effective in establishing excellent protein quality in these two populations and additional cycles of selection are being conducted to improve the agronomic performance.

To further fill the need for varieties adapted to temperate areas, the variety Colus developed at Purdue from Colombian and U.S. germplasm has been converted to opaque-2 and is undergoing selection. Ranges in protein and lysine as a percent of protein for Colus cycle one, self-pollinated one generation were 9.4 to 15.7% and 3.63 to 5.07% with mean values at 12.3 and 4.28%, respectively (Table 12).

Considerable effort is being concentrated on the development of special varieties and source breeding materials using the sugary-2 opaque-2 double-mutant. Selection and improvement based on yield, kernel vitreousness, and protein quality of sugary-2 opaque2 varieties, Temp HA sugary-2 opaque-2 and Temp HB sugary-2 opaque-2 is in progress. Full-sib family selection and evaluation of 270 families based on the yield, kernel vitreousness and protein quality have been completed for cycle one and these values are shown in Table 13.

Table 13

Variation in protein quality of two sugary-2 opaque-2 temperate varieties.

	Temp HA C_1 S_1	Temp HB C_1 S_1
Percent Protein		
Mean	12.5	13.1
Range	8.5-16.5	10.4-16.1
Lysine/Protein		
Mean	4.29	4.30
Range	3.53-5.67	3.57-5.54

Ranges in protein and lysine as a percent of protein for Temp HA sugary-2 opaque-2 were 8.5 to 16.2% and 3.53 to 5.76% with mean values of 12.5 and 4.29%, respectively. Ranges in protein and lysine as a percent of protein for Temp HB sugary-2 opaque-2 were 10.4 to 16.1% and 3.57 to 5.54% with mean values of 13.05 and 4.30%, respectively. The protein quality is maintained in sugary-2 opaque-2. There is a range in variability and an examination of the distribution show them to be normally distributed. Kernel vitreousness was markedly improved in the sugary-2 opaque-2 kernels compared to those of opaque-2.

Grain Yields in High Lysine Hybrids. A uniform test of experimental opaque-2 hybrids was organized and conducted in four states (IL, IA, IN, MO) in the U.S. corn belt (4). The results are given in Table 14.

The results of this study indicate the yields of opaque-2 hybrids range from 50 to 94 quintels per hectare (140 bushels/A). A number of these experimentals gave excellent yields that would be quite competitive with some of the better normal commercial hybrids. We have known that the opaque-2 gene interacts with different genetic backgrounds, therefore, as opaque-2 hybrids or varieties are reconstituted some perform poorly with regard to yield and/or grain quality, while others are more nearly equal to normal hybrids. The protein content and quality in the uniform test of experimental opaque-2 hybrids was also quite good with ranges of 8.62 to 10.74 protein percent, 3.94 to 4.74 for grams lysine per 100 grams protein and .38 to .50 for lysine as percent of sample. In general, we observed that the yields of opaque-2 hybrids ranged from 80-90% or more of normal hybrids on the average.

Table 14

1973 Data on opaque-2 hybrid uniform test+

Pedigree		Yield Ave. q/ha‡	L/P	Pedigree		Yield Ave. q/ha	L/P
Mo 2^{Rf} x MO 20 W	o_2	85.8	4.40	W64 x W117	o_2	50.5	4.74
Mo 2^{Rf} x K41	o_2	80.2	4.06	(N28 x N31) x R Mo 17	o_2	85.0	4.56
Mo 2^{Rf} x 33-16	o_2	49.5	4.06	(A632 x B14A) x C123	o_2	50.0	4.28
Mo 2^{Rf} x K55	o_2	90.2	4.32	($R177^6$ x $C123^6$) x 00387-1	o_2	85.5	4.59
(Mo 2^{Rf} x K55) x (Mo 20 W x K41)	o_2	74.0	4.28	($B14^6$ x $B37^6$) x 00374-A	o_2	80.0	4.44
K55 x K41	o_2	76.8	4.54	($B14^6$ x $B37^6$) x 00368-A	o_2	79.5	4.69
Mo 17 x N28	o_2	86.2	4.50	($R177^6$ x $C123^6$) x 00402-A	o_2	80.8	4.51
Va 35 x Mo 17	o_2	70.8	3.94	($R177^6$ x $C123^6$) x 00397-A	o_2	76.2	4.55
R182 x N28	o_2	87.0	4.50	($B14^6$ x $B37^6$) x 00379-A	o_2	86.2	4.72
C123 x N28	o_2	81.0	4.22	(B37 x H84) x 00379-A	o_2	92.0	4.64
R177 x N28	o_2	82.5	4.46	$B37^{Cm}$ s x $N28^{Ht}$ check		87.8	3.21
R177 x Va 43	o_2	86.8	4.63	$H49^{Cm}$ sx $N28^{Ht}$ check		103.5	3.07
Va 43 x N28	o_2	94.0	4.49	(B37 x B14A) x B57	o_2	71.2	4.51
(R802 x R177) x C123	o_2	75.8	4.28	A619 x A632	o_2	60.8	4.66

+Conducted in four states (Illinois, Iowa, Indiana, Missouri)

‡q = quintel = 100 kilo

Source: Bauman, et al (4)

In Table 15 are shown the grain yields of a commercial and opaque-2 counterpart grown in four different years. The results indicate that opaque-2 ranged from 86 to 92% of its normal counterpart. The overall average of the ten tests show that the opaque-2 grain yields were 90.7% of the normal counterpart hybrid. The data also indicated the variation that one commonly observes in grain yields associated with year to year variations in different environments.

Table 15

Grain yeilds (q/ha) of a commercial normal and opaque-2 counterpart grown in four different years.

Year	Number of tests	Genotype	Yield q/ha	Yield % of normal
1	4	Normal	112.8	
		Opaque-2	103.2	91.4
2	4	Normal	108.2	
		Opaque-2	99.0	91.5
3	1	Normal	92.4	
		Opaque-2	82.2	89.5
4	1	Normal	123.6	
		Opaque-2	106.2	86.0
Overall Mean of 10 Tests				90.7

Six related single crosses from a diallel among Oh43, W64A, B37, and C103 inbreds and the three-way hybrid [(Oh43 x B37) x C103] each near-isogenic for the opaque-2, sugary-2 opaque-2, and their normal counterparts were evaluated for their agronomic yield in a replicated yield trial on the Purdue Agronomy Farm in only one season (9). Three normal commercial crosses and their opaque-2 counterpart hybrids were also grown as checks. The results are shown in summary form in Table 16. The six related single crosses and the three-way normal hybrid range from 86.4 to 112.2 quintels per hectare; the opaque-2 hybrids ranged from 67.8 to 97.8 quintels per hectare; and the sugary-2 opaque-2 hybrids ranged from 67.8 to 85.8 quintels per hectare. The commercial normal hybrids ranged from 102.6 to 123 quintels per hectare and their counterpart opaque-2 hybrids ranged from 81.6 to 106.2 quintels per hectare. Overall the opaque-2 hybrids ranged from 74 to 94% of their normal counterpart hybrids, while sugary-2 opaque-2 hybrids ranged from 74 to 85% of their normal counterpart hybrids. The mean protein percent over all hybrids for the normal, opaque-2, sugary-2 opaque-2 near-isogenic varieties was 9.52, 9.83 and 10.58%, respectively; while mean lysine as a percent of protein values were 2.99, 4.29, and 4.50 for normal, opaque-2, and sugary-2 opaque-2, respectively.

Table 16

Summary of grain yields and protein quality

Hybrids	Yield q/ha	Yield % of normal	Protein %	L/P
	-------Range-------			
6 Single crosses & 3-way normal	86.4-112.2	---	9.0-9.5	2.71-3.04
6 single crosses & 3-way Opaque-2	67.8-97.8	74-94	9.1-10.4	4.09-4.46
6 Single crosses & 3-way Sugary-2 Opaque-2	67.8-85.8	74-85	9.6-12.0	4.36-4.60
Commercial Normal	102.6-123.0	---	9.2-10.1	3.03-3.20
Commercial Opaque-2	81.6-106.2	77-86	9.1-10.0	4.22-4.55

Source: (9)

Dry Matter Accumulation. With respect to agronomic effects of opaque-2, dry matter accumulation essentially ceases at forty-five days, about seven to ten days earlier than normal (Figure 1). There is a significant hybrid x genotype interaction which indicates that opaque-2 behaves differently in various hybrids at maturity. Hence, there might be a possibility to obtain opaque-2 hybrids that yield as much as their normal counterparts (3, 10). The black layer (indication of physiological maturity) occurs at the same time as in normal. Opaque-2 generally has a higher moisture content at maturity. And the cob weight is about 10% less than normal. In almost all opaque-2 expressions or effects there are significant interactions indicating selection should be effective.

Likewise with respect to the agronomic effects of sugary-2 opaque-2 a serious drawback is that the yields of this double-mutant are lower than opaque-2, and like opaque-2 and other types of maize with modified amino acid balance, are less than in the normal counterpart hybrids or varieties (1, 9). Shown in Figure 2 are the results of a dry matter accumulation study comparing the normal, opaque-2, sugary-2 opaque-2, and sugary-2 genotypes in isogenic hybrid backgrounds. Illustrated is the grain weight per ear accumulation of these genotypes averages over four hybrids. Significant differences in grain weight of the ear were not found until thirty-eight days after pollination. There were no significant differences in those sampling dates after fifty-two days. Hence, the differences among genotypes that were first found at thirty-eight days were fixed by fifty-two days after pollination. The grain weight at maturity of opaque-2 and sugary-2 opaque-2 hybrids were significantly less than the normal counterpart hybrids. There was a significant hybrid x genotype interaction which indicates that the genotypes behave differently in various hybrids. The grain weight of sugary-2 was not significantly less than normal in some hybrids. Further developmental studies are in progress to investigate the compensating effects in endosperm metabolism and constraints on dry matter accumulation. This is an extremely important area of investigation in order to enhance our progress in improving grain production and nutritional quality.

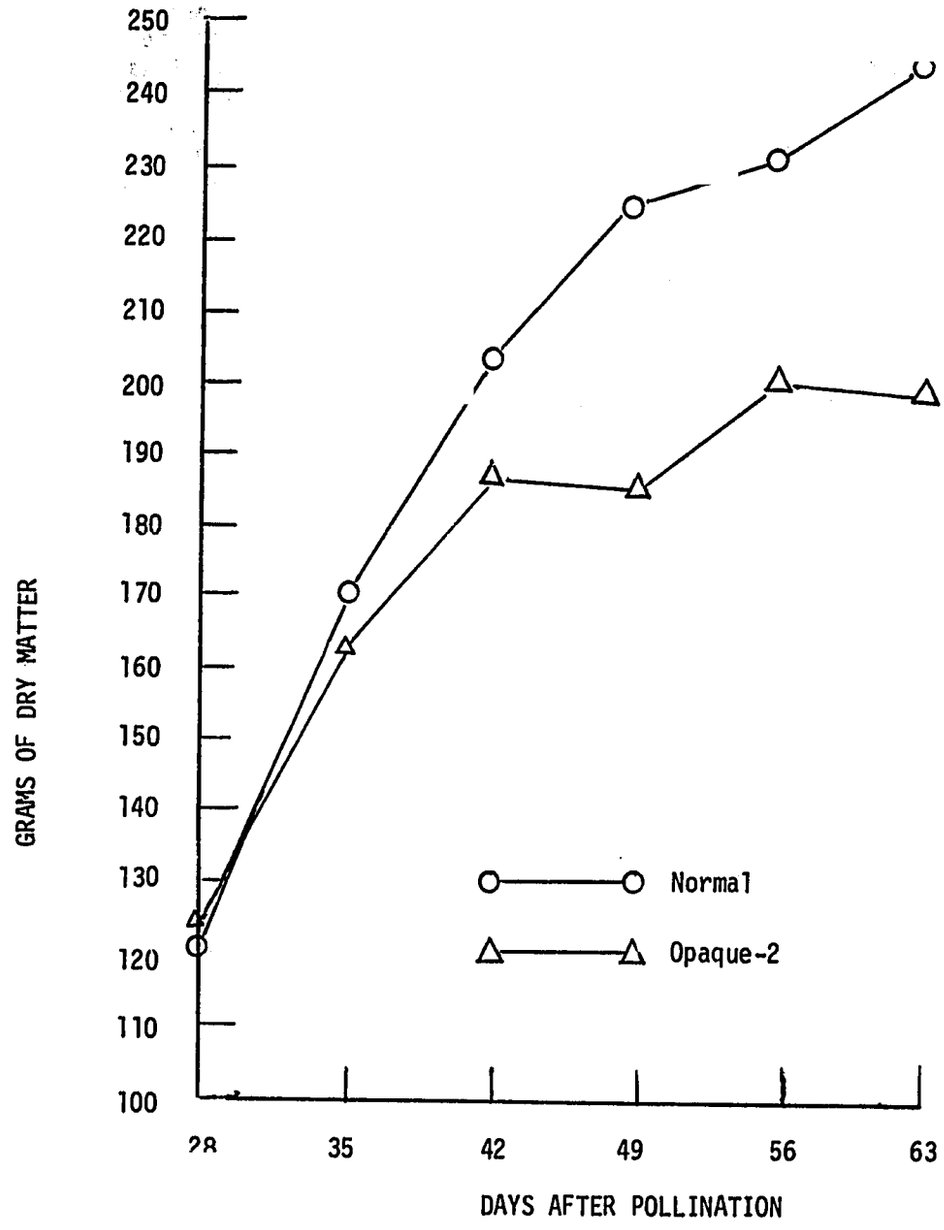

Figure 1. Comparison of kernel weight of opaque-2 and normal variations of B14 x B37.

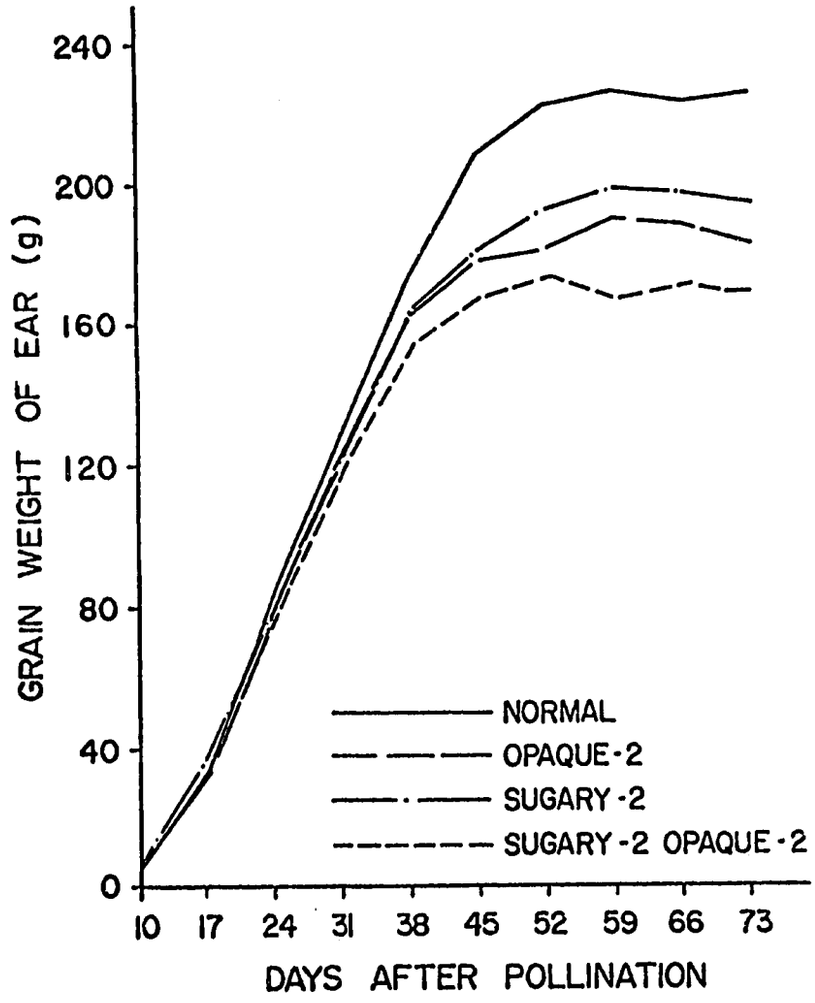

Fig. 2 Grain weight per ear accumulation of $\underline{su}_2$, $\underline{su}_2\underline{o}_2$, $\underline{o}_2$ and normal averaged over hybrids.

Grain Yields and Protein Quality in Commercial Opaque-2 Hybrids. Additional grain yield and protein quality data were generously supplied by Dr. Paez and Dr. Snyder. These data represent information and experience with the development of commercial opaque-2 hybrids. Table 17 shows the grain yields and ear weights of opaque-2 and normal hybrids during three-year tests.

Table 17
Yields and ear heights of opaque-2 and normal hybrids during 3-year period (6 entries, 6 locations).

	Yield bu/a	Ear wt.	Plants/A	Yield Adj to 20,000
Opaque-2	134.9	.47	19,968	134.28
Normal	151.5	.51	20,710	145.71
O_2 vs N	89.0%			93.0%
		% of normal		
Range				
Opaque-2	125-150	(83-94)		
Normal	134-168			

Courtesy Co. A

These data are from six entries tested at six locations with three replications at each location. The opaque-2 hybrids were about 10% lower in yield than their normal endosperm counterparts. The opaque-2 ranged in yield from 83 to 94% of the normal hybrids in the test. Table 18 shows the grain yields and ear weights of opaque-2 and normal hybrids during a two-year period, representing nineteen entries tested over four locations with three replications at each location.

Table 18
Yield and ear heights of opaque-2 and normal hybrids during 2-year period (19 entries, 4 locations).

	Yield bu/a	Ear wt.	Plants/A	Yield Adj to 20,000
Opaque-2	145.7	.52	19,528	148.57
Normal	160.0	.56	19,755	160.00
O_2 vs N	91.0%			93.0%
		% normal		
Range				
Opaque-2	122-161	(86-100)	12 of 19 entries 90%	
Normal	134-181		of normal or greater	

Courtesy Co. A

Again the opaque-2 hybrids were about 9-10% lower in yield than were their normal endosperm counterparts. In these particular tests the opaque-2 ranged from 86-100% of the normal hybrids. Twelve of the nineteen entries were 90% of the normal hybrid counterpart or greater. Table 19 illustrates the variation in protein and lysine contents among some commercial normal and opaque-2 hybrids.

Table 19

Lysine (%), protein (%) and grams of lysine per 100 grams of protein (L/P) and yield for selected entries from Expt. 1975.

E#		% Lysine+	% Protein+	L/P+	Yield‡ bu/A
6	+	.29	9.99	2.88	132.4
9	+	.27	9.63	2.84	133.6
16	+	.31	10.46	2.95	128.3
19	+	.29	9.58	2.99	138.4
3	Com. o_2	.38	9.93	3.80	129.3
4	Com. o_2	.35	9.56	3.65	118.0
5	Com. o_2	.37	9.55	3.87	137.0
7	Com. o_2	.32	8.52	3.76	139.7
1	o_2	.38	10.03	3.82	113.6
13	o_2	.38	10.09	3.74	127.3
17	o_2	.40	10.50	3.76	133.5
2	o_2	.38	9.88	3.86	123.5
8	o_2	.39	10.59	3.66	124.9

+ = Average for 5 reps.
‡ = Average over 3 locations.
Courtesy Co. B.

Entry #7 is of particular interest because of the low protein and lysine contents and the fact that it also was the highest yeilding hybrid of all the entries (both normal and opaque-2) in the trial. However, the lysine as a percent of protein value was not as low as entry #4 which exhibited one of the lower yields in the comparison. Table 20 shows a comparison of grain yields for normal and opaque-2 counterparts of some selected commercial hybrids.

Table 20

Comparison of yeilds for normal and opaque-2 counterparts of commercial hybrids.[1]

Hybrid	Yield (Bu/A) and % Moisture at Harvest		
	1974	1975a	1975b
D+	109.6 (25.4%)	150.4 (21.5%)	----
Do_2	98.7 (24.7%)	138.8 (20.3%	----
B+	94.0 (27.8%)	163.0 (23.5%)	----
Bo_2	85.7 (28.7%)	145.4 (24.5%)	----
1+	102.1 (33.3%)	----	133.6 (18.3%)
$9o_2$	91.8 (33.7%)	----	113.6 (17.2%)
Eo_2	----	----	133.5 (15.5%)
$13o_2$	----	----	127.3 (16.3%)
Ave. (Normals)	99.3 (29.0%)	152.1 (22.1%)	133.2 (18.7%)
Ave. (All Entries)	95.9 (29.6%)	135.6 (22.5%)	122.7 (19.1%)

[1]1974: 25 entries (+, o_2 and wx); open-pollinated; 3 replications each at 2 locations.

1975a: 25 entries (+ and o_2); opaque-2 border and normal entries detassled; 2 replications each at 2 locations.

1975b: 25 entries (+ and o_2); opaque-2 border and normal entries detassled; 2 replications each at 3 locations.

Courtesy Co. B.

Once again there was about a 10% reduction in yield with near isogenic counterparts in both open-pollinated (1974) and controlled pollination trials (1975a and 1975b). It is interesting to note that the hybrid Exp. opaque-2 and entry #13 opaque-2 have yeilds equivalent to a commercial hybrid in the same test (1975b) and the average for four normal endosperm hybrids in the same test. Table 21 shows a summary for grain yield of commercial yield tests for one year over three locations.

Table 21

Summary of commercial screening yield tests (1975) at 3 locations.

E#		Location 1 Yield bu/A	Location 2 Yield bu/A	Location 3 Yield bu/A	Mean Yield bu/A
Ave		94.7	164.9	108.6	122.7
LSD		28.6	15.6	14.8	22.0
CV		14.7	4.9	6.6	10.9
6	+	123.7	166.1	107.6	132.4
9	+	119.2	168.4	113.2	133.6
16	+	123.9	161.0	110.2	128.3
19	+	116.6	180.0	118.8	138.4
3	Com. o_2	107.8	161.6	118.6	129.3
4	Com. o_2	95.7	153.4	104.8	118.0
5	Com. o_2	118.7	174.3	118.1	137.0
7	Com. o_2	118.1	176.5	124.6	139.7
17	Ex o_2	98.2	180.0	122.5	133.5
13	Com. o_2	74.4	186.1	121.3	127.3
1	Com. o_2	90.7	146.0	104.2	113.6
14	Ex o_2	108.2	139.5	98.5	115.4
11	Ex o_2	87.4	144.2	84.6	105.4
8	Ex o_2	01.5	171.0	102.4	124.9
12	Ex o_2	95.5	147.0	87.0	109.8
15	Ex o_2	87.4	156.6	111.9	118.6
2	Ex o_2	97.5	162.8	110.3	123.5
10	Ex o_2	87.1	149.9	97.1	111.3
18	Ex o_2	59.4	179.0	109.7	116.0
21	Ex o_2	72.9	164.7	111.5	116.4
25	Ex o_2	79.3	179.9	110.0	123.0
24	Ex o_2	81.6	157.7	111.5	116.9
20	Ex o_2	46.8	154.8	101.1	100.9
23	Ex o_2	80.2	175.9	106.6	120.9
22	Ex o_2	95.6	185.2	119.9	133.5

Courtesy of Co. B.

These data indicate the variability among hybrids and the variability within hybrids over locations. In general, the opaque-2 hybrids did not perform well at location one, but some opaque-2 out-yielded the normal hybrids at locations two and three. From these and the previous data shown it is apparent that the opaque-2 gene performed differently in respect to yield and quality traits in different backgrounds.

Summary. There is evidence that through selection, opaque-2 hybrids with acceptable yield, improved protein quality and other agronomic traits can be developed. This is a matter of the breeding objectives and developing the proper selection indices along with needed breeding resources to accomplish the task.

Modified (vitreous) opaque-2 and sugary-2 opaque-2 modified varieties with their improved kernel density have not necessarily resulted in higher yielding potential because of increased density. However, through proper monitoring for yield and protein quality, varieties can be selected with good yield (perhaps not equal to normal), resistant to ear rots and grain insects and good protein quality. The data indicate that random mating selected modified and non-modified opaque-2 populations for several generations without selection resulted in little or no change in nutritional value and the degree of modification was maintained. This indicates that modified opaque-2 varieties (except for outcrossing to normal) will tend to retain their desirable characteristics when grown by the farmer for successive generations. The identification of a simply inherited gene modifier of the soft endosperm of opaque-2 and the sugary-2 modification of opaque-2 both, without serious reduction in lysine levels, should greatly simplify breeding of more acceptable vitreous opaque-2 varieties with good nutritional quality.

There is some question as to whether consistent grain yields comparable to the best yielding normal varieties can be obtained with the opaque-2 gene system due to the trade-off and compensating effects among storage metabolites and possible differences in energy transfer mechanisms, utilization of photosynthate or other constraints associated with the opaque-2 gene.

Dudley (6) has recently reported a unique study of the interrelationships among oil, protein, intrinsic energy contents, grain yield and other agronomic traits. He studied the performance of crosses among nine strains, Illinois High Oil (IHO), Reverse High Oil (RHO), Switchback High Oil (SHO), Illinois Low Oil (ILO) Reverse Low Oil (RLO), Illinois High Protein (IHP), Reverse High Protein (RHP), Illinois Low Protein (ILP), and Reverse Low Protein (RLP). These materials represent the widest range of oil and protein percentages presently known. Table 22 shows the correlation among traits related to calorie production using means of crosses of strains selected for percent oil with those selected for percent protein.

Table 22

Correlations among traits related to calorie production using means of crosses of strains selected for percent oil with those selected for present protein.+

	Trait					
	Cal/ kernel	k cal/ ha	% Oil	% Protein	g/100 kernels	Grain yield
Cal/g dry matter	-.74*	-.45*	.94*	.51*	-.22	-.61*
cal/kernel		.47*	-.78*	-.30	.99*	.58*
k cal/ha			-.32	-.67*	.49*	.98*
% Oil				.23	-.85*	-.49*
% Protein					-.36	-.70*
g/100 kernels						.62*

*Significant at the .05 probability level.

+Adapted from J. W. Dudley et al.

Grain yield was negatively correlated with both percent oil and percent protein. The two highest yielding crosses, RLP x ILO and RLP x LRO, had almost normal levels of protein (approximately 10% but slightly lower than average levels of oil. Grain calorie production per hectare closely paralleled grain yield. However, production of oil or protein per hectare was influenced by both grain yield and concentration of oil or protein in the grain (i.e., IHO had the highest g.c.a. effect for kg oil/ha and IHP had the highest g.c.a. for kg protein/ha). The caloric content of the grain as cal/g dry matter, on the other hand, was correlated with percent oil and to a lesser degree with percent protein and negatively correlated with grain yield.

These data point out the fact that the optimum combination of protein, oil, and grain yield depends on the objectives of a breeding program. For example, where production of protein or oil per hectare is a primary consideration, intermediate levels of protein or oil with higher yields may be the preferred approach. For maximum production of calories per hectare, selection based primarily on grain yield with some increase in percent protein is appropriate. The study indicated a substantial amount of genetic variability remaining (for yield, oil and protein) even after forty-eight generations of forward selection. Thus, given the right selection indices it is certainly possible to move protein values up perhaps one to three percentage points along with improved grain yield.

The acceptable loss of calories per acre as a trade-off for increased amounts of high-quality protein is a function of demand for the two components. In the U.S. current use of opaque-2 corn is restricted because of present yield differentials compared to normal corn and a very atypical corn-soybean oil meal price relationship, with the meal being low in price relative to corn. This economic situation certainly may not prevail in the future. In fact, with increasing population, protein supplements could become much higher in relative price compared to corn. The situations in the U.S., for example, are much different from those of other parts of the world, where high-quality protein supplements for animal feeding are scarce, or in parts of the world where maize is widely used as human food. Thus, in regions where maize is an acceptable food, yield may be a secondary consideration.

A large research effort is currently devoted to improving yield and agronomic characteristics of conventional varieties of corn. A research program of similar magnitude (or integrated research) will be required to produce "protein" varieties that are more nearly competitive with conventional varieties. Equality of yields need not necessarily imply that all biosynthetic pathways involved are also equivalent. Our current constraints to yield or potential yields of our normal varieties as well as "improved protein" types could be limited by inefficiencies in any one or more of many areas such as: 1) energy transfer mechanisms, 2) net assimilation rates, 3) translocation and utilization of photosynthate, 4) nutrient uptake and use, and other areas. We need to develop effective cooperation between breeding and biochemistry-physiology, and research these potential constraints of yield to realize a substantial boost in the slope of yield trends. Though research in this area may be difficult and costly, it offers promise of being highly productive. Recognizing the relative efficiency (C_4 plant) and production capability of corn, the breeding approach to improve quantity and/or quality of protein will be difficult but offers tremendous potential.

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GENETIC IMPROVEMENT OF MAIZE

Federico Poey

The principal objectives of the International Wheat and Maize Improvement Center (CIMMYT) are to develop and introduce improved maize and wheat germplasm to the less developed countries (LDC) of the tropical world and to assist them in increasing food production.

With regard to corn, a serious effort is underway to improve also the protein value of the grain through the use of the opaque-2 mutant.

Subsistence crop economy in large areas of the majority of the LDC's justifies the use of breeding technology that produces open-pollinated varieties. It is assumed that this type of variety allows more adaptation flexibility in addition to the fact that once introduced in an area, the seed can be selected continuously by the farmer.

The breeding system used is based on family selection (progeny from one ear) and recombination of the best ones based on an international evaluation program. Specific populations composed of varied sources of germplasm are recombined at the CIMMYT's experiment stations in Mexico by a half-sib scheme where families, after detasseled, are pollinated by plants derived from a composite represented by all families. This scheme allows gene recombination while inferior families are discarded. Once the population is considered stabilized, sets of 250 families are yield tested in collaborating countries. Data from these tests are computed and the best 100 families are identified. Selected families are then recombined among themselves by hand pollination in reciprocal crosses to produce 250 family sets that will be tested in another improvement cycle. The best families generate new experimental varieties at each cycle.

Parallel to this scheme, each population is backcrossed to the opaque-2 mutant and recombined with the base population after recovering the homozygous O2 genotypes. Protein quality and quantity of the endosperm is analyzed in order to eliminate inferior values. Protein quality analyses used include rapid methods for tryptophan and lysine. The ninhydrin method is also used specifically to identify opaque-2 phenotypes.

In addition to this population CIMMYT is developing various white and yellow opaque-2 populations which are handled on similar family based schemes where protein values are monitored after selecting families for yield and agronomic characteristics.

Experimental varieties are then developed for each population based on families selected on different locations and tried out in locations of potential use.

National programs in various countries then utilize these materials to develop local varieties or to recombine them in other fashions to produce hybrids or new open pollinated varieties.

Protein and amino acids are analyzed at the Protein Quality Laboratory of CIMMYT, at the rate of 20,000 yearly. The laboratory also serves as a training site for technicians from collaborating countries.

The introduction of opaque-2 corn faces the problem of the associated soft endosperm characteristic which results in a different phenotype generally not accepted by the direct consumer market. Efforts to select modifying types that closely resemble the normal type of corn are underway. Although commonly resulting in erratic lysine and protein values, close monitoring of the selections have succeeded in fixing a protein quality comparable with the soft endosperm O2 phenotypes.

Major problems in the introduction of opaque corn to farmers are those related with soft endosperm phenotype acceptance of the farmer and second in importance is the yield loss resulting from this characteristic.

BREEDING MAIZE FOR PROTEIN QUANTITY AND QUALITY[1/]

M. S. Zuber

It has been well documented that normal corn grain is deficient in the amino acids lysine and tryptophan. These two amino acids are essential in the diets of monogastric animals. For their optimum growth and performance, protein from normal corn grain must be supplemented. Humans, especially infants, on diets consisting of high amounts of normal corn, have shown irregular growth and symptoms of malnutrition.

Therefore, when Mertz, Bates, and Nelson (3) reported that the opaque-2 gene, and later the floury-2 gene, increased the lysine content over that found in normal corn, it aroused tremendous interest among corn breeders, nutritionists, and economists. It was one of the first indications that it might be possible to alter the amino acid composition in corn through genetics. This discovery also stimulated research on protein quality in other crops.

After the discovery that the opaque-2 gene increased lysine content in corn, many breeders introduced this gene into elite normal inbred lines, mostly by the backcross method. The first opaque-2 hybrids resulting from crosses of these converted lines have not been overwhelmingly accepted by the U.S. farmer. These hybrids were rejected primarily for their 15 to 20 percent lower yield than their normal counterparts and secondly for more kernel ear rots, insect damage, and kernel breakage due to the soft endosperm.

To overcome the deficiencies of opaque-2, breeders have been focusing their attention on selection for higher test weight and modified kernel types in the opaque-2 types. The modified kernel types have more vitreous endosperm and may range from slight mottling, half and half, to a nearly normal endosperm type. The frequency of the modified kernel types will depend upon the background genotype. Some genotypes may have few if any modified kernel types whereas in other genotypes their occurrence is more frequent.

Our experience at the University of Missouri has shown a large range in the lysine content of the modified-endosperm opaque-2 types. We have found a low negative correlation of about 0.52 between modified types and lysine content with the full opaque having the highest lysine content However, it is possible to find nearly normal endosperm types equal to the full opaque in lysine content. Therefore, to successfully select a nearly normal endosperm opaque-2 type with high lysine content, it is necessary to monitor for lysine content through the development and selection process. To date, most corn breeders do not have facilities available for large numbers of lysine and protein determinations. Until breeders have facilities to permit a large number of lysine and protein determinations to be made at a low cost, progress in the improvement of th opaque-2 types will be slow.

At the University of Missouri we have used two approaches in identifying hard endosperm types in the opaque-2 background. In addition we have been very fortunate that our Experiment Station Laboratory, under the direction of Dr. C. W. Gehrke, has developed and perfected new

[1/] Cooperative investigations of the Department of Agronomy, University of Missouri Agricultural Experiment Station. Journal Series Number 7662 and the Agricultural Research Service, US Department of Agriculture.

automated methods for determining lysine and protein. They provide us with 2,000 determinations per year which have greatly enhanced our protein quality research.

One approach we have used involved a microscopic examination of endosperm tissue for the presence or absence of zein bodies. Opaque-2 kernels with high lysine have no visible zein bodies at 400X magnification. Individual kernels can be examined and those selected can be planted for propagation. We have classified kernels for zein body number and found a negative 0.65 correlation between estimated zein body numbers and lysine content (1). We have used this method successfully to identify normal appearing kernels in the opaque-2 background with high lysine.

The second approach involved measuring kernel hardness either by determining the amount of pressure necessary to crush a single kernel or by measuring the amount of kernel breakage in a 100 g lot when subjected to a Steinlite Corn Breakage Tester*. By this method the kernel is destroyed and cannot be propagated. We have used this method for identifying S_0 and S_1 ears with above average kernel hardness in opaque-2 material. Selected ears must have lysine determined to be sure the hard kernel types have a suitable level of lysine content.

By use of these two methods we have developed opaque-2 hybrids with 85 percent vitreous endosperm compared with 90 to 95 percent for normal corn. These hydrids have lysine contents equal to the opaque-2 types and preliminary yield trials indicate yields are equal to their normal counterparts.

In our opinion, it will be possible to overcome the deficiencies of the opaque-2 phenotypes by selecting for the modified kernel types. The task will be time-consuming, as monitoring for lysine content will be necessary during the breeding process.

If breeders were successful in developing high-lysine modified endosperm opaque-2 hybrids equal in yield to their normal counterparts, this question arises: Would the U.S. farmer grow them?

Whether he grows them will depend on whether it will be more profitable to grow high-lysine corn rather than normal corn. Several factors may affect his decision, such as: Whether he receives a premium for higher lysine content when he markets the crop, and if he feeds the crop to swine, whether it would be a cheaper source of lysine than purchasing a protein supplement. In the past, farmer interest in high-lysine corn has also been related to the price of soybean meal.

In the second approach we have investigated the possibility of increasing lysine by cyclic selection in normal corn in the absence of endosperm mutants (4). If successful, a high-lysine normal corn would eliminate most of the problems associated with the nonvitreous opaque-2 types.

* Mention of a company name, trademark, or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or the Missouri Agricultural Experiment Station, and does not imply its approval to the exclusion of other products that may also be suitable.

In the cyclic selection program, we used three open-pollinated varieties: Logan County Composite, Reid Yellow Dent, and Midland Yellow Dent. Each cycle required two generations where in the first generation 100 ears were selfed. Lysine and protein were determined for each ear. Ten percent of the ears with highest lysine were selected and kernels from selected ears were planted and plants were randomly mated. Seed produced from this generation was used for starting the subsequent cycle.

We have completed three cycles of selection and the results are shown in Tables 1, 2, and 3 for Logan Composite, Reid, and Midland respectively.

Table 1

Results of Recurrent Selection for High Lysine in Normal-Endosperm Logan Composite

	Percent Lysine			Percent Protein		
	Diff. Yrs.	1974	1975	Diff. Yrs.	1974	1975
Original Pop.	0.27	0.34	0.35	13.1	11.6	12.3
2nd Cycle	0.40	0.35	0.41	13.7	13.0	13.9
3rd Cycle	0.44	0.42	0.43	14.4	13.2	14.7

Table 2

Results of Recurrent Selection for High Lysine in Normal-Endosperm in Reid Yellow Dent

	Percent Lysine			Percent Protein		
	Diff. Yrs.	1974	1975	Diff. Yrs.	1974	1975
Original Pop.	0.26	0.29	0.33	12.3	10.8	12.2
2nd Cycle	0.34	0.31	0.35	12.3	11.7	12.0
3rd Cycle	0.36	0.34	0.36	12.0	12.1	12.3

Table 3

Results of Recurrent Selection for High Lysine in Normal-Endosperm in Midland Yellow Dent

	Percent Lysine			Percent Protein		
	Diff. Yrs.	1974	1975	Diff. Yrs.	1974	1975
Original Pop.	0.26	0.32	0.36	13.4	10.5	12.5
2nd Cycle	0.35	0.32	0.38	13.6	12.9	13.9
3rd Cycle	0.34	0.32	0.39	13.5	11.6	13.9

Evaluation of progress by comparing the original population with advanced cycles indicated lysine was increased in all three populations, but more so in Logan Composite than in the other two. Lysine content for Logan Composite third cycle was in the opaque-2 range (Figure 1).

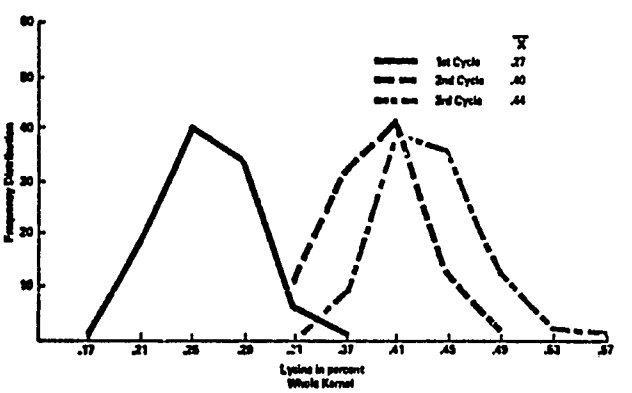

Figure Frequency distribution for 1st, 2nd and 3rd cycle of recurrent selection for high lysine on a whole kernel basis. Logan Composite.

If cyclic selection really increased lysine content in normal dent corn, the question arose as to whether or not the genetic system involved would affect the lysine level of opaque-2 phenotypes (2). To answer this question we selected two high-lysine and two low-lysine families from the first cycle of recurrent selection for higher lysine content in Logan Composite. The frequency distribution for the two high-lysine and low-lysine parents are shown in Figure 2.

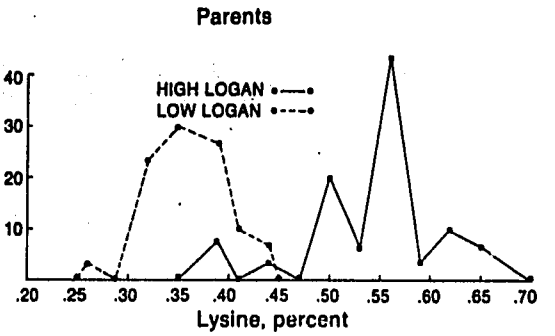

Figure 2. Protein content frequency distribution for 1st, 2nd and 3rd cycle of recurrent selection for high-lysine on a whole kernel basis in Logan yellow dent.

These families were selfed and in the S_2 generation 60 plants were selfed and outcrossed to two inbred line opaque-2 testers. The following year each S_3 selection was grown adjacent to its F_1 topcross. All plants were selfed. F_2 seed from the F_1 topcross was separated into normal and opaque phenotypes. Lysine and protein were determined for each S_4 line and its corresponding normal and opaque phenotype F_2 testcross.

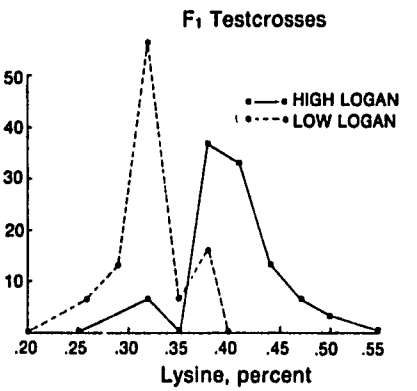

Figure 3. Frequency distribution for grams of lysine per 100 grams of protein on a whole kernel basis for the 1st, 2nd and 3rd cycle of recurrent selection in Logan Composite.

The results showed that lysine levels from the low and high families were transmitted to the F_1 opaque-2 testcrosses (Figure 3) and to the normal and opaque-3 segregates of the F_2 populations (Figure 4).

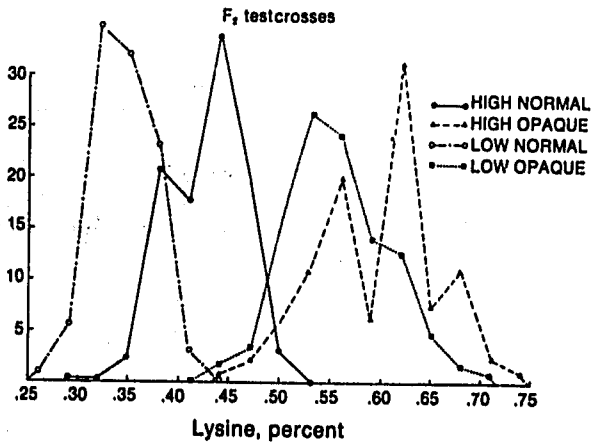

Figure 4. Frequency distribution for 1st, 2nd and 3rd cycle of recurrent selection for high lysine on a whole kernel basis. Midland Yellow Dent.

Some of the recovered opaque-2 phenotypes from the F_2 generation involving the high-lysine family had 30 to 50 percent more lysine than the opaque-2 tester (Table 4).

Table 4

Lysine Content of Opaque-2 Lines Derived from Crosses of O_2 With High-Lysine, Normal-Endosperm Families Selected From the First Cycle of Recurrent Selection for High Lysine in Logan Composite

	Percent Lysine		
Line	S_1	S_2	S_3
264-1	0.68	0.60	0.60
264-2	0.76	0.62	0.63
264-5	0.62	0.49	0.64
264-6	0.66	0.60	0.55
267-7	0.66	0.62	0.63
O_2 Check	**	0.50	0.50

The results suggest that at least two genetic systems are involved in lysine synthesis. One system is associated with the opaque-2 gene per se and a second system involves genes associated with the high- and low-lysine, nonopaque normal families obtained from the first cycle of recurrent selection.

The two genetic systems appear to complement each other in an additive manner. Therefore, high-lysine selections in normal corn can be used as donors for upgrading both lysine and protein of opaque-2 corn.

Selected high-lysine opaque-2 lines derived from testcrosses involving high-lysine normal-endosperm families were grown to determine the stability of lysine levels in subsequent generations. Lysine content for several selections through three generations of selfing is presented in Table 4. In general, the lysine level was 10% higher than the opaque-2 check.

CONCLUSIONS

Although the endosperm mutant genes opaque-2 and floury-2 nearly double the lysine content over normal corn, farmers in the U.S. have not accepted

these high-lysine hybrids due to lower yields, more kernel rots, and higher kernel breakage than for normal dent corn. Many of these defects are due to the soft endosperm associated with these two genes. Some of these deficiencies can be overcome by selecting more normal-vitreous type endosperms. These types can be identified by measuring kernel crushing strength, kernel breakage, or by examining endosperm tissue microscopically for the absence of zein bodies. Not all vitreous endosperm-opaque-2 types are high in lysine, therefore, lysine content should be monitored during the breeding and selection process. Several opaque-2 hybrids with vitreous endosperms have been developed at the Missouri Agricultural Experiment Station. These hybrids are equal in lysine content to the regular opaque-2 types and yield compares favorably with the normal checks.

Increasing lysine and protein content in normal corn by recurrent selection has been demonstrated. Increasing lysine content in some populations by this method would appear to be more successful than in others.

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INTERACTIONS BETWEEN YIELD, PROTEIN AND LYSINE IN CEREALS

Edwin T. Mertz

The identification of high lysine mutants in maize (11, 12, 13), barley (8, 15) and sorghum (14, 16) during the last twelve years has forced cereal breeders to look at two additional variables in their breeding programs, namely, the quality and quantity of seed protein. Since the first limiting amino acid in all cereals of economic importance is lysine (9), lysine as percent of the total protein has become the primary chemical measure of seed protein quality. Man and animals also have specific requirements for total protein. Thus, the total percent of protein in the seed is another important chemical measure of the nutritional value of the seed.

The plant breeder who attempts to breed for more protein quantity and quality in cereals is faced with an unfavorable interaction between these two variables and yield.

Yield and Percent Protein. Dudley et.al. (5) studied the performance of crosses among nine normal (non-opaque) strains, Illinois High Oil, Reverse High Oil, Switchback High Oil, Illinois Low Oil, Reverse Low Oil, Illinois High Protein, Reverse High Protein, Illinois Low Protein, and Reverse Low Protein. A discussion of all major interrelationships in their data is given in Dr. Glover's paper (6). Grain yield was negatively correlated with percent protein (-0.70, significant at the .05 probability level). Data presented by other speakers at this conference suggest the same negative correlation between yield and percent protein in other cereal grains (sorghum, barley, millets, rice and wheat). In wheat, however, the negative correlation appears to be reduced by genes for high protein (10).

In opaque-2 and floury-2 maize, yield and protein are negatively correlated as reported by Dudley et al. (4). In two opaque-2 and two floury-2 synthetics, yield and percent protein were negatively correlated (phenotypic correlation significant at the 0.05 probability level). These authors concluded that "---selection for the percentage of protein--- could result in an appreciable reduction in yield."

Protein and Lysine Interactions. Bauman et al. (1) presented correlations for several characters measured on 44 opaque-2 maize ears. The following correlations were reported for protein and lysine (Table 1).

It is obvious that a strong negative correlation exists between lysine as a percent of kernel protein (a good chemical measure of nutritional value) and total protein.

Table 1

Correlations Between Protein and Lysine

Characters	Correlation Coefficient
Protein (% of kernel) and lysine (% of kernel)	+ 0.70**
Protein (% of kernel) and lysine (% of kernel protein)	- 0.52**
Protein (% of kernel) and protein (% of endosperm)	+ 0.97**
Lysine (% of kernel) and lysine (% of endosperm)	+ 0.76**

**Significant at the 1% level.

This presents the plant breeder who is selecting for useful opague-2 types with a dilemma. Should he breed for maximum yield, which presumably leads to seeds with lower levels of total protein (but protein of better quality), or sacrifice yield for more total protein of poorer quality? In studies carried out at Purdue (7) and reported in more detail by Glover at this conference (6), weanling rats were fed opaque-2 maize as the only protein source. Maize samples used were S_2 families taken out of the high and low (modified selections) lysine intercrosses developed from HMO opaque-2 synthetic. One low protein selection, LP-HL2, contained 9.2% protein, 4.1 grams lysine per 100 grams protein, 0.38 percent lysine in the seed, and a mean protein efficiency ratio (PER) of 2.66. A high protein selection, HP-LL1, contained 11.48% protein, 3.5 grams lysine per 100 grams protein, 0.36 percent lysine in the seed, and a mean PER of 2.20. When fed as the only food except for added mineral and vitamins, both gave the same feed efficiency ratio (grams gained per grams of food consumed).

These data suggest that the rat was able to utilize for growth a low protein maize with good protein quality as efficiently as a high protein maize of lower protein quality. Since the low protein maize should be higher yielding than the high protein maize, one could conclude that the breeder should breed for maximum yield in his opaque-2 selection program.

The danger here, however, is that total protein could fall to levels at which protein itself becomes the limiting nutrient. To avoid this pitfall, the breeder must work closely with the nutritionist in any breeding program involving high lysine cereals.

Nitrogen Fertility. Ample evidence exists in the literature for a strong dependence of both yield and total protein content on availability of nitrogen in the soil. Obviously, this variable must be controlled in any

Table 2

Energy and Nitrogen Requirements for One Per Cent Increase in Protein

Crop	Nitrogen requirement*		Per cent increase in nitrogen requirement for one percentage point increase in protein	Equivalent glucose required
	Standard cereal**	One percentage point increase in protein		
Wheat	16.0	17.0	6	1
Rice NO_3 as N source	9.7	10.8	11	1
Rice NH_3 as N source	10.4	11.7	12	1
Maïze	11.3	12.3	9	1
Barley	11.5	12.3	10	1
Sorghum	13.6	14.6	7	1
Oats	14.8	15.8	7	1
Rye	16.0	17.0	6	1

*Milligrams of nitrogen per gram of photosynthate, assuming the cereal protein is 16% N by weight.

**Assumed standard cereal composition of Spector (17).

studies on interactions between yield and protein content. Other speakers at this conference have shown the effects of limited nitrogen fertilization on grain protein and grain yield.

Energy Requirements for Higher Protein and Lysine Levels in Grains. Bhatia and Rabson (2) recently examined the requirements for additional photosynthate and nitrogen in order to raise protein and lysine levels in cereals. They used the analysis systems of Penning de Vries (3). de Vries, after extensive examination of biochemical pathways and the energy requirements of the component reactions involved, concluded that in plants under aerobic conditions, 1 gram of glucose can produce 0.83 g of carbohydrates, or 0.40 g of proteins (nitrate nitrogen source) or 0.33 g of lipids. He assumed that the pathways in crop plants would be not significantly different from the known pathways in microorganisms.

Table 2 summarizes Bhatia and Rabson's calculations on the percent increase in nitrogen requirement and energy requirement to obtain a one percentage increase in total protein in the seed.

Table 2 shows that the net overall increase of photosynthates (equivalent glucose) required for production of all grains with a one percentage point increase in protein would be roughly one percent. The nitrogen requirement, however, would be increased in the range of 6 to 12 percent depending on the cereal grain under consideration. This must be supplied as extra fertilizer or by remobilization of nitrogen internally in the plant.

Bhatia and Rabson (2), point out that if the genetic obstacles to introducing symbiotic bacteria into cereals are overcome, there will still be an energy cost for symbiotic nitrogen fixation and this will compete with grain filling for available photosynthates, just as in the legumes.

These authors also calculated the substrate and energy requirements for normal and opaque-2 maize, as well as for normal and high lysine barley. The requirements were dependent on the biosynthetic pathways for the different amino acids. They calculated a maximum increase of about 2.5 percent in glucose (photosynthate) requirement because of the change in amino acid pattern for 1508 barley mutant when compared with a recently released normal barley cultivar. Since total protein normally remains the same or falls slightly in the high lysine counterpart, no additional glucose would be required.

In most cases, the observed typical yield reductions in improved (high lysine) cultivars of maize, sorghum and barley exceed the predictable loss in grain yield (about 2.5 percent due to amino acid pattern change) based on the calculations of Bhatia and Rabson (2). However, the highest yielding opaque-2 hybrids approach or may exceed the 97.5 percent ceiling which they propose for 1508 barley mutant. When yields of opaque-2 hybrids fall below about 95% of the normal counterpart, one must assume factors other than a change in amino acid composition, such as impaired endosperm development.

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RESULTS OF LYSINE FORTIFICATION OF WHEAT PRODUCTS IN SOUTHERN TUNISIA*

Mohamed el Lozy, M.D., and George R. Kerr, M.D.

By the mid nineteen-sixties, a considerable body of evidence had accumulated suggesting that the fortification of cereal products with essential amino acids might perhaps be of world health importance by improving the food supply of the poorer countries. This evidence was far from conclusive, however, and could be interpreted in many different ways. Thus Howe et al. (18) suggested that: "The most rapid and least expensive way of correcting these deficiencies is by mandatory supplementation of each cereal..." while Hegsted (15), basing his conclusions on the same body of evidence, wrote: "Until these data are available, amino acid supplementation of cereals must be considered an expensive gamble that developing nations can ill afford." The controversey persits: in 1974 Altschul (1) wrote: "But, fortification with amino acids will probably become an essential and regular adjunct of human food systems," while Miller and Paine answered, "We conclude that amino acid fortification should be rejected on nutritional and logistic grounds" (23).

This extreme disagreement is due to the fact that there was no clear evidence either for or against a beneficial effect in people living under "real life" conditions. Arguments were therefore based partly on general nutritional theory, and partly on extrapolation from animal experiments whose relevance was, and still is, strongly debated.

It therefore became clear that the only way to resolve the controversy was by a series of well designed field trials of sufficient scale and duration and under sufficiently realistic conditions for the results to be convincing to all (1, 13, 15, 19). Consequently, in the late sixties, the United States Agency for International Development (AID) supported the setting up of three large scale studies in Tunisia (fortifying wheat), Thailand (fortifying rice), and Guatemala (fortifying corn).

The reasons for choosing Tunisia have been clearly explained by Rosenfield (24): "To review some of the project's history, in the spring of 1967 discussions were first held in Washington to develop a preliminary listing of countries where amino acid fortification might make a meaningful contribution toward reducing the incidence and severity of protein malnutrition. Tunisia was given high priority on this listing because its major source of protein and calories was wheat. Dr. Henry Sebrell, Mr. Otho Skaer of the Great Plains Wheat Company, and Dr. Aaron Altschul visited Tunisia in May 1967 to assess what might be done. After a series of meetings with representatives of the government, nutritionists, and millers, there was general agreement that amino acid fortification was a particularly desirable approach for Tunisia.

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While anxious to undertake country-wide fortification of wheat, the government of Tunisia first wanted to evaluate under realistic conditions the economic and health aspects of such action. Therefore, government officials asked the US AID Mission to support a large-scale evaluation in which the Tunisian government would participate. This request led to the development of the project by personnel of AID, USDA, and the Tunisian government and Dr. Fredrick Stare and his associates at the Department of Nutrition of the Harvard School of Public Health."

The project began in September 1969 and it was one of the initial responsibilities of the then recently formed Institute of Nutrition and Food Science. In fact, the project played a key role in the formation of the institute.

Early in the planning stage it was clear that the project would have to be in the southern, non-wheat growing area of the country, to ensure that the populations receiving fortified wheat products did not consume any locally grown, non fortified products. In addition, the South is even more heavily dependent on wheat products as a main source of both protein and energy than the country as a whole. Southern Tunisia also appeared to be an area where the effects of any improved diet would be relatively easy to demonstrate. There was great poverty and the companions of poverty: a high infant and child mortality rate, small and stunted children and a high degree of iron deficiency anemia. In addition, it was noted that there was little or no evidence of the more severe, and intractable, forms of protein calorie malnutrition (marasmus or kwashiorkor) which would probably have been unaffected by fortification.

After visits to the whole area by Drs. Stare, Brown, McGandy and Gershoff of Harvard's Department of Nutrition, Drs. Elizabeth Boutourline and Gino Tesi of Florence, Italy, and Dr. Nessib Hemaidan of AID/Tunis the area around the Chott el Djerid, a large salt lake that is dry during most of the year, was selected (Figure 1), and three delegations (equivalent to counties) bordering on the lake were chosen.

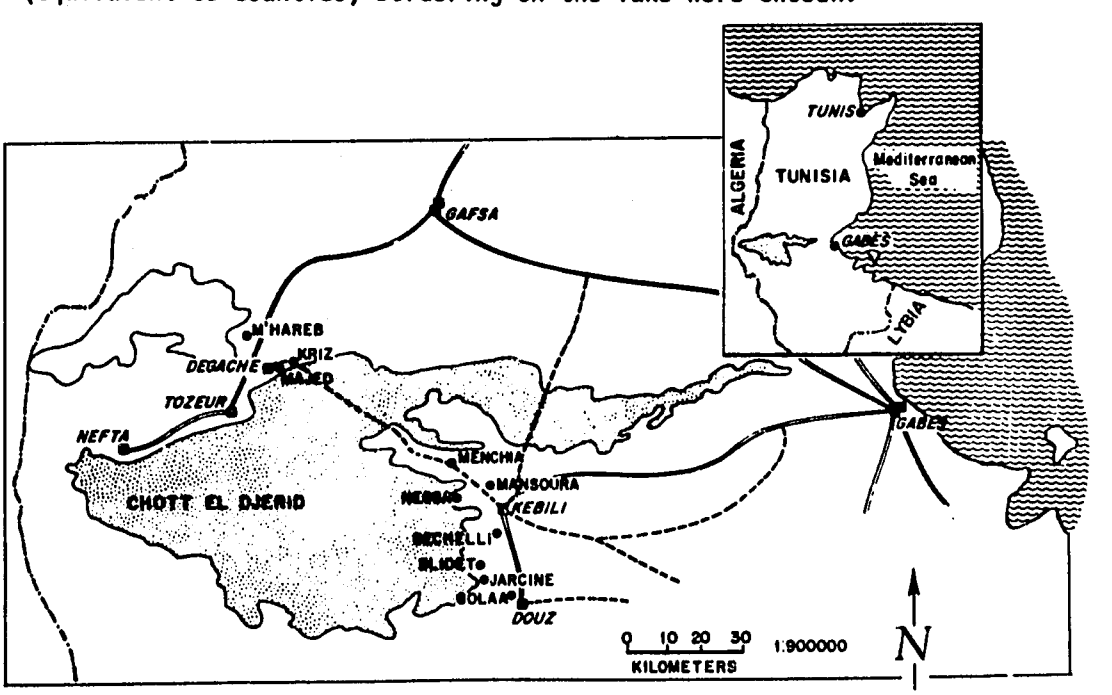


Figure 1: Map of Tunisia and Study area.

The delegation of Degache, north of the lake, was to be the control area, and the families in these villages were to receive their usual, unfortified wheat products. All wheat products going to the other two delegations were to be fortified, those going to Kebili with a vitamin/iron mixture and those going to Douz with the same mixture plus lysine.

In each of the delegations three villages (four in Kebilli) were chosen on the basis of socioeconomic, demographic, and racial similarity (Table 1). These 10 villages had about 3,000 children under five years of age and a total population of approximately 16,500. From the fall of 1970 to the spring of 1975 all the preschool children in these ten villages were examined every six months, a total of ten times. At each examination anthropometric measurements were taken, clinical examinations were performed, and, if the parents consented, blood and urine samples were obtained for a variety of nutrition-related analyses.

Table 1. Population and annual per capita income in the study village at the start of the study.

Delegation	Village	Families	Population	Per Capita Income ($)
Degache	Kriz	391	2059	75
(control)	Majed	344	1800	73
	M'hareb	352	1661	64
Kebilli	Negga	251	1446	78
(vitamin/	Mansoura	465	2464	77
iron)	Bechelli	147	842	65
	Menchia	317	1802	81
Douz	Golaa	472	2693	82
(lysine)	Blidet	176	950	53
	Jarcine	139	855	67

The anthropometric measurements included height, sitting height, leg length, weight, arm, head and chest circumferences, four skinfolds, number of teeth erupted, and an x-ray of hand and forearm, from which skeletal maturation and the lengths of the radius and ulna were determined. The anthropometric measurements were performed by trained personnel under the direction of the field director, Dr. Elizabeth Boutourline. Before each of the semiannual examination periods, the measurers were put through a training program in a village not included in the main study, in which each child was measured by all the measurers and differences in technique were noted and corrected. The x-rays were scored according to the Tanner-Whitehouse system by Dr. Boutourline, and in the initial stages, sets of x-rays were sent to Dr. Tanner in London for evaluation. As almost total agreement was found, this second evaluation was dropped.

The clinical examination involved most of the signs suggested by Jelliffe (19), and was performed by a nurse trained to detect them and supervised by a physician. The blood samples were analyzed for hemoglobin, packed cell volume (PCV), serum proteins, and serum albumin, and the urine was analyzed for riboflavin and creatinine.

In addition to these recurrent studies of children, several other studies were performed. A complete census was performed prior to the beginning of the study and was updated before each of the semi-annual measuring periods. In addition, three socioeconomic studies were carried out, at the beginning of the study (1970), in the middle of the study (1973), and after termination of the fortification (1975). A quantitative dietary study was carried out in 1971-72, and two questionnaire surveys related to food purchasing and consumption were completed in 1970 and 1975, and a study of birth weights and of the neonatal mortality was carried out in 1974-75.

The anthropometric indices of nutritional state of the preschool children in the study area before the start of fortification have been described (2, 3, 4, 5, 11, 22). All indices, except dental eruption, show a clearcut retardation in the comparison to Western standards. Table 2 summarizes the results in the three areas.

Table 2. Anthropometric measures as percentages of standards in the three areas before the start of fortification

Measurement	Control	Vitamin/Iron	Lysine
Weight	82.7	88.6	83.9
Height	92.7	95.7	92.2
Arm circumference	86.3	87.2	87.5
Nutritional Status Index	91.0	93.3	91.2

The Nutritional Status Index (NSI) is an index obtained by combining several anthropometric measures (weight, height, and arm, head, and chest circumferences) each expressed as a percentage of an appropriate standard (in this case, the Denver standards) (14). It is clear that, in almost all cases, the children in the vitamin/iron area were bigger to begin with than those in the other two areas. The children in the other two areas, on the other hand, were very similar.

Table 3 gives the percentages of children in the three areas with weights below 80% of standard (underweight) (27) and with heights below 90% of standard (stunted) (20). Here again we see that the vitamin/iron region fares better, before the start of fortification, than the other two regions.

Table 3. Percent of children underweight and stunted at the start of fortification.

	Control	Vitamin/Iron	Lysine
Underweight	42	28	39
Stunted	30	20	36

Clinical examination along the lines recommended by Jelliffe (2) and the I.C.N.N.D. (16) revealed a high incidence of non-specific signs, and a very low incidence of signs suggestive of clinically manifest PCM. (6, 8).

Laboratory data before the start of fortification showed a high incidence of mild anemia, with low mean hemoglobin and PCV levels (Table 4). In addition, study of a small sample of older children showed a very high incidence of intestinal parasites, and a variable incidence of urinary schistosomiasis (13).

Table 4. Hematological findings before the start of fortification.

	Control	Vitamin/Iron	Lysine
Hemoglobin	10.9	10.8	10.2
Packed cell volume	33.8	34.4	33.6
Mean corpuscular hemoglobin concentration	32.1	31.4	30.1

Table 1 shows the extremely low per capita income in the area. This led to a diet based largely on wheat products which were found to supply 81% of the protein and 66% of the energy in the diet (25). (Table 5) Furthermore, 66% of the families consumed less energy than recommended by the FAO/WHO Expert Committee (21) and 51% consumed less protein than recommended. Calculation of the amino acid composition of the diet (Table 6) showed clearly that, irrespective of the scoring pattern used, the biological value of the protein was limited by its lysine content (Table 7) (9).

Table 5. Daily per capita consumption of foods, and energy, protein, and lysine content and cost (6).

Food	Amount g/day	Energy Kcals/day	Energy MJ/day	Protein g/day	Lysine mg/day	Cost*** (¢)
Cereals	310	1060	4.54(64)***	33.8(81)	769(60)	6.8
Potatoes	30	10	0.05(1)	0.3(1)	16(1)	0.3
Sugar	32	130	0.54(8)	--	--	0.7
Pulses	10	30	0.14(2)	2.2(5)	144(11)	0.5
Vegetables	93	30	0.12(2)	1.0(2)	43(3)	1.8
Fruits	51	90	0.38(5)	1.1(3)	35(3)	0.7
Meat	13	30	0.12(2)	2.3(6)	204(16)	3.5
Fish	1	--	0.00(0)	0.2(-)	16(1)	*
Milk	20	10	0.05(1)	0.7(2)	54(4)	*
Oil	37	280	1.17(16)	--	--	1.3
TOTAL		1670	7.11	41.6	1281	16.9

*These items, together with tea, coffee, tomato concentrate form a miscellaneous group which together accounts for 1.3¢ a day.

**Cost is calculated on basis of 1972 conversion; $1 US = 0.47 Tunisian Dinar.

***Figures in parentheses indicate percent of total dietary protein, energy and lysine derived from food classes.

Table 6. Essential amino acid concentration (mg/g protein) in wheat protein, total dietary protein using unfortified wheat products, and total dietary protein after lysine fortification of wheat product to a final concentration of .2%.

	Amino acid concentration of wheat protein	Amino acid concentration of total dietary protein using unfortified wheat products	Amino acid concentration of total dietary protein using lysine-fortified wheat products	Amino acid concentration in reference protein (7)
Isoleucine	40(100)	40(100)	39(98)	40
Leucine	77(110)	75(107)	74(106)	70
Lycine	23(42)	31(56)	45(82)	55
Total sulphur containing	44(126)	41(117)	40(114)	35
Total aromatic	79(132)	77(128)	76(127)	60
Threonine	29(72)	31(78)	30(75)	40
Tryptophan	12(120)	12(120)	12(120)	10
Valine	45(90)	45(90)	45(90)	50

*Figures in parentheses indicate concentration of amino acid as percent of that in reference (FAO/WHO 1973) protein.

Table 7. First and second limiting amino acids of total dietary protein as determined by five reference proteins, and the effect of lysine fortification of wheat products.

	Total dietary protein with unfortified wheat products			Total dietary protein using lysine fortified wheat products			
	1st limiting amino acid (% of amount in reference protein)	2nd limiting amino acid (% of amount in reference protein)	Per capita consumption reference protein diet (gm/d)*	1st limiting amino acid (% of amount in reference protein)	2nd limiting amino acid (% of amount in reference protein)	Per capita consumption reference protein diet (gm/d)*	Increase in amount of reference protein in diet (%)
FAO/WHO (1973)	Ly (56)	Th (77)	23.1	Th (76)	Ly (82)	31.8	38
Breast milk	Ly (47)	Tr (69)	19.2	Tr (68)	Ly (68)	28.5	48
Cow's milk	Ly (39)	Th (70)	16.3	Ly (58)	Th (69)	24.2	48
Whole egg	Ly (44)	Th (65)	18.1	Ly (64)	Th (64)	27.0	49
FAO (1957) modified	Ly (55)	Th (73)	22.7	Th (72)	Ly (80)	30.2	33

*Per capita consumption of total dietary protein x concentration of most limiting amino acid compared to that in reference protein.

Hence it was clear that the original impression that this was a suitable area in which to study the effectiveness of lysine fortification was confirmed. The diet and health of the population was clearly poor, but not so poor as to preclude improvement.

After one year of baseline data collection, during which the data shown above were collected, the fortification program began. As noted above, the delegation of Degache was the control area, and continued to receive unfortified wheat products. All wheat products shipped to the other two delegations were processed in specific mills under control of project personnel. All wheat products going to the Kebili delegation were enriched with iron, vitamins A and D, niacin, thiamine, and riboflavin (Premix A) while those going to the Douz delegation were enriched with the same mixture plus lysine (Premix B, Table 8). Neither mix affected the color, taste, or cooking properties of the wheat products.

Table 8. Composition of premixes and concentration of added nutrients in flour.

	Premix A[1] (Kebili)	Premix B[2] (Douz)	Concentration in Flour (/100 g flour)
Thiamine	2.05%	0.19%	0.85 mg
Riboflavin	1.27%	0.12%	0.54 mg
Niacin	14.41%	1.35%	6.38 mg
Vitamin A	11.10%	1.04%	1,000 IU
Vitamin D_2	0.28%	0.03%	200 IU
Iron	12.64%	1.18%	5.59 mg
Lysine (added)	0.00%	60.00%	300 mg
Tri-Calcium Phosphate	1.00%	1.00%	
Non-gelatinized wheat starch	57.25%	35.09%	

[1]Premix A is formulated to be added to the millstream at a rate of 0.75 oz/100 lb flour or 0.04687%.

[2]Premix B is formulated to be added to the millstream at a rate of 8 oz/100 lb flour or 0.5%.

The premixes were all made by Pillsbury Company in Mineapolis from edible grade lysine donated by the Ajinomoto Company of Tokyo and vitamins donated by Merck and Hoffman-LaRoche. In addition to preparing the premixes, testing them for key nutrients and shipping them to Tunis, the Pillbubry Company also provided the iron (metalic iron) used in the fortification. In addition, Pillsbury sent a mill engineer to Tunis to help select the mills to be used, ordered and supervised the installation of the fortification machinery, and periodically checked on its operation. Without the thorough and enthusiastic cooperation of Pillsbury the study would have been far more difficult and we appreciate their cooperation.

A technician from the project was always in the mill during any production run of fortified wheat products, checking the calibration of the feeders, seeing that the bags were properly color-coded, taking samples of flour to the laboratories to check that they did in fact contain the fortificants, and checking that the consumption of premix corresponded to the amount required for the amount of wheat milled.

As a final check, samples of wheat products on sale in the village stores were periodically bought and sent for analysis to the laboratory in Tunis, and occasionally to the laboratories of the Pillsbury Company in Minneapolis. This rather tight control system did not totally prevent errors, but those that did occur were immediately detected and corrected. We believe that these controls were as effective as is possible outside a laboratory study.

Over the course of the study the income in all three areas increased, the increase being least in the control area and almost identical in the two other areas. At the same time the cost of living was increasing, the increase being believed to be similar in all three areas. As a result, the control area showed a small increase in real income (Table 9) while the other two regions showed a more substantial increase.

Table 9: Mean family income (Dinars/month).

	Control	Vitamin/iron	Lysine	Cost of living index
1970	13.97	14.97	15.41	100
1971	21.43	28.66	25.36	121
1975	25.16	34.86	33.71	151

Figure 2 shows the evolution of the Nutritional Status Index over the duration of the study.

The most striking feature is the seasonal pattern, with values in the fall (odd-numbered examinations) lower than those in the spring (even-numbered examinations). This suggests slower growth in summer due to summer diarrheas, but we do not have the morbidity data required to substantiate this hypothesis. Furthermore, it is obvious that the control and lysine areas move in parallel, the children in the lysine area were bigger than those in the control area at the start of the study and remained so, by the same amount, throughout the entire period of fortification. Hence it is clear that the fortification did not have any effect on the nutritional state of the children, as estimated by anthropometric methods. A worrying observation is that the children in the vitamin/iron area showed a gradual deterioration during the last two years of the study. It is unlikely that this decline can be explained by economic factors, as this is the region showing the greatest increase in income, while the region with the least increase in income (the control area) showed no change.

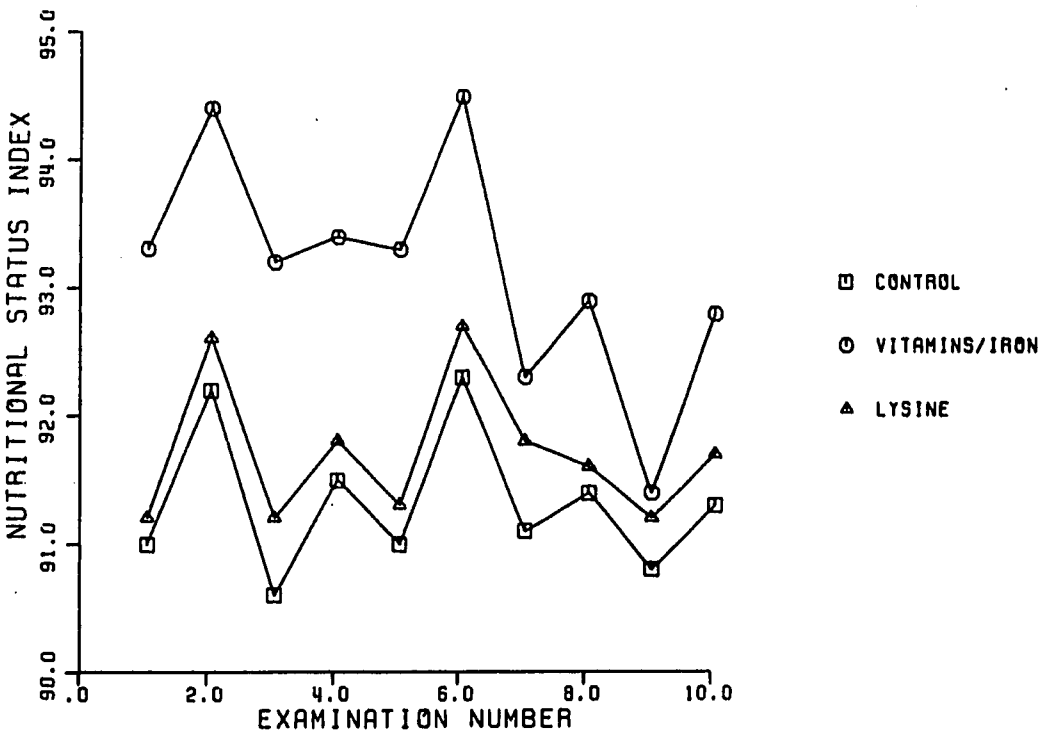


Figure 2: Evolution of the Nutritional Status Index over the duration of the project.

Figure 3 shows the weights as percentage of standard over the duration of the study, and their behavior is identical to that of the Nutritional Status Index. Similarily, Figure 4 shows the proportion of children underweight (below 80% of standard) and it is a mirror image of Figure 3.

Since height is an intrinsically less labile measurement than weight (weight can be lost, but height cannot) Figure 5 shows very much attenuated seasonal changes. In addition we see the same parallelism of behavior between the control and lysine fortified regions, and the same deterioration in the vitamin/iron region. Indeed, the deterioration in height appears to begin earlier than that in weight. Figure 6 shows the corresponding mirror image pattern of percentage of subjects stunted over the duration of the study.

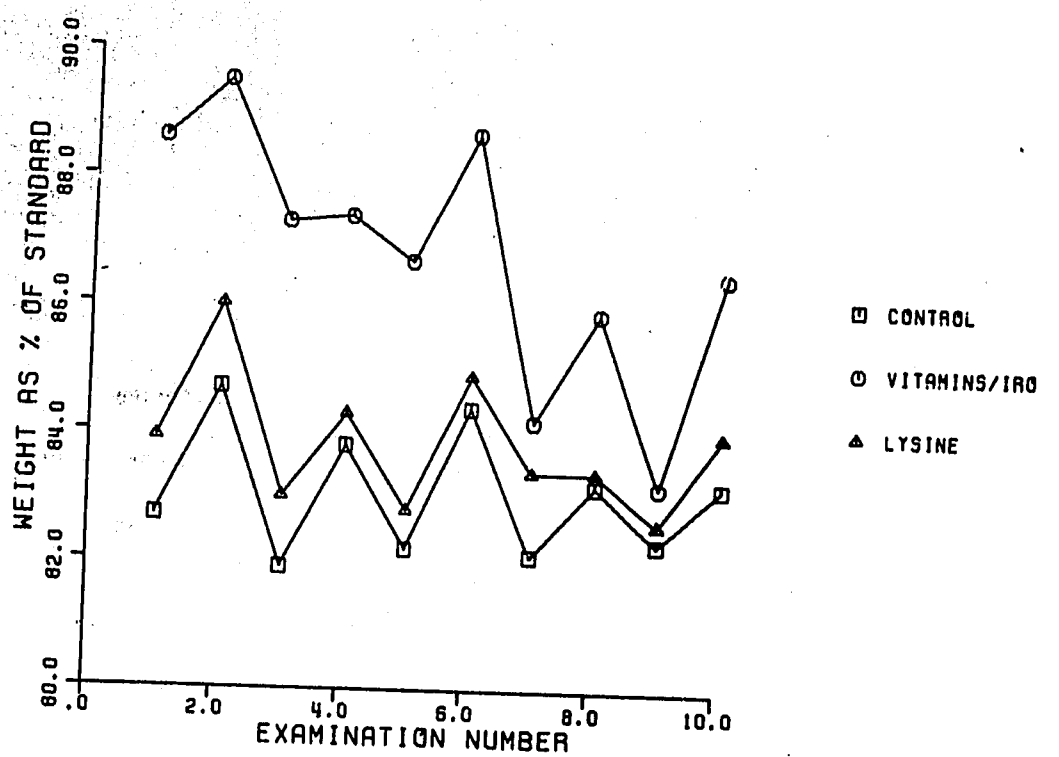
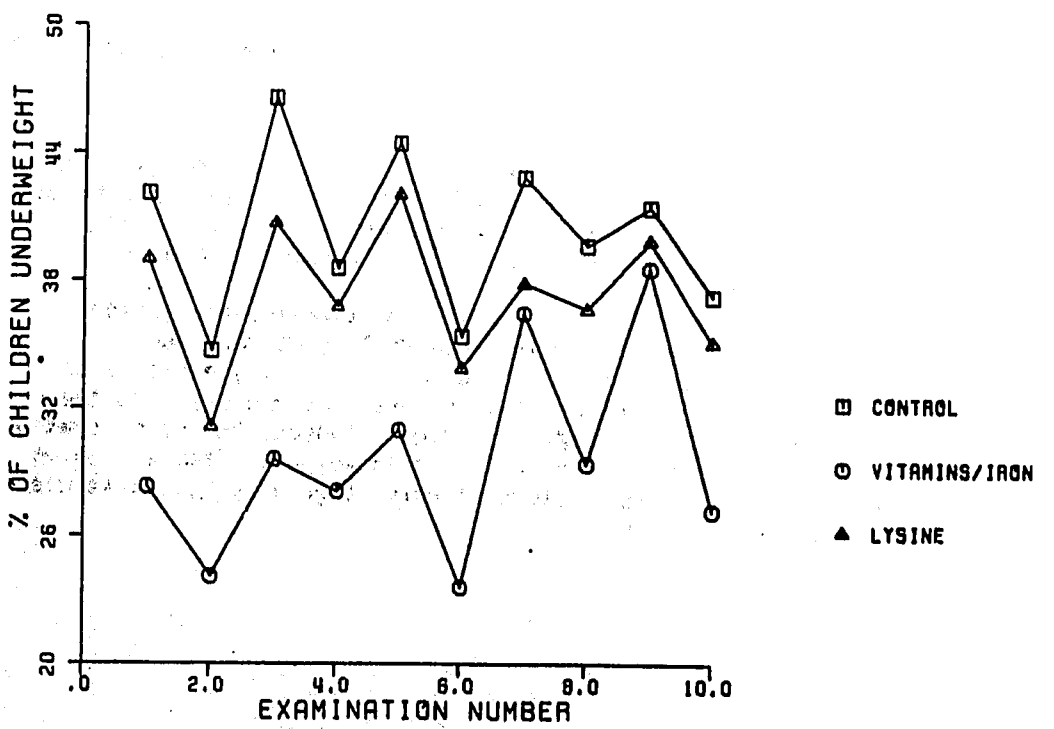


Figure 3: Weight as percent of standard at each examination period.



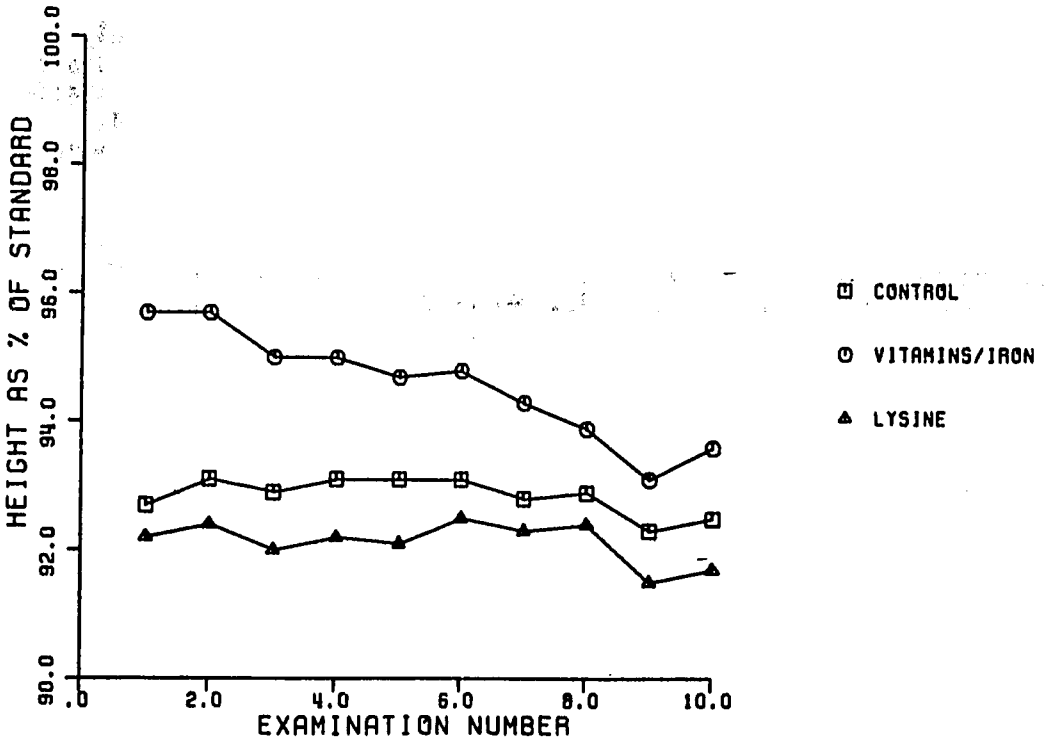


Figure 5: Height as percent of standard at each period.

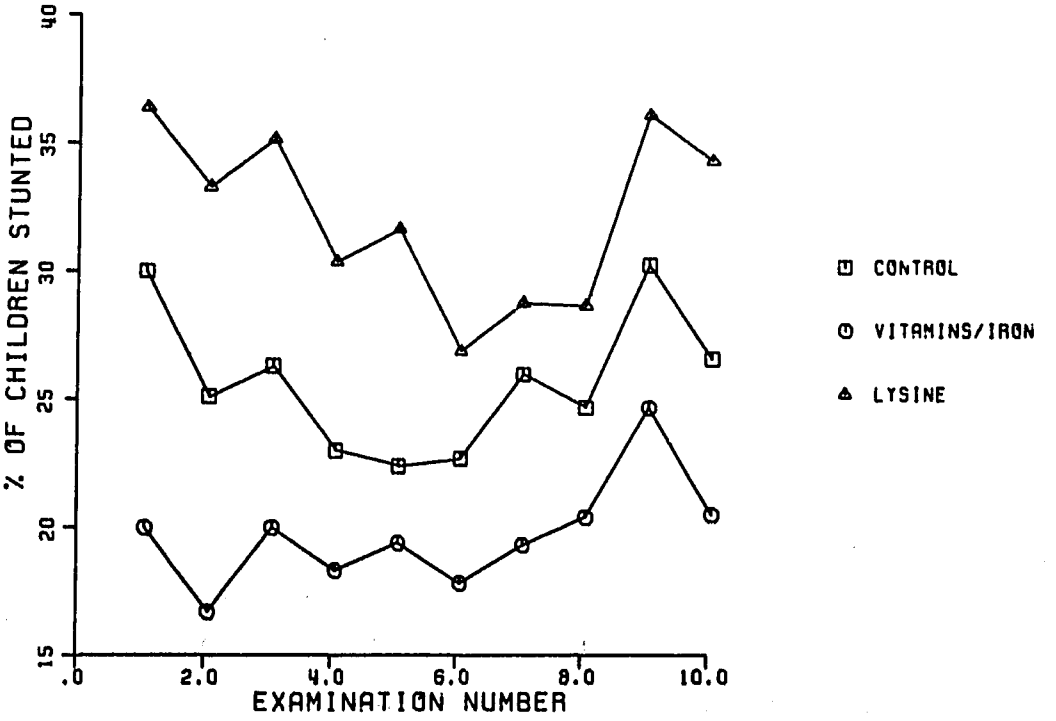


Figure 6: Percentage of children stunted (below 90% of standard height) at each examination

Tables 10 and 11 show the weight and height increments, respectively, during the last year of the study as a function of the age at the start of that year. The differences in growth in height are not statistically significant between the three areas, and those in weight are barely significant, with growth being greatest in the control area and similar in the two other areas.

Table 12 shows some of the results of the newborn study carried out during the last year of the project, and it is clear that there are no differences in any of the variables measured.

Table 10: Weight increments (kg/year) of children from the three areas during the last year of the study.

Age last birthday*	Boys Control	Boys Vitamin/iron	Boys Lysine	Girls Control	Girls Vitamin/iron	Girls Lysine
0	2.77	2.42	2.44	2.81	2.47	2.37
1	2.26	2.48	2.71	2.35	2.40	2.50
2	3.92	2.04	2.51	4.72	2.49	2.18
3	2.10	1.65	1.88	1.93	1.80	1.92
4	1.74	1.68	1.68	1.65	1.70	1.85
5	1.98	2.07	1.81	2.11	1.60	1.85

*At the beginning of the one year growth period.

Table 11. Height increments (cm/year of children from the three areas during the last year of the study.

Age last birthday*	Boys Control	Boys Vitamin/iron	Boys Lysine	Girls Control	Girls Vitamin/iron	Girls Lysine
0	11.3	11.4	10.2	12.1	19.2	10.6
1	8.0	8.3	8.6	10.2	8.3	7.9
2	7.6	8.6	7.5	8.5	8.2	7.6
3	7.6	6.9	6.8	9.6	7.2	8.2
4	6.4	7.0	6.3	6.5	6.2	6.3
5	6.1	7.1	7.4	6.5	6.2	5.7

*At the beginning of the one year growth period.

Table 12. Results of newborn study.

	Control	Vitamin/iron	Lysine
Birth weight	2971	2923	2932
Birth length	47.5	48.0	47.9
Weight gain in first month	659	740	654
Length gain in first month	3.8	3.9	3.7
Perinatal mortality (%)	5.9	10.2	10.4

Figure 7 shows the changes in the packed cell volume over the period of the study. The values initially decreased in all three areas to reach a minimum at the time of the fourth examination, then rose to a peak at the time of the fifth examination, and then fell back to the starting level showing no net change over the duration of the study. During the first three and last four examinations the differences between the values obtained in the three regions were small, with the vitamin/iron area tending to be highest and the lysine (actually vitamin/iron/lysine) area the lowest. During the fourth, fifth and sixth examinations the differences increased, with the lysine area passing from the lowest in the fourth examination to the highest in the fifth, then falling to the lowest at the time of the seventh examination.

Figure 8 shows the different behavior of the hemoglobin levels. The values in all three areas again tended to be similar to each other, except during the first two examinations, when levels in the lysine area were much lower than in the other two, and in the fifth when the control area had lower levels. Like the packed cell volume, the hemoglobin rose markedly in all three areas between the forth and fifth examinations, but unlike the packed cell volume it reamined elevated throughout the rest of the study. We have no explanation for the different behavior of these two hematological indices, but as the patterns of change in the three areas were essentially similar there is again no evidence of any effect due to the fortification.

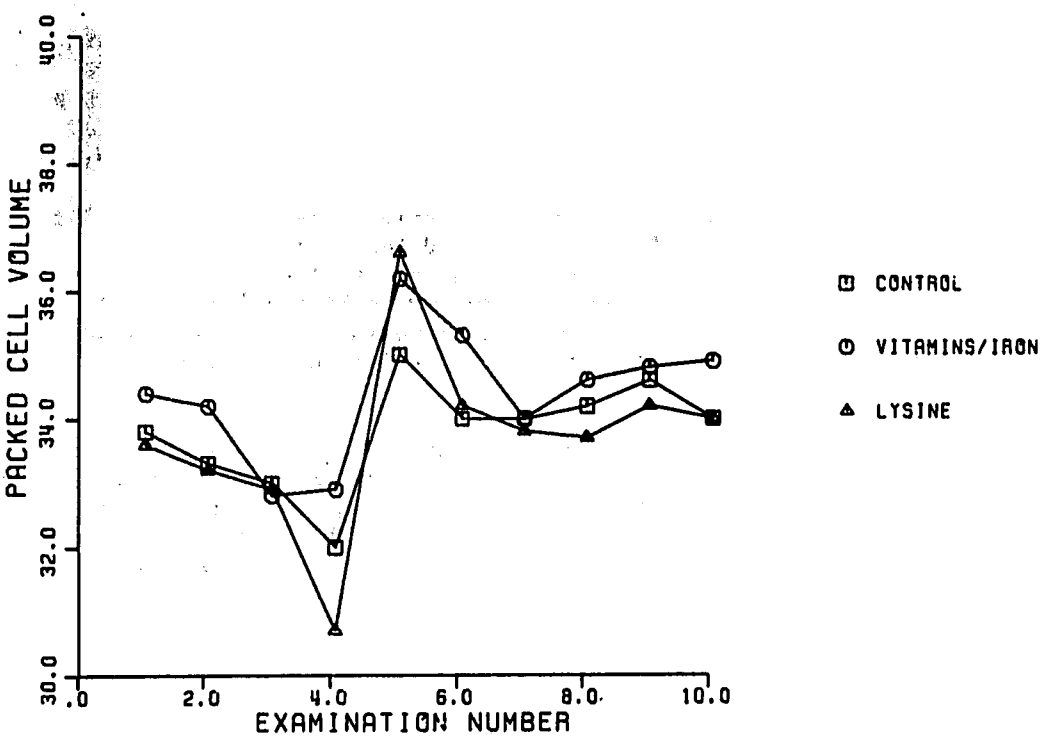


Figure 7: Packed cell volume at each examination.

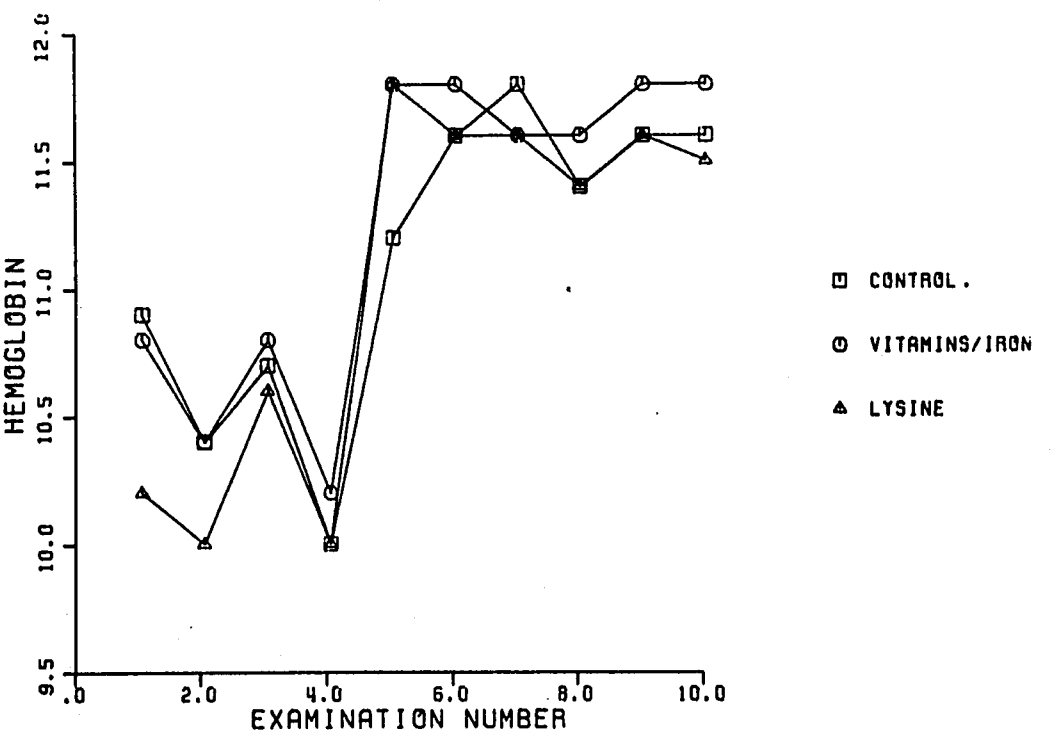


Figure 8: Hemoglobin at each examination.

Figure 9 shows the evolution of plasma protein levels during the study. The results of the first three examinations are not comparable to those of the later examinations, as during the course of the third examination a different (more sensitive) analytical method was introduced. From the time of the fourth examination onwards, the levels in the control area were slightly lower than those in either the vitamin/iron or lysine area, with no difference between the latter two.

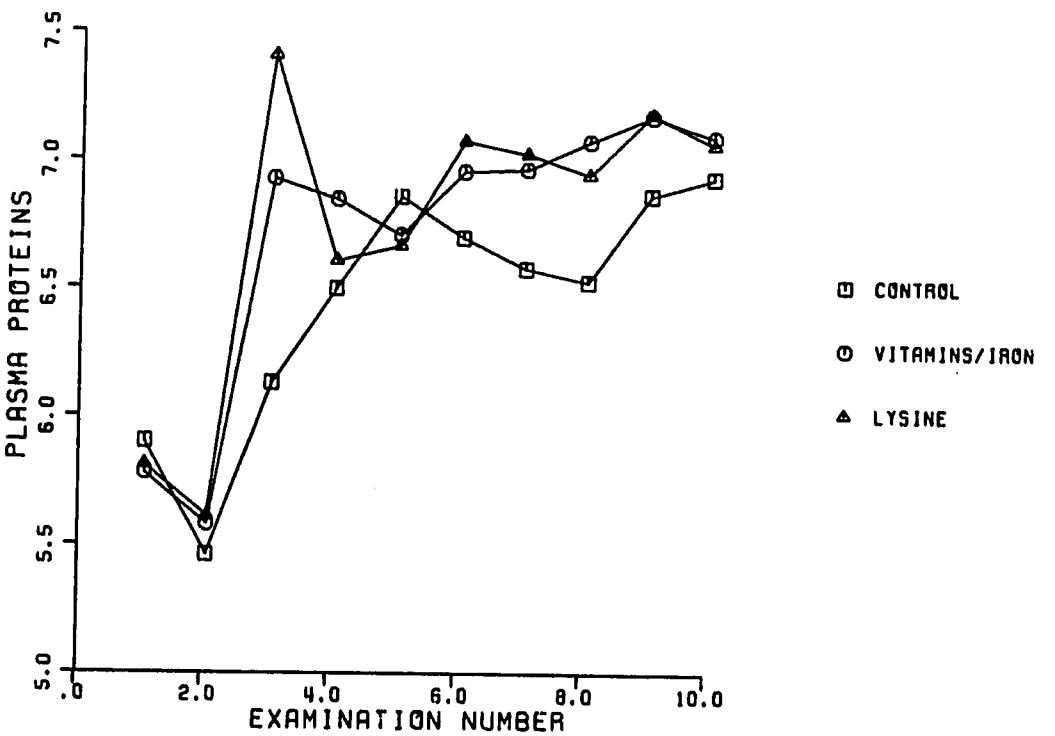


Figure 9: Plasma proteins at each examination.

Table 13 summarizes some of the laboratory results at the end of the study, and once again it is clear that there are no differences between the three regions.

Table 13. Selected laboratory findings in the three areas at the end of the study.

	Boys			Girls		
	Control	Vitamin/iron	Lysine	Control	Vitamin/iron	Lysine
Hemoglobin	11.6	11.8	11.6	11.6	11.7	11.3
PCV	34.1	34.9	34.2	34.2	34.5	33.7
MCHC	34.1	33.7	33.9	33.9	33.9	33.8
Total serum protein	6.9	7.1	7.0	7.0	7.1	7.0
Serum albumin	3.9	4.0	4.0	3.9	4.0	4.0
Serum globulin	3.0	3.1	3.1	3.0	3.1	3.0

DISCUSSION

It is clear that this study failed to demonstrate any improvement following fortification. Several possible explanations may be considered:

1. It is possible that no nutritional intervention can, in isolation, have any effect without correction of the numerous problems (health, sanitation, population pressure, ignorance, poverty, etc.) which afflict a developing society. If this is correct, nutritional interventions should only be carried out as part of an integrated, multifactorial approach. Such interventions would be more complex than simple nutritional ones, but they may well represent the only effective approach.

2. It is possible, that the low caloric intakes may be responsible for the lack of response, with the added lysine being used for energy. There is no doubt that our study population had low intakes of both energy and protein, and it is not clear which is the main deficiency. We hope to carry out a small study in the same region to determine the effect of combined lysine and energy supplementation.

3. It is possible that protein quality is a less important factor than has been believed in the past. It is fairly clear that adults require less essential amino acids per gram of protein than do infants in the first few months of life, but it is not yet clear how the change from infant to adult pattern occurs. Recently, el Lozy and Hegsted (10) have formulated a theoretical model which suggests that by the age of two years, the amino acid requirements of a child are fairly close to those of adults. There are little data available at present to substantiate or refute this hypothesis, though the results of Holt and Synderman (17) on the phenylalanine requirements of phenylketonuric infants do behave as the model predicts. There is little doubt that there is an urgent need for good studies of the amino acid requirements of children at different ages.

4. It might be possible that the failure to demonstrate an effect was due to a specific, uncorrected, dietary deficiency which masked the effect of the fortification. The most often mentioned possibility was zinc deficiency (7). While the zinc intake of our study population was below the Recommended Allowances (12) we do not really know enough about human zinc requirements to ascribe the lack of response to inadequate zinc intake. Furthermore, a small study of hair zinc levels in relation to anthropometric measurements revealed very little relation between the two. In the control area the correlation between hair zinc and the NSI was 0.25, while the lysine area it was -0.04. While the first correlation is indeed statistically significant, it merely signifies that 6% of the subject to subject variability in NSI is related to zinc levels. We, therefore, feel that to ascribe the lack of response to zinc deficiency is, at this stage, mere speculation.

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PROTEIN IMPROVEMENT OF WHEAT BY BREEDING

V. A. Johnson
and
P. J. Mattern

Wheat breeders have been more concerned with increasing the yield of wheat than with modification of its nutritional quality. Thus, the thrust of most breeding programs is to make wheat more productive. The serious shortage of calories as well as protein in many developing countries and the need to satisfy both requirements to overcome malnutrition lend strong support to increasing yields as the necessary central objective of breeding programs. They do not, however, dismiss the need for, nor lessen the value of nutritional improvement of wheat in the fight against world hunger. The Agricultural Research Service and University of Nebraska have engaged in cooperative research on wheat protein improvement since 1955. Since 1966 the research has been supported in large part by funds from the Agency for International Development, U.S. Department of State. In this report we will discuss some of our major findings and examine the outlook for breeding improvement of wheat protein.

Protein Content

The strong effect of production environment on the protein content of wheat is well known. As a general rule protein is highest in low rainfall environments that do not support high grain yields. High rainfall or irrigation situations are likely to produce high yields with depressed protein content of the grain. In the high plains winter wheat area of the USA where historically N-fertilization was not practiced for economic reasons, relatively low yields of low protein wheat are occurring with increasing frequency.

The strong effect of production environment on the protein content of wheat tends to mask genetic effects and has led frequently to the erroneous conclusions that the genetic effects are nonexistent. Others believe that there is a basic incompatibility between protein and yield in wheat. Our research results largely refute both of these ideas.

There is much evidence of existence of substantial genetic variability for protein content in wheat grain (10). Atlas 66 is probably the most widely utilized genetic source of high protein. At Nebraska we have succeeded in transferring the Atlas 66 genes to a productive, high quality hard red winter wheat variety which was released to growers of four states in 1976 under the name Lancota. A linked gene for leaf rust resistance also was transferred from Atlas 66 to Lancota. In Nebraska performance trials in 1973 and 1974 Lancota was equal to the popular Centurk and Scout varieties in yield and it produced grain with 1.5 percentage points higher protein content than Centurk and Scout 66 (Table 1).

Table 1. Performance of Lancota in Nebraska statewide trials in 1973 and 1974.

Variety	C.I. No.	Grain yield (q/ha)			Protein content (%)[1]		
		1973	1974	2-yr. $\bar{x}$	1973	1974	2-yr. $\bar{x}$
Lancota	17389	28.6	32.6	30.6	15.5	15.0	15.3
Centurk	15075	29.3	32.6	31.0	13.9	13.7	13.8
Scout 66	13996	29.3	31.3	30.3	13.9	13.8	13.9

[1]Dry weight basis

We believe that Lancota has genetic potential to produce grain with 2 percentage points higher protein than other varieties grown in Nebraska. In 1975 Lancota and Centurk were evaluated in 16 paired plots at Lincoln, Nebraska. The results are reported in Table 2. Lancota was consistently somewhat higher yielding than Centurk and it produced grain that was consistently 2 percentage points higher in protein than Centurk.

Table 2. Grain yield and protein content of Centurk and Lancota winter wheat varieties in paired plot comparisons at Lincoln, Nebraska, 1975.

Centurk				Lancota			
Protein	Lysine		Yield	Protein	Lysine		Yield
	Adj.	Unadj.			Adj.	Unadj.	
%	%	%	q/ha	%	%	%	q/ha
15.3	3.3	3.1	40.7	16.6	3.0	2.8	45.7
15.6	3.1	2.9	36.2	17.6	2.9	2.7	40.8
16.1	3.1	2.9	37.0	17.8	2.9	2.6	37.7
16.1	3.1	2.9	33.8	17.1	2.9	2.7	38.0
16.1	3.0	2.9	34.1	17.9	2.9	2.7	33.5
15.3	3.2	3.0	36.6	17.9	2.9	2.7	39.2
15.9	2.9	2.7	33.2	17.4	2.9	2.7	38.2
15.4	3.2	3.0	35.2	17.1	2.9	2.7	35.2
16.0	2.8	2.6	35.3	17.7	3.0	2.8	34.9
15.8	3.2	3.0	32.4	17.7	2.9	2.7	28.9
15.3	3.3	3.1	38.3	16.7	3.0	2.8	43.0
15.6	3.2	3.0	35.6	17.7	2.9	2.6	37.3
15.0	3.1	3.0	37.9	17.3	2.8	2.5	40.0
15.6	3.2	3.0	38.8	17.6	2.9	2.7	36.5
14.9	3.0	2.9	37.6	17.8	2.9	2.7	38.7
15.2	3.2	3.1	42.9	17.7	2.8	2.6	39.9
16.2	3.1	2.9	34.7	17.6	2.7	2.5	37.3
15.6	3.2	3.0	25.2	17.5	2.9	2.7	29.9
$\bar{x}$=15.6	3.1	2.9	35.9	17.5	2.9	2.7	37.5

The old Indian variety Nap Hal, PI176217, maintained in the USDA World Wheat Collection, also has been utilized extensively in the Nebraska program and elsewhere as a genetic source of high protein. It has the disadvantage of possessing numerous undesirable agronomic traits which make it unattractive to many breeders as a high protein parent in new crosses. However, we have succeeded in transferring the protein genes of Nap Hal to experimental wheats with much improved agronomic characteristics and productiveness (2).

Several years ago we crossed Nap Hal with Atlas 66 to ascertain whether its genes for high protein were the same as those carried by Atlas 66. If the same, segregation for protein in the F_2 would not be expected. The identification of F_2 progenies significantly higher in grain protein content than either Nap Hal or Atlas 66 and much higher than ordinary varieties suggested different genes for protein in the parent varieties that together function additively (4). We have confirmed this in subsequent studies of lines from this cross in the F_3-F_6 generations (2).

Lines have been recovered that approach the Atlas 66 parent in yield and are 3 to 4 percentage points higher in the protein content of their grain than either Atlas 66 or Nap Hal (Table 3). They range from 4 to 7 percentage points higher in protein than the Centurk variety but are less productive than Centurk.

Table 3. Grain yield, protein, and lysine content of selected varieties and F_7 lines from Nap Hal/Atlas 66 grown at Yuma, Arizona in 1975.

Population	No.	Protein (%)	Adj. lysine (% of protein)	Grain yield (bu/a)
Nap Hal/Atlas 66	13	16.8 (16.7-19.7)	3.3 (3.2-3.4)	56 (40-89)
Nap Hal	3	13.0 (12.7-13.4)	3.3 (3.2-3.4)	43 (25-52)
Atlas 66	3	14.4 (14.0-14.7)	3.1 (3.0-3.1)	94 (79-108)
Centurk	3	10.5 (10.0-10.8)	2.9 (2.7-3.0)	86 (60-102)
Lancota	3	13.9 (12.9-15.3)	3.0 (3.0-3.1)	117 (65-144)

Other hybrid combinations also have provided evidence of the existence of many different genes for high protein in wheat. One of these, Favorit/Cirpiz/Yang Kwang/At66/Cmn/Velvet, is characterized in Table 4.

Table 4

Promising high protein experimental winter wheats grown in single plots at Lincoln, Nebraska in 1975.

Name or Pedigree	Plot no.	Plant height	Seed rating	Yield	Protein	Mixing Time	Mixing Tolerance
		In.		q/ha	%		
Lancota	$\bar{x}$ of 18	38	VG	37.5	17.5	3-1/3	3
Centurk	$\bar{x}$ of 18	40	G	35.9	15.6	3-2/3	4
Lca/K43902-Mironofskaya-808-Akrain	5032	41	VG	36.5	17.4	2-2/3	4+
NE701134/Aurora	5058	39	Ex	42.4	18.0	2-1/3	3
NE701152/Sort 315-16	5316	36	VG	42.8	17.6	2-1/3	2
Favorit/Cirpiz/Yang Kwang/At 66/Cmn/Velvet	11345	34	VG	39.6	19.1	3-2/3	4
"	12291	32	VG	41.0	19.8	3-2/3	4
	12293	33	VG	41.1	19.3	3-2/3	4
"	12297	32	VG	42.0	19.1	3	3+
"	12312	32	VG	39.0	19.1	4	4

Several selections from this cross produced grain with more than 19% protein compared to 17.5% for Lancota and 15.6% for Centurk at Lincoln last year. Equally important, they are moderately hardy winter wheats; they exceeded Lancota and Centurk in yield; were 6 to 8 inches shorter in height; and produced very good seed with good dough development properties. We are using these lines extensively in new crosses and probably will enter one of them in the IWWPN for international evaluation.

The bio-energy implications of changing cereal grain protein concentration by plant breeding recently has been examined by researchers at the IAEA in Vienna, Austria (1). They have shown that increased imputs of carbon assimilates and nitrogen are necessary to increase protein concentration in cereal grains while maintaining high yields. Such imputs presumably would be increasingly difficult to achieve in the new high yielding varieties when the environment for full expression of their high yield potential is provided. However, most of the world's wheat production is from rain-fed areas where environmental constraints cause yields to be relatively low and the genetic yield potential of the varieties grown is seldom fully expressed. In these sub-optimal environments increased bio-energy requirements of higher grain protein should not constitute a breeding barrier.

Recent research by K. D. Wilhelmi of our group involved the study of nitrogen relations in the high protein Lancota and two other varieties during the spring growing season. Lancota was significantly higher yielding and its grain was significantly higher in protein than the

Lancer variety. Lancota exhibited measurable NO_3 reductase activity later in the season, it absorbed more soil nitrogen, and it translocated a larger portion of plant nitrogen to its grain than the varieties with which it was compared.

In earlier research at Nebraska the internal N-transport system of the plant was implicated in the high grain protein phenomenon. This caused us to postulate that phenotypic expression of high protein genes could be expected on low fertility as well as high fertility soils. We were able to substantiate this in the International Winter Wheat Performance Nursery and in subsequent Nebraska experimentation (3). CI14016, a high protein wheat, maintained its 2 percentage points protein superiority over Lancer in a wide array of soil fertility levels during a 2-year period at Nebraska sites (Table 5).

Table 5. Mean yield and protein responses of CI14016 and Lancer wheats to nitrogen fertilizer at selected low fertility test sites in Nebraska in 1969 and 1970.

Nitrogen applied	Grain Yield		Protein Content	
	Lancer	CI14016	Lancer	CI14016
kg/ha	q/ha	q/ha	%	%
0	25.9	25.8	10.8	12.5
22.5	29.9	27.4	11.2	13.3
45.0	31.4	29.5	11.8	14.0
67.5	31.1	30.6	12.6	14.9
90	31.1	30.3	13.2	15.4
112.5	30.7	30.5	13.6	15.8
135	30.2	31.1	14.0	16.3
$LSD_{.05}$	1.1	1.1	0.3	0.3

Insurmountable genetic linkages of high protein genes in wheat with undesirable agronomic and quality traits have not been uncovered in our research. Within the yield ranges normally encountered in the bread wheat area of the USA, higher grain protein content has not proven to be incompatible with high yield. Lancota is an example. Neither have we found incompatibility of the protein genes from Atlas 66 with acceptable milling and bread-making characteristics. Again the Lancota variety serves as an example. Commercial millers and bakers have rated the processing qualities of Lancota as entirely acceptable to excellent in all respects. It has been suggested that short stature in wheat may be incompatible with high protein (5). We have been unable to demon-

strate this within the plant height range of our experimental materials. Note in Table 4 that the very high protein selections from Favorit/Cirpiz/Yang Kwang/Atlas 66/Cmn/Velvet are shorter than Lancota and Centurk by 6 to 8 inches.

The utility of genes for high protein with which we are working in Nebraska appears to be high. Baked products and pasta, the forms in which most wheat is consumed in the U.S., are prepared from the starchy endosperm fraction of the wheat kernel. We find that the effect of the protein genes from Atlas 66 resides entirely in the starchy endosperm and, therefore, is transmitted in its entirety to white milled flour (Table 6).

Table 6
Protein and lysine contents of whole grain, andosperm, and non-endosperm fractions of 4 wheat varieties grown at Yuma, Arizona in 1973.

Variety	Protein content[1] (%)			Lysine content (% protein)		
	Whole grain	Endosperm fraction	Non-endosperm fraction	Whole grain	Endosperm fraction	Non-endosperm fraction
Nap Hal	19.6	18.9	24.5	3.1	2.5	4.6
Atlas 66	19.4	19.3	19.8	2.8	2.5	4.4
CI13449	15.5	14.5	19.6	3.1	2.8	4.4
Centurk	15.4	15.0	19.6	3.0	2.5	4.5
$LSD_{.05}$	1.0	0.9	1.0	NS	NS	NS

[1] Dry weight basis

In the case of Nap Hal the effect is found both in the starchy endosperm and non-endosperm (bran) milling fractions. Since endosperm constitutes more than 80% of the wheat kernel, it is unlikely that high protein from any source, as measured by whole kernel analysis, would be confined to the bran milling fraction.

Lysine

Since 1966 we have analyzed all of the wheats in the World Collection for protein and lysine except the most recent accessions. The genetic variability for lysine that we have uncovered in wheat is less than that identified in corn, barley, and sorghum. Two wheats appear to have particular promise as genetic sources of elevated lysine. They are Nap Hal and CI13449. We are using both extensively in our breeding program. Each consistently produces lysine levels that average from 0.3 to 0.5 percentage point or 10 to 16% higher than ordinary varieties. We consider this to be of sufficient magnitude to be useful to breeders for nutritional improvement of wheat.

The elevated lysine of CI13449 resides mainly in the starchy endosperm of the grain, whereas that of Nap Hal is in the aleurone layer (2). Further evidence of different genes for lysine in the two varieties was provided by the progeny from a cross of Nap Hal with CI13449. Lines significantly higher and lower in lysine than either parent variety were identified. In several of the high lysine lines high productivity was combined with the Nap Hal level of protein (Table 7).

Table 7. Grain yield, protein, and lysine content of selected varieties and F_6 lines from Nap Hal/CI13449 grown at Yuma, Arizona in 1975.

Population	No.	Protein (%)	Adj. lysine (% of protein)	Grain yield (Bu/a)
Nap Hal/CI13449	18	12.7 (12.0-14.8)	3.5 (3.4-3.6)	86 (37-125)
Nap Hal	4	13.0 (12.7-13.6)	3.3 (3.2-3.4)	38 (24-48)
CI13449	4	10.5 (10.2-11.3)	3.2 (3.1-3.3)	92 (76-118)
Centurk	4	10.1 (9.8-10.4)	2.9 (2.8-3.1)	68 (49-82)
Lancota	5	13.1 (12.7-14.2)	3.1 (2.9-3.3)	126 (121-135)

Relationship of Protein and Lysine

This was studied in 12,600 common wheats from the World Collection (9). Increases in protein content from 10% to 15% result in depression of lysine per unit protein. Protein increases above the 15% level have little or no further effect on lysine level. Lysine expressed as percent of grain weight increases as protein increases.

We believe that the genetic variation for lysine in wheat, if utilized by breeders, would permit high protein varieties to be developed that are as high or higher in lysine level than low protein varieties. Evidence of this is provided in Tables 8, 9, and 10.

Table 8. Experimental lines with combined high protein-high lysine grown in a replicated yield trial at Yuma, Arizona in 1975.

Variety or line	Plot number	Plot yield	Protein content	Lysine per unit protein
		g	%	%
Centurk	1	751	11.0	3.1
CI13449	243	1110	11.8	3.4
Nap Hal/CI13449	245	1090	12.9	3.5
"	252	892	13.0	3.3
NB69851//At 66/Cmn/CI13449	25	817	12.9	3.2
Nap Hal/Atlas 66	183	524	16.0	3.0
"	296	472	17.6	3.0

Table 9. Experimental lines with combined high protein and normal lysine grown at Lincoln, Nebraska in 1975.

Variety or line	Plot no.	Plot yield	Seed rating	Protein content	Lysine per unit protein
		g		%	%
Centurk	9081	309	Good	14.5	2.9
CI13447//Lcr/At 66/Cmn	9075	258	Good	17.3	3.0
At 66/Cmn//CI13449	9147	309	Good	16.3	3.0
Nap Hal/Atlas 66	9167	200	Medium	19.1	3.0

Table 10. Average protein and lysine content of selected high protein-high lysine experimental lines grown at 11 test sites in 10 countries in 1975.

Variety or Pedigree	Plot No.	Protein %	Lysine % of Prot.
Centurk	$\bar{x}$ of 2 plots	14.3	3.0
Nap Hal/At 66	219	17.5	3.0
"	180	17.5	3.0
"	220	16.9	3.0
Nap Hal/CI13449	171	16.8	3.2
"	194	16.4	3.2
"	173	16.2	3.2
"	207	16.0	3.2
$LSD_{.05}$		1.2	0.11

Dissemination of Germplasm and Research Information

The International Winter Wheat Performance Nursery was organized in 1968 by Nebraska under its AID contract for protein improvement of wheat. One hundred ten experimental lines and new productive varieties from 24 countries have been evaluated to date in the nursery. Test sites currently total 67 in 37 countries. The nursery has aided in identification of useful winter varieties for production or breeding purposes in developing countries. An example was the early identification of the Russian variety Bezostaya 1 as a productive useful variety in Turkey and Afghanistan based on its performance in the IWWPN.

Since 1974, Nebraska has distributed promising experimental lines from its protein improvement program to many developing countries for local evaluation and use. Both winter and spring types have been involved. In 1976 winter lines were distributed to cooperators in 18 countries. Spring habit lines were sent to 12 countries. Some of the lines are being used extensively in local breeding programs. We also encourage wheat breeders in developing countries to provide us with seed of their most productive new wheats which we cross with our best high protein-high lysine lines. We screen the resulting hybrid populations for presence of the protein and lysine genes and return this material to the breeders for local agronomic evaluation in the developing countries. The procedure permits utilization of the high protein-high lysine genes in developing countries that do not possess laboratory capabilities for protein and lysine determinations. Nebraska will aid these countries in the analyses for protein and lysine of progenies from the crosses. The Nebraska group has filled several hundred requests for seed of high

protein-high lysine lines received from breeders and other researchers throughout the world. The contribution these materials ultimately will make to wheat improvement in these countries will not be known for several years.

Nebraska informally cooperates with CIMMYT and Oregon State University by providing high protein-high lysine lines for use as parents in spring x winter wheat crosses made in Mexico. Productive winter segregates from the crosses are selected at Oregon State University; spring wheat selections are made in Mexico. Winter selections from Oregon will be analyzed in early generations by the Nebraska laboratory to assure retention of genes for protein and lysine in productive new wheats resulting from the spring x winter program.

Three International Wheat Conferences have been organized by Nebraska and ARS under the AID contract for protein improvement of wheat. The First International Winter Wheat Conference was held in Ankara, Turkey in 1972. In 1974, a Latin American Conference was held at Porto Alegre, Brazil and in 1975, the Second International Winter Wheat Conference took place in Zagreb, Yugoslavia. Proceedings of all three conferences were printed and disseminated to wheat researchers throughout the world (6, 7, 8). A large number of requests for publications and reports from the Nebraska project have been filled.

Outlook

Elevated protein content of wheat is compatible with improved productivity within normal ranges of wheat yields. There is sufficient genetic variability for lysine in wheat to overcome the normal depression of lysine associated with higher levels of grain protein. Although protein depression in combination with high grain yields can and does occur in some production environments, varieties that produce more protein in their grain than comparably productive-ordinary varieties in these environments have been developed. Insurmountable linkages of high protein genes with genes conditioning undesirable agronomic and quality traits in wheat have not been identified.

There are numerous genetic sources of elevated protein in wheat. These can be effectively utilized in breeding programs if selection for high protein genes is done in early generations. The absence in some developing countries of laboratory capabilities to screen breeding populations for improved protein constitutes a constraint to wheat protein improvement in these countries. The procedure instituted by the Nebraska group offers a means of circumventing this constraint until the needed laboratory capabilities are developed in these countries.

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RICE FORTIFICATION IN THAILAND

Stanley Gershoff

As a nutritionist and a member of a school of public health for 24 years, the motivation that I had in getting into the kind of study that I'm about to discuss, is the hope that we might develop information which would enable us to produce low cost, practicable programs which would improve the health and nutritional status of people. The lack of urgency that one finds, after all of these years, is very disturbing. Professor Newman, a few minutes ago, said that studying pigs is easier than studying people because you can kill the pigs. In fact, we kill millions of people all the time by neglect and we never do that with pigs.

Ten years ago nutritionists felt that the major nutrition problem in the world was protein-calorie malnutrition, and for many of them, it was capital P for protein and small C for calories. Since protein seemed to be the major problem, many people started worrying about what might be done about it. Among the methodologies that were proposed was fortification. It made a lot of sense. It made sense because the grain crops that made up a major part of the diet for people in developing countries had protein of substandard value which could be improved by the addition of amino acids. At that time, nutritionists, and the other health workers, could have been classified into three groups. There was a very strong group which said that fortification was the way to go, either nutritionists were scientists, or they were not; we could analyze proteins, we knew what their amino acid composition was; we didn't have to go out in the field to test fortification programs. If we did, we were likely to get negative results and that would set back fortification.

There was another group that said fortification would not work, and we ought to forget the whole thing. I think that most of us felt that it was one of many logical approaches to protein malnutrition but that before we went out and spent literally billions of dollars, fortification programs ought to be field tested. It was easy to demonstrate in animal studies that the addition of lysine and threonine markedly improved the biological value of rice protein.

Thailand was picked as a location to study rice fortification for a number of reasons. One was the fact that severe protein calorie malnutrition was prevalent in such countries as Biafra during the war, or in Bangladesh, where people were starving to death and dying in large numbers of acute infectious disease, did not exist in Thailand. There is no reason to expect that the provision of a little lysine or Vitamin B1 will appear effective if the diet supplies only a small part of the caloric or protein needs.

There was another reason, and that was that in Thailand, there was a cadre of health workers and nutritionists who were capable, a government that was sympathetic to doing things for their people, and a population that was very much concerned about the health of their children.

In rat studies, if lysine is added in progressive amounts to a diet containing rice as the sole protein source, one gets a very nice growth response at the lower level of supplementation, but as more and more lysine is added, an imbalance occurs which is accompanied by a decrease in growth. For our rat studies, Thai rice was fed with a variety of lysine and threonine mixtures. Without any lysine or threonine, about 4-1/2% of the 7% protein in the Thai rice was utilized. By adding 0.2% lysine and 0.1% threonine to rice the biological value of its protein could be raised to 100%.

We were concerned about the amino acid composition of the Thai diet as a whole. Records were collected of the food consumed in many families with a combined total of 145 people. The rice was analyzed in Bangkok with an amino acid analyzer for amino acid content, and then tables which had been published were used to attempt to estimate the amino acid profile of the Thai's diet in the villages in which the work was conducted. I learned something. For years, I had been teaching students that when you mix up a variety of foods, the amino acid content of the different proteins in the diet supplement each other. We found, however, that when 70% or more of the calories are supplied from a single source, like rice, the addition of small amounts of foods with proteins of high biological value has little effect on the biological value of the protein of the whole diet. The results of our Thai diet analyses were such that using the amino acid storage patterns of the FAO/WHO Bulletin of 1973 on energy and protein requirements, it was clearly indicated that for infants and 10-12 year olds lysine and threonine were limiting amino acids. For adults, there appeared to be no problem with the amino acid score of the protein of the Thai diets.

The study in Thailand was started about six years ago. We did an eighteen-month pilot study and I was very grateful for it because all kinds of things happened in the pilot study that were of use to us when we got into the full field study. As an example, we discovered that with storage in a hot, humid climate our rice fortification grains (RFG) stuck to each other. When the boxes of RFG were opened we found rocks. Changes had to be made in the coatings of the RFG.

The Thai government has a law against the importation of rice or rice products. The initial rice fortification grains that we started with had a rice flour filler, and these could not be brought into the country. This necessitated a change in carrier, so corn starch was used as the filler, but this resulted in texture and palatability problems.

The rice fortification grains were really a mini-pasta made by the Ajinomoto Company. It made a dough-like mixture to which the desired nutrients could be added. After the grains were extruded, a coating was applied so the simulated grains would not fall apart during the cooking process. The fortified material was added to rice at the village mills at a level of 1%. The rice being consumed in the area where the work was conducted was glutenous, and was steamed so that leaching of nutrients was not much of a problem as demonstrated by laboratory animal and analytical tests. There was no riboflavin in the initial fortified grains. As the study went on, it was felt that riboflavin was required and the fortified grains, which had been hard to find in rice, became easily visible. The yellow color of the fortified grains was not objectionable to the people involved.

The study was done in twenty-nine villages extending over a one hundred kilometer area with a population of approximately 13,500 people. The study was started with 1,265 children 6 months to 5 years of age and finished with about 2,250 children 6 months to 9 years, because most of those started continued throughout the study, and those born during the course of the study were added as they reached the appropriate age. The villages were divided into five groups. One group of villages acted as controls and in their villages there was no intervention except for the semi-annual physical examination.

Another group of villages received a placebo added to their rice. Another group of villagers had a fortification grain that contained those nutrients which we were most concerned about other than the amino acids. These were thiamin, riboflavin, Vitamin A and iron. Another group received the vitamins and iron plus lysine at a .2% level, and threonine at a .1% level.

Day care centers were built in some of the villages. These were built for a variety of reasons. Initially, they were justified as a demonstration to the villagers of our interest in them. We did not have nutrition programs in the day care centers, because they would have interfered with the fortification study. We were very interested in the day care centers as possible delivery systems for a variety of health programs. I understand that since we have left, our day care centers have continued to operate with the financial support of the villagers.

The use of the fortificants was voluntary. Some of the participants used them occasionally. The day care centers could be used without using fortified rice and the fortified rice could be used without using the day care centers.

Medical examinations were conducted twice a year. At each examination, reclining lengths and weights, head, arm and chest circumferences, skinfold measurements and hemoglobins and hematocrits were obtained. A physical examination similar to those that are usually done on nutrition surveys was done; medical histories were obtained and reproductive histories of the mothers were taken. In the winter period hand-wrist x-rays and fecal samples for parasite examinations were obtained. The major interest initially in getting some parasite data was not because of the intention to do a parasite study, but because of concern that there might be a very unequal distribution, and the possibility of all the ascaris in the raw control villages and all the hookworm in the lysine/threonine villages. In addition to this, morbidity data were collected every fifteen days on every single child in the study for a three-year period. It is not necessary to discuss the details of doing this, but it was really an heroic effort and it worked out very well. We also were concerned about who was eating the fortified rice and who might not be eating it. This was very important because we didn't have the kind of control, that they had in the Tunisian study where people could not remove the fortificants from the flour. The children who went to the day care centers had their lunches examined every day. With riboflavin in the RFG it was easy to determine whether their rice was fortified. Each home was visited by our workers at

least once every fifteen days and the combination of looking in their pots, our mill records and our conversations with the householders enabled us to determine who was using RFG and who wasn't. With the use of IBM machines, of course, it was possible to split the population up into those who never used the RFG and those who used it 10% of the time, 20, 30 and so on.

The study was continued for four years. At the end of the third year, a study of the data provided an indication of what was happening to those children who were using the fortification mix over 70% of the time and it appeared at that time that there was a small, but statistically significant improvement in every parameter that was studied. It turned out later that this was wrong, that there was a secular change in the entire population. I'm not going to show you the many tables I have of data. The fact of the matter is that when we analyzed the data at the end of four years, there was absolutely no significant change or suggestion of a change in anything that we measured.

Why not? After all, we provided lysine and threonine. We provided the three vitamins that they seemed to need. There is no question that the children were retarded in growth and development. One of the things that the epidemiologists are likely to say when you tell them that your nutrition intervention didn't work, is, "of course it didn't work. You had no control over infectious disease." In our study, we obtained morbidity data on every child for three years unless they weren't in the study that long.

When we looked at the morbidity data, to our surprise, the children were not very sick. When we divided the children 1 to 5, into tertiles according to the amount of morbidity, the third of the children with most morbidity showed no difference in growth than the third with the least morbidity. The question might be asked whether I am saying that the children who are sick don't lose weight or grow just as fast as the children who are not sick. In 1974 we measured the lengths and weights of several hundred children every month. When a child didn't gain or lost weight during a month or didn't grow, almost invariably the child was sick that month or the previous month. With the exception of grave illness following most periods of sickness, catch-up growth was such that the growth deficit was made up within a few months.

Why did we fail to obtain the desired effect from fortification? There are three possibilities. One is that we missed a nutrient of importance. Frankly I am skeptical about this possibility. At this meeting the question of possible zinc deficiency in Tunisia and other countries was raised. I did zinc analyses on the Tunisian children's hair and over 400 samples of the Thai children's hair. The amount of zinc in the hair varied enormously. We were unable to find any evidence that the children with really low hair zincs were different than those with high hair zincs using anthropometric and morbidity data. Nevertheless, in any future study that we may be fortunate to do, we will try to take into consideration those nutrients which we haven't looked at. The one nutrient that I would be concerned about might be calcium. Calcium is very low in Thai diets and it bothers me, since we have x-ray evidence of rickets and poor bone development.

A second possibility has to do with enteric parasites. I was surprised that with large numbers of children with parasites that we saw very little diarrhea and very few children who felt ill.

The explanation which we favor is that the children did not get enough calories. Ordinarily when one talks about populations not getting enough calories it is taken for granted that they do not have enough calories. Our children appeared to have plenty of food. They would come to the day care centers with large lunches from home, which they rarely consumed completely. There was no evidence of their being ravenously hungry. Their diets contained less than 10% fat. As you know, cooked rice contains over 70% water. They ate glutenous rice which I find a little hard to chew, and I imagine would rapidly fill one's stomach. We studied the diets of two dozen children who were followed for three days by a nutritionist who recorded everything they ate. Sixteen of these children, by our calculations, ate a mean of about 64% of what they should have been getting according to the FAO standards. The results of another study involving a larger number of children in another area of Thailand, also indicate Thai children do not get enough calories even though adequate food is available. Given this kind of a situation where the traditional foods are available but the children do not meet their caloric needs, what can one possibly do? It seems to me that the logical intervention would be to raise calories by increasing the amount of fat in the diet and in any future study, we would. The problem is that fat might be looked at as empty calories and if one increases calories by increasing the amount of dietary fat, other limiting nutrients in the Thai diet such as protein, Vitamin B1 or B2, may become a problem.

We must conclude from the results of this large study that our understanding of the nutritional causes of malnutrition in countries like Thailand is inadequate and that further studies are essential before any intelligent large scale programs to protect against chronic malnutrition in rice-eating populations, can be implemented. Furthermore, it is clear that amino acid fortification of rice diets at this time would be foolish. Finally, the hypothesized value of producing new rice strains with high protein levels or protein with increased biological value has been diminished.

CURRENT STATUS OF BREEDING HIGH PROTEIN RICE

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Presented by Charles N. Bollich

Introduction

Rice is the staple food in tropical Asia and is consumed mainly as milled rice. As with the protein of other cereals, lysine is the first limiting essential amino acid of rice protein. But the lysine content of milled rice protein is high (3.4 to 4.0 g/16 g N). Among cereal proteins, rice protein has one of the highest nutritive quality, but its content in the grain is low, limiting the nutritional value of rice to humans.

In more than 10,000 varieties from the International Rice Research Institute (IRRI) world collection of rice examined, the lysine content of protein varied by only 0.5 percentage points from the mean lysine content at any protein level. This small variation is not unexpected, since at least 80% of the protein of milled rice is glutelin (alkali-soluble protein) which has an aminogram similar to that of milled rice protein.

The two objectives of the protein program of IRRI are to raise the protein content of improve rice varieties by at least two percentage points (from about 7% to 9% for milled rice or from 8% to 10% for brown rice) and to determine the nutritional utility and other aspects of increased protein. Considerations of high yield and good grain quality have priority over protein improvement.

Evaluation

Progress is difficult to measure in the improvement of protein content because of the variability associated with this characteristic of rice. The evaluation procedure used at IRRI consists of selecting for agronomic types, disease and insect resistance, and other essential traits in the early generations.

The protein per seed (P/S) values are determined to identify promising early selections. The lines with high P/S values are promoted to replicated yield trials, while those with low values are discarded. Although a few lines are found each season which exhibit both improved yield and protein content, they do not all give consistent performance for these traits from season to season. However, those lines that consistently have a higher protein content with no sacrifice in yield content than the control varieties in the previous season, are advanced to the agronomy evaluation trails at three levels of added nitrogen fertilizer. Finally, the better selections are evaluated through cooperative trials established in 1975 in Indonesia, Thailand, and at IRRI. This permits an evaluation of the stability of performance for both the protein content and yield potential over several locations.

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A total of 276 varieties and lines were evaluated for protein content in the replicated yield trials at IRRI during both the 1975 dry season and wet season. Many lines that exhibited a high protein content in 1974 did not perform well during 1975. During both the dry and wet seasons of 1975, only a few lines performed better than the average-protein check IR26 in yield and the high-protein check line IR480-5-9 in protein content.

Over four seasons, the most promising entry in the agronomy advanced evaluation trials is the improved line IR2153-338-3, which has a one percentage point higher content of protein than IR8 and IR26, with mean yields comparable with those of IR8 (Table I).

TABLE I

Brown rice protein content[a,b] and rough rice yield[b] of varieties and lines grown during five seasons (mean of three nitrogen levels). IRRI Agronomy Department, 1974 dry season - 1976 dry season.

Variety or line	Protein (%)		Yield (t/ha)	
	Range	Mean	Range	Mean
IR8	7.4-8.4	7.9	4.2-5.5	4.9
IR26	7.7-8.4	8.0	4.4-7.3	5.6
IR480-5-9	8.4-9.3	8.8	3.7-5.2	4.3
IR2153-338-3	8.7-9.1	8.9	3.7-6.8	4.9

a/ N x 5.95.

b/ At 14% moisture.

Yields of IR26, however, averaged 0.7 t/ha higher. IR26 appears to have not only a higher yielding ability and pest resistance than does IR8, but also its protein content is comparable with other improved rice varieties. IR2153-338-3 appears to have an advantage of one percentage point in protein content at the same yield level as IR8 and a spectrum of disease and insect resistance equal to that of IR26. However, for farmer acceptance, the yields of IR2153-338-3 must be equal to those of IR26 without sacrificing protein content. Although its grain yield was higher, the content and the properties of protein of IR2153-338-3 milled rice were similar to those of IR480-5-9 rice.

Diallel Selective Mating As A Breeding Technique

All outstanding high-protein lines were crossed with the best lines, possessing resistance to pests and diseases and tolerance to various adverse environmental conditions. A modified diallel selective mating scheme at the rate of two cycles a year was continued to concentrate genetic factors for protein content with the above traits. The effectiveness of this scheme to concentrate genetic factors for protein content was evaluated in 1975. Based on P/S values, agronomic type, and pest reactions, 64 promising uniform F_3 and F_4 selections were selected from the first and second mating cycles. These were then compared with their parents for yield and protein content in replicated trials. Highly significant differences were found in the percent protein of the grain, P/S, and yield. The tendency for an increase in the protein content of the rice grain to be associated with a decreased yield potential was less with the progeny than with the parent lines. The protein yield of fourteen of the sixty-four progeny lines was superior to that of their parents. Diallel selective mating appears to be a promising tool for increasing the protein content of rice while maintaining a high yield level.

Utilization of High Protein Rice

An increased protein content has been found to be associated with a higher proportion of brown rice protein residing in the endosperm (milled rice) and improved grain resistance to abrasive milling. High protein rices, such as IR480-5-9, have similar grain quality, including whiteness of cooked rice as rices with an average protein content; also, they have only a slightly lower lysine content of protein. Biological tests in cooperating laboratories with *Streptococcus zymogenes*, weaning rats, and humans verified that protein quality of milled rice was predictable from amino acid scores based on lysine. The content of utilizable protein of milled rice was affected more by the protein content than by the protein quality.

Replacing average-protein rice with an equal weight of high-protein rice improved the nitrogen retention in humans in studies where adults were fed a rice diet and preschool children were fed rice-based diets. A one-year study is planned for 1977 by IRRI to determine the effect of replacing average-protein rice with high protein rice on the height and weight of preschool children fed a rice-based diet.

Critical Problems in Protein Studies That Require Further Investigation

More information on the factors which affect protein level in the grain (genetics, environment, and others) is needed to reduce the variability and increase the consistency of performance for grain protein from season to season in order to improve the efficiency of the evaluation system. Efforts at IRRI to develop a seedling test for high protein content of grain have been unsuccessful, because a high protein content of grain appears to be related to a more efficient breakdown and translocation of leaf nitrogen to the developing grain rather than to a faster rate of nitrogen uptake by the plant.

Varietal difference in the properties of cooked rice gruel from rices of various amylose content (1 to 37% of starch) needs to be measured and compared because amylose content determines the volume expansion during cooking. This is especially important in weaning food used for children, since bulk is an important dietary factor for humans and for children, in particular. In addition, the cause of the relatively poorer digestibility of protein of rice and other cereals as compared to wheat protein in humans needs to be investigated.

The inheritance of protein in triploid endosperm tissue seems to be complex and needs to be studied in detail. Plans are underway at IRRI to undertake such genetic studies.

GENE SOURCES FOR HIGH-LYSINE BARLEY BREEDING

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and
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Introduction:

Our approach to improving the protein quality of barley is a concerted effort of the Plant and Soils and Animal and Range Sciences Departments. Our endeavors include field testing and selection followed by chemical and animal evaluation of known high-lysine cultivars, crossing these with barleys having desirable agronomic characteristics and searching for new sources of high-lysine genes, including chemically induced and spontaneous mutants. Our project has the major objective to improve the nutritive quality and productivity of barley for semi-arid regions of the world. This is a joint task assumed by barley breeders, plant physiologists and pathologists, cereal chemists, biochemists, and animal nutritionists.

This paper will review our research efforts in chemical and animal evaluation of some of these high-lysine cultivars and will present evidence of spontaneous mutations that improve protein quality in certain cultivars. In addition we will give a brief review of the progress in selection, development and discovery of high-lysine sources in barley over the past six years (Table 1).

Hiproly

Present status: Hiproly barley was found in the world barley collection by the Swedish scientists Hagberg and Karlson (12) and Munck et al. (22). The high-lysine character is due to a single recessive gene (20,24) or a linked gene complex (17) located in the seventh chromosome. The gene increases the lysine level (g/16g N) in the endosperm by approximately 30%. Hiproly was introduced from Ethiopia as a part of a sample from which two morphologically similar lines were propagated (23), one of which is Hiproly (CI 3947) and the other is low in lysine and named Hiproly Normal (CI 4362). These barleys have been tentatively designated Hiproly isogenes, both being of erectoid type with naked seeds. Hiproly also possesses the genetic trait for shrunken endosperm having a 1000 kernel weight of about 40g (24) compared to a 1000 kernel weight of 50g for Hiproly Normal.

Compared to Hiproly Normal and other barleys commonly produced commercially, Hiproly has an increased content of lysine, aspartic acid, methionine and a decreased content of glutamic acid and proline in the protein (21). Kjeldahl protein of the two Hiprolys is 2-6 percentage points higher

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TABLE 1. BARLEY CULTIVARS HAVING A GENETIC CHARACTER FOR HIGH-LYSINE AND THEIR NORMAL ISOTYPES.

Cultivar	No. observations per mean	%Protein[a]	%Lysine[a]	Original Reference
Hiproly	8	18.6	4.15	12
Hiproly Normal[b]	8	17.2	3.08	
Bomi/Riso 7	1	11.8	4.07	9
Bomi/Riso 8	1	12.5	4.76	9
Bomi/Riso 9	1	13.0	3.79	9
Bomi/Riso 13	1	12.7	4.68	9
Bomi/Riso 1508	3	12.4	4.82	15
Bomi[b]	3	12.7	3.59	
Carlsberg/Riso 29	3	12.2	4.52	6
Carlsberg/Riso 56	1	12.9	4.63	6
Carlsberg/Riso 86	2	13.2	4.44	6
Carlsberg II[b]	3	11.6	3.68	
Notch - 1	-	15.7	4.00	3
Notch - 2	-	14.6	3.96	3
NP - 113[b]	-	11.7	3.38	
CI 7115	-	-	3.6-4.1	29 27
High-amylose Glacier	3	11.2	3.91	
Glacier[b]	3	11.1	3.60	
Betzes seg1 seg1	3	14.6	3.99	c
Betzes[b]	3	13.0	3.48	

a. Montana Agricultural Experiment Station data with the exception of Notch - 1, Notch - 2, NP - 113 and CI 7115.

b. Normal isotype

c. Unpublished data. Montana Agricultural Experiment Station.

than the protein of normal barleys (21). An increase in Kjeldahl nitrogen in barleys generally results in a lower percentage of lysine in the protein (g/16g N) (10), (23). Hiproly does not follow this convention but rather shows a near linear increase in lysine (g/16g N) with increased protein (23).

Hiproly and Hiproly Normal are not very desirable agronomic types due to low yield, weak straw, poor threshing characteristics and in the case of Hiproly, the shrunken endosperm. Hiproly will yield only about 30% of the accepted standard and Hiproly Normal only about 40%. However, the genetic trait of high-lysine can be selectively bred into other cultivars (24).

Nutritional evaluation: Our nutritional evaluation of Hiproly and Hiproly Normal has included rats, poultry and pigs in addition to chemical and physical measurements. Chemical analysis of the Hiproly isotype confirmed earlier reports. In 26 comparisons, Hiproly and Hiproly Normal barley pairs contained an average of 18.5% (14.0 to 20.5%) and 17.2% (13.2 to 19.3%) protein, respectively. Eight pairs were analysed by chromatography (28) for total amino acids. Hiproly contained an average 4.15% (4.01 to 4.41%) lysine (g/16g N) and Hiproly Normal 3.08% (2.9 to 3.26%).

Fractionation of the protein complex in Hiproly and Hiproly Normal showed the salt soluble fraction accounted for 41.8 and 43.5% of the total lysine, respectively (11) (Table 2).

TABLE 2. PERCENTAGE OF OSBORNE FRACTIONS AND THEIR LYSINE CONTENT OF HIPROLY AND HIPROLY NORMAL BARLEYS

	Hiproly			Hiproly Normal		
	Albumins + Globulins	Hordein	Glutelin	Albumin + Globulin	Hordein	Glutelin
Percent of total protein	27	33	35	26	36	33
Percent lysine in protein	5.6	1.6	3.8	4.0	0.6	2.7

The salt soluble and the glutelin fraction together accounted for 78.7% of the total lysine in Hiproly and 80.6% in Hiproly Normal. The salt soluble and glutelin proteins contained 1.6 (5.6 vs 4.0) and 1.1 (3.8 vs 2.7) percentage points more lysine, respectively, in Hiproly than in Hiproly Normal (7). The higher lysine content of Hiproly (4.02%) in comparison to Hiproly Normal (2.80%) was due largely to the higher lysine content of its salt soluble and glutelin proteins. Although the hordein fraction represented 35.1% of the total protein in Hiproly and 36.2% in Hiproly Normal, it only accounted for 14.8 and 9.5% of the total lysine, respectively (11). It is interesting though

that Hiproly exceeded Hiproly Normal in lysine content in the hordein fractions as well as in the salt and alkaline soluble fractions. These findings are in agreement with the report of Ingversen and Køie (14). These authors concluded that at least one lysine rich protein has been increased in the Hiproly glutelin fraction.

The Hiproly pair investigated in the fractionation studies were fed to inbred weanling rats (26) and crossbred pigs (25) in growth and digestibility studies. A naked Compana (CI 16135) was employed as a control in the rat studies and Unitan (CI 10421), Compana (CI 5438) and Glacier (CI 9676) were compared as controls in the pig studies.

TABLE 3. THREE WEEK PERFORMANCE OF RATS FED THREE BARLEYS AND CASEIN IN ISONITROGENOUS-ISOCALORIC DIETS: TRIALS I AND II[a]

Diet	Trial	Naked Compana CI 16185	Hiproly Normal CI 4362	Hiproly CI 3947	Casein-cornstarch
No. rats[b]	I	15	15	15	15
	II	15	15	14[c]	14[c]
Avg gain, g	I	38.5[f]±2.7	40.9[f]±2.6	57.7[e]±2.5	67.0[d]±2.5
	II	40.2[e]±2.3	40.3[e]±2.4	68.2[d]±2.4	65.6[d]±2.7
Avg feed, g	I	216.4[d]±8.7	213.4[d]±8.4	242.5[d]±8.0	222.4[d]±8.4
	II	241.6[e]±8.4	238.9[e]±8.8	280.9[d]±8.6	236.8[e]±9.3
Avg feed/gain	I	5.73[d]±0.3	5.32[d]±0.3	4.34[e]±0.3	3.35[f]±0.3
	II	6.17[d]±1.9	6.03[d]±2.0	4.15[e]±1.9	3.70[e]±2.1
Avg PER	I	1.44[g]±.05	1.61[f]±.05	1.98[e]±.05	2.97[d]±.05
	II	1.55[e]±.10	1.64[e]±.10	2.45[d]±.10	2.65[d]±.10

[a]Least squares means with standard errors. Newman et al. (26)
[b]Average initial weights were 52.0 g and 58.3 in trials I and II, respectively.
[c]One rat died in each group.
[d,e,f,g]Means in the same line bearing different superscript letter, differ significantly (P<.01)

Rats fed Hiproly barley gained faster (P<.01), were more efficient (P<.01) and produced higher PER values (P<.01) than rats fed the Hiproly Normal and naked Compana barleys in two growth trials (Table 3). Consumption of the Hiproly diets was increased over the other two barley diets but the difference was statistically significant (P<.01) only in one trial. Hiproly Normal produced a higher PER in rats than the naked Compana although the difference was significant (P<.05) only in one of two trials.

Casein-fed rats in trial I were superior in growth and efficiency to all barley-fed rats. However, performance of rats fed Hiproly barley and the cornstarch-casein diet in trail II did not differ significantly. Rats fed casein, Hiproly Normal and Compana diets tended to eat similar amounts of feed, whereas, rats fed Hiproly consumed more feed in each trial. However, the increase in feed consumption was significant only in trial II.

These findings confirm the results of Munck et al.(24) who reported higher PER values for Hiproly diets as compared to a reference barley diet fed to mice. Protein efficiency ratios of rats fed the Hiproly Normal and naked Compana diets in this study were comparable to PER values reported for rats fed barley diets in the experiments of Howe (13). The PER values of rats fed Hiproly diets in our study are similar to those reported by Howe et al. (13) when normal barley was supplemented with 0.2% L-lysine or a mixture of 0.2% L-lysine, 0.2% DL-methionine and 0.5% threonine, respectively. This indicates that the protein of the Hiproly barley is similar in nutritional value to normal barley supplemented with free amino acids.

Greater digestion and utilization of nitrogen were observed in rats fed cornstarch-casein diets. These differences were significant ($P<.05$) for all diets tested except for apparent biological value (ABV) determinations obtained with mature rats (Table 4).

TABLE 4. NITROGEN DIGESTIBILITY AND APPARENT BIOLOGICAL VALUE OF COMPANA, HIPROLY NORMAL, HIPROLY AND CASEIN-CORNSTARCH DIETS[a]

Protein source No. rats/ Mean	Rat age	Naked Compana CI 16185	Hiproly Normal CI 4362	Hiproly CI 3947	Casein-cornstarch
		Nitrogen digestibility[b]			
8	4-week	$79.6^{e}\pm1.1$	$78.7^{e}\pm1.1$	$72.1^{f}\pm1.1$	$95.8^{d}\pm1.1$
6	8-week	$80.7^{e}\pm1.2$	$76.3^{f}\pm1.2$	$73.2^{f}\pm1.2$	$93.1^{d}\pm1.2$
8	Mature	$78.5^{e}\pm1.5$	$75.2^{ef}\pm1.5$	$72.9^{f}\pm1.5$	$89.8^{d}\pm1.5$
	Average	79.6	76.7	72.7	92.9
		Apparent biological value of protein[c]			
8	4-week	$66.6^{e}\pm2.7$	$57.2^{f}\pm2.7$	$51.0^{f}\pm2.7$	$82.5^{d}\pm2.7$
6	8-week	$46.4^{e}\pm3.1$	$41.3^{e}\pm3.1$	$46.4^{e}\pm3.1$	$62.7^{d}\pm3.1$
8	Mature	$26.7^{d}\pm3.0$	$29.8^{d}\pm3.0$	$26.5^{d}\pm3.0$	$35.6^{d}\pm3.0$
	Average	46.6	42.5	41.3	60.3

[a]Least squares means with standard errors. Newman et al. (26)

[b]$\frac{\text{Nitrogen intake - Fecal nitrogen excretion}}{\text{Nitrogen intake}} \times 100$

[c]$\frac{\text{Nitrogen Intake - (Fecal nitrogen excretion + Urinary Nitrogen excretion)}}{\text{Nitrogen intake}} \times 100$

[d,e,f]Means in the same line bearing different superscript letters, differ significantly ($P<.05$).

Nitrogen digestibility of the Hiproly diet was significantly lower ($P<.05$) than that of the Compana barley diet for rats at all ages and lower ($P<.05$) than that of the Hiproly Normal barley diet when fed to 4-week-old rats. Mean nitrogen digestion coefficients for Hiproly diets fed to 8-week-old and mature rats were less than those for Hiproly Normal diets but differences were small and lacked statistical significance

Apparent biological value (ABV) of protein followed the general pattern of nitrogen digestibility. Cornstarch-casein diets were of superior nutritional value to all barley diets ($P<.05$) except when fed to mature rats. Compana barley fed to 4-week-old rats had a higher ABV that was significantly ($P<.05$) greater than values obtained with the other barleys. Variation was apparent in the 8-week-old and mature rat ABV determinations, but differences were not significant between barleys.

The nitrogen balance data are not in complete agreement with rat gain, feed efficiency and PER measurements made in the two growth trials. Although, the ABV of Hiproly barley protein was low in comparison with the other two barleys, rats grew faster, more efficiently and had higher PER values when fed Hiproly in the growth trials.

We have completed five trials with pigs in studying the feed value of the Hiproly isotypes. In the first study weanling pigs were fed 14.6% protein vitamin-mineral fortified diets prepared with Hiproly (17.9% CP), Hiproly Normal (17.4% CP) or Compana barley (15.1% CP) with and without supplemental protein in a 3 x 2 factorial arrangement. Six pigs were fed each diet ad libitum as a group. Pigs weighed an average 20.0 kg initially and were terminated when a pen averaged 47.6 kg. The three protein supplemented diets contained equal levels of soybean meal and meat meal, 3 and 1%, respectively, by weight. Diets were equalized in protein level by substituting dextrose for barley. Pigs fed Hiproly diets gained weight faster ($P<.01$) than pigs fed the other barleys regardless of protein supplementation (Table 5).

TABLE 5. GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY OF PIGS FED PROTEIN SUPPLEMENTED AND UNSUPPLEMENTED HIPROLY, HIPROLY NORMAL AND UNITAN BARLEYS[a]

Cultivar	Hiproly		Hiproly Normal		Unitan	
Supplement[b]	-	+	-	+	-	+
No. pigs	6	6	6	6	6	6
Avg daily gain, kg	.65[c]	.65[c]	.41[e]	.50[d]	.41[e]	.52[d]
Avg daily feed, kg	1.92	1.95	1.53	1.67	1.54	1.69
Avg feed efficiency	2.93	3.00	3.75	3.37	3.71	3.27

[a]Average initial and final weights were 20.0 and 47.6 kg, respectively.
[b]- = no supplement and + = protein supplement added.
[c,d,e]Means with different superscript letters are significantly different ($P<.01$).

Added protein improved the growth rate of pigs fed Hiproly Normal and Compana barley diets whereas supplemental protein had no apparent effect on gains of pigs consuming the Hiproly diet. Pigs consumed an average 20.4% more of the Hiproly diets and were 18.9% more efficient compared to pigs receiving the other cultivars. Supplemented protein appeared to increase feed intake and improve feed efficiency of pigs allotted to Hiproly Normal and Compana diets but had no major effect on these parameters of pigs fed Hiproly. Digestion coefficients for nitrogen obtained on Hiproly diets with 45 kg pigs tended to be less than coefficients of digestibility obtained with reference barleys though the difference was not significant.

A second study compared the growth of pigs from approximately 48 to 90 kg when fed vitamin-mineral fortified Hiproly Normal and Hiproly diets containing 14% protein. Protein in these two diets was totally supplied by the cereals. Two control groups were fed vitamin-mineral fortified barley diets with or without supplemental protein. The supplemented control diet was balanced for 14% protein with soybean meal and meat meal. The control diets were prepared with Unitan barley (CI 10421) which is a six-row commercial variety and in this instance contained 10.7% CP. Hiproly fed pigs gained faster ($P<.01$) than pigs fed either the Hiproly Normal or the unsupplemented Unitan diet and equal to the protein supplemented Unitan control diet (Table 6).

TABLE 6. GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY OF PIGS FED HIPROLY, HIPROLY NORMAL AND COMPANA BARLEYS[a]

Cultivar	Hiproly	Hiproly Normal	Compana - protein	Compana + protein
No. pigs	6	6	6	6
Avg daily gain, kg	.83[b]	.54[c]	.50[c]	.89[b]
Avg daily feed, kg	2.92	2.24	2.31	3.18
Avg feed efficiency	3.55	4.22	4.77	3.54

[a]Average initial and final weights were 48 and 90 kg, respectively.
[b,c]Means with different superscript letters are significantly different ($P<.01$).

The pigs in the Hiproly group appeared to consume more feed and utilize it more efficiently than those on the Hiproly Normal and unsupplemented Unitan diets. However, pigs fed the supplemented Unitan diet tended to eat more and were equally efficient compared to those fed the Hiproly unsupplemented diet. The percentage of lean meat in the carcasses tended to be best in pigs fed Hiproly and the supplemented Unitan control diets.

In the third study, weanling pigs averaging 23.6 kg were used to compare Hiproly, Hiproly Normal and Glacier barleys in diets formulated to contain equal amounts of protein (16.0%), lysine (.70%) and methionine plus cystine (.50%). Soybean meal was included in the Hiproly Normal and

Glacier diets at the expense of barley to equalize protein level to that in the Hiproly diet with no supplemental protein. L-lysine and DL-methionine were added to balance the amino acids in the Hiproly and Hiproly Normal diets to the levels calculated for the Glacier barley-soybean meal diet. There were no significant differences in rate of gain due to diet (Table 7).

TABLE 7. GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY OF PIGS FED HIPROLY, HIPROLY NORMAL AND GLACIER BARLEYS BALANCED FOR PROTEIN, LYSINE AND METHIONINE PLUS CYSTINE[a]

Cultivar	Hiproly	Hiproly Normal	Glacier
No. pigs	12	12	12
Avg daily gain, kg	.66	.72	.73
Avg daily feed, kg	2.17	2.38	2.46
Avg feed efficiency	3.31	3.35	3.44

[a]Average initial and final weights were 23.6 and 102.5 kg, respectively.

Pigs fed the Hiproly Normal and Glacier barley diets tended to eat more and those on the Glacier diet appeared to utilize their feed less efficiently than those fed the Hiproly diet.

An additional two trials were conducted to study the effect of replacing soybean meal with Hiproly or Hiproly Normal barleys in diets prepared with low-protein barley. Unitan (10.5% CP) was fed in one trial and Glacier (10.5% CP) was fed in the second study. Control diets were prepared to contain 16% protein for the growing period (25 to 46 kg pig wt.) and 14% protein for the finishing period (46 to 95 kg pig wt.) using a blend of the low-protein barleys and 47% protein soybean meal. In each trial two diets were prepared with either Hiproly or Hiproly Normal blended with the low-protein barley. The high-protein barleys replaced approximately 50% of the soybean meal in growing diets and all of the soybean meal in finishing diets. Calculated lysine and methionine + cystine levels were equalized to that of the control barley-soybean meal diets using L-lysine and DL-methionine.

TABLE 8. GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY OF PIGS FED BALANCED DIETS PREPARED WITH BLENDS OF HIPROLY OR HIPROLY NORMAL BARLEYS WITH UNITAN BARLEY[a]

Cultivars	Hiproly + Unitan	Hiproly Normal + Unitan	Unitan
No. pigs	12	12	12
Avg daily gain, kg	.83[b]	.80[b]	.75[c]
Avg daily feed, kg	2.73	2.60	2.50
Avg feed efficiency	3.27	3.28	3.34

[a]Average initial and final weights were 25.1 and 95.1 kg, respectively.
[b,c]Means with different superscript letters are significantly different ($P<.05$).

In the first study, no differences were noted in growth rate of pigs fed the diet mixtures containing Hiproly or Hiproly Normal and Unitan barley. (Table 8). However, both groups fed the mixed barley diets gained faster ($P<.05$) than those fed the control diet. Though the control pigs tended to eat less, differences in feed consumption and efficiency were not statistically significant ($P>.10$) between diet groups. In the second study there were no differences in gain, feed consumption or efficiency due to treatment (Table 9). Diet did not significantly influence carcass leanness in either trial.

TABLE 9. GROWTH RATE FEED CONSUMPTION AND FEED EFFICIENCY OF PIGS FED BALANCED DIETS PREPARED WITH BLENDS OF HIPROLY OR HIPROLY NORMAL WITH GLACIER BARLEY[a]

Cultivars	Hiproly + Glacier	Hiproly Normal + Glacier	Glacier
No. pigs	9	9	9
Avg daily gain, kg	.78	.80	.78
Avg daily feed, kg	2.50	2.71	2.58
Avg feed efficiency	3.34	3.42	3.35

[a]Average initial and final weights were 22.9 and 93.8 kg, respectively.

Variety development: Ultimately we are interested in developing high-lysine lines and populations of high agronomic qualities, not lines and populations with shrunken endosperm. Our experience with Hiproly crosses indicates plump high lysine lines can be obtained. In Table 10 are shown some data on a few of the better lines.

TABLE 10. PRELIMINARY INFORMATION ON SELECTED HIGH LYSINE - HIGH YIELDING LINES FROM HIPROLY CROSSES

Selection and generation	% Protein	% Lysine in Grain	% Lysine in Protein	Kernel wt. (mg)
Betzes x Hiproly, F_8	16.2	.595	3.67	38.7
Betzes x Hiproly, F_8	18.5	.680	3.68	-
Betzes x Hiproly, F_8	18.0	.680	3.78	-
Compana x Hiproly, F_8	17.9	.660	3.69	38.2
Compana x Hiproly, F_8	17.7	.660	3.73	-
Hiproly x Bonus T5-7g,F_6	16.4(GQA)	.670	4.09	41.4
Hiproly x Bonus T3-7c,F_6	17.1(GQA)	.595	3.48	34.8
Hiproly x Bonus T3-7c,F_6	16.7(GQA)	.613	3.67	31.9

Many of the lines seem to have acceptable agronomic type for this early in the breeding program. A male sterile facilitated recurrent selection population consisting of plump high lysine lines only, was planted this spring. Sieving before replanting should insure stabilizing the plumpness trait. The genetic base of this population is being widened.

Another approach to improving high lysine lines in regard to the shrunken endosperm character is to increase kernel weight while ignoring the shrunken appearance.

In addition, theoretically, it seems possible that suppressor genes of the shrunken endosperm character might exist or could be induced. This spring we treated with the mutagen DES and planted seed lots of Hiproly, Nupana plus sex1 sex1 with the hope of inducing such suppressor genes. We also have populations of Washonupana, Nupana and Nubet in the field this summer that have grown from DES treated seed. We will be looking for the induction of plump high lysine mutants by employing a light table to select opaque kernels for further evaluation and sieving to select for plump mutants.

If high lysine barley is to be used extensively it seems important that the gene or genes used should show stability for high lysine production in as many environments as possible. We have recently summarized our data (26 environments) for Hiproly and Hiproly Normal (Table 11). We may conclude that the high protein and high lysine of Hiproly express well over a range of environments. What the response will be in higher yielding genotypes is less certain.

Use of a recently available double translocation will permit us to transfer the Hiproly gene into a large number of genotypes without extensive testing for lysine.

High Amylose Glacier

Present status: In 1967 Merritt (18) described a barley with an amylose content of approximately 44% of the total starch. This barley was designated Glacier Ac 38 since it was shown to be a mutant of the six-rowed variety Glacier (CI 9676). The biosynthesis, genetic control and structure of the starch has been thoroughly investigated (2, 19, 31). In addition to the starch mutation, high-amylose Glacier has a greater percentage of lysine in the protein than the normal Glacier. This was first reported by Pomeranz et al. (27) and has been further verified in our laboratory (5). The genetic trait for high-lysine in high-amylose Glacier has not been thoroughly investigated.

Nutritional evaluation: In our evaluation of the Glacier isotypes, we have shown that the mutant consistently contains more lysine ($P<.05$) than Glacier as well as more arginine, cystine/2, threonine and tryptophan and less proline and glutamic acid (5). A study of the Osborn fractions indicate that the mutant Glacier contains slightly more of the albumin/globulin fraction and less of the prolamin fraction than the parent Glacier.

Table 11. Protein, lysine and agronomic comparisons between Hiproly Normal and Hiproly.

Character measured	number of comparisons	Hiproly Normal (x) mean	Hiproly Normal (x) range	Hiproly (y) mean	Hiproly (y) range	Difference + $\bar{x} - \bar{y}$	byx ‡	r^2 §
Kjeldahl Protein, %	26	17.20	13.2-19.3	18.45	14.0-20.5	-1.24**	0.65**	.530**
G.Q.A. Protein, %	26	16.55	12.5-18.8	17.17	12.7-19.6	-0.62**	0.64**	.520**
Udy Protein, %	26	16.08	11.5-19.7	19.63	14.6-21.8	-3.55*	0.47**	.388**
Transmittence, %	26	39.4	33.5-45.0	46.6	41.0-51.8	-7.26**	0.29**	.090
Lysine in barley, %	26	.448	.360-.515	.581	.415-.670	-.133**	0.99	.455**
Lysine in protein, %	26	2.61	2.18-3.09	3.15	2.53-3.46	-.539**	0.14**	.016
Yield, Q/ha	8	21.9	10.8-35.8	14.9	8.6-27.2	6.98**	0.75	.886**
Plump, on 6/64 sieve, %	26	73.7	15.8-95.3	17.7	1.0-47.3	55.0**	0.25**	.141
Thin, thru 5½/64 sieve, %	26	12.1	1.3-57.2	46.0	13.0-93.6	-33.9**	1.16	.416**
Kernel weight, mg.	26	49.2	39.9-57.0	38.4	32.9-45.1	10.8**	0.48**	.501**
Test weight, Kg/hl	8	71.4	67.7-77.5	66.1	58.2-71.6	5.35**	0.78	.652
Plant height, cm.	7	74.7	40.6-101.6	72.2	43.2-96.5	2.5	0.90*	.992**
Date headed, days from 1/1	6	175.5	175. -179.	178.5	175-182	-3.0	0.38	.223
Lodging, %	2	46.0	3.0-90.0	25.0	0.0-50.0	21.0	0.54	1.000

\+ Significant differences by paired t-test are starred.
‡ Significant differences from a slope of 1.0 are starred.
§ Significant correlations of paired values are starred.

TABLE 12. PERFORMANCE OF WEANLING RATS ON GLACIER AND HIGH-AMYLOSE GLACIER BARLEYS

Cultivar	Glacier	High-amylose Glacier
No. rats	28[a]	30
Avg gain, g	73.2[c]	86.9[b]
Avg feed, g	394.0[c]	438.1[b]
Avg feed efficiency	5.49	5.11[c]
Avg PER	2.01[c]	2.16[b]
Digestible N, %	81.4[b]	75.1[c]
Retained N, %	59.7[b]	59.6[b]

[a]Two rats died in this group.
[bc]Means in the same line bearing different superscript letters are significantly different; gain, feed, digestible nitrogen ($P<.01$) and feed efficiency and PER ($P<.10$).

Weanling rats were fed isonitrogenous, isocaloric diets prepared from the mutant and normal Glaciers. Rate of gain and feed consumption were greatest ($P<.01$) in rats fed the mutant barley (Table 12). These rats also tended to have greater feed efficiency and PER ($P<.10$) compared to rats fed Glacier diets. Digestible nitrogen was greatest ($P<.01$) on normal Glacier diets although there was no significant difference in nitrogen retention between barleys.

TABLE 13. PERFORMANCE OF PIGS FED GLACIER OR HIGH-AMYLOSE GLACIER BARLEYS WITHOUT SUPPLEMENTAL PROTEIN

Cultivar	Glacier	High-amylose Glacier
No. pigs	20	20
Avg daily gain, kg	.68[a]	.65[a]
Avg daily feed, kg	3.25[a]	3.06[b]
Avg feed efficiency	4.81[a]	4.73[b]

[a,b]Means with different superscript letters are significantly different ($P<.10$).

Pig diets fortified with vitamin and minerals but no protein supplement were prepared with the mutant and normal Glacier and fed to pigs weighing 58.5 kg initially to market weight (98.9 kg) (5). Gains did not differ between diets although pigs fed Glacier tended to consume more feed but were less efficient ($P<.10$) (Table 13). Carcass of pigs fed the mutant barley were leaner than those fed the normal Glacier. No significant differences in pig performance were observed in later studies when the two barleys were equally supplemented with soybean meal protein as well as minerals and vitamins (Table 14).

TABLE 14. PERFORMANCE OF PIGS FED GLACIER OR HIGH-AMYLOSE GLACIER BARLEYS WITH SUPPLEMENTAL PROTEIN

Trial	2		3	
Barley	Glacier	High-amylose Glacier	Glacier	High-amylose Glacier
No. pigs[a]	16	16	16	16
Avg daily gain, kg	.73	.73	.76	.75
Avg daily feed, kg	2.13	2.25	2.66	2.76
Avg feed/gain ratio	2.86[c]	3.13[b]	3.50[c]	3.68[d]

[a]Average initial and final weights in trials 2 and 3 were 17.5 and 96.2 kg and 21.9 and 98.5 kg, respectively.
[bc,de]Means in the same line, within trial having different superscript letters differ significantly: $P<.01$ and $P<.10$, respectively.

Variety development: High Amylose Glacier has a somewhat smaller seed than Glacier (46.9 mg. and 49.0 mg. respectively, as an average from 17 environments). However, seed that weighs 47 grams per 1000 can hardly be considered small.

We are attempting to determine if the high lysine trait can be separated from the high amylose starch characteristic. It has been determined that the high amylose starch (50:50) is less desirable than the normal (25:75) and waxy starches (0:100) (5).

Shrunken Betzes (segl segl)

Present status: Betzes segl segl occurred as a spontaneous mutant in the variety Betzes. The mutant was described by Jarvi and Eslick (16).

Nutritional evaluation: In earlier unpublished rat feeding trials Betzes with 13.2% protein in the barley and 3.57% lysine in the protein had an adjusted PER of 1.39 whereas Betzes segl segl with 15.4% protein and 3.86% lysine in the protein had adjusted PER of 1.62. Both barleys were fed in an 8% protein ration.

In recent trials Betzes (12.7% protein) and Betzes segl segl (14.8% protein) were compared chemically and in rat growth trials. Their respective adjusted PER were 2.05 and 2.00 (Presented in Table 18, page 17).

The Betzes segl segl mutant, based on pairs grown in 18 environments, had higher protein and higher lysine in the barley and in the protein than did its' normal isotype whether lysine was determined on the amino acid analyzer, by UDY, or by microbiological assay. (Table 15). However, the Betzes segl segl contained more hordein in the protein than did Betzes.

Table 15. Protein, Lysine and Agronomic Comparisons Between Betzes and Betzes segl segl

Character Measured	Number of Comparisons	Betzes(x) mean	Betzes(x) range	Betzes segl segl (y) mean	Betzes segl segl (y) range	Difference $\bar{x}-\bar{y}$ +	byx ‡	r^2 §
Kjeldahl Protein, %	18	14.59	12.3-18.6	15.93	12.8-18.6	-1.34**	0.65**	.768**
GQA Protein %	18	13.64	9.2-21.7	14.64	10.2-16.4	-1.01*	0.40**	.464**
Udy Protein, %	18	14.55	10.9-17.6	17.10	14.6-19.1	-2.55**	0.52**	.526**
Transmittence, %	18	44.6	40.0-51.5	47.2	41.0-53.0	-2.52**	0.58	.314*
Lysine in Barley, %	18	.436	.34-.53	.518	.40-.59	-0.082**	0.34**	.212
Lysine in Protein, %	18	3.05	2.62-3.59	3.25	2.65-4.08	-0.203**	0.58*	.404**
Yield, Q/ha	9	32.8	11.5-49.2	10.1	4.9-31.9	22.7**	0.56*	.677**
Plump, on 6/64 sieve, %	19	46.8	0.0-74.3	3.9	0.0-31.6	42.9**	0.10**	.113
Thin, thru 5½/64 sieve, %	19	27.8	6.5-91.1	88.7	39.5-100.0	-60.9**	0.11**	.023
Kernel Weight, mg	17	34.3	21.9-40.1	18.8	12.4-22.1	15.4**	-0.05**	.005
Test Weight, Kg/hl	8	63.2	59.2-67.7	42.4	30.3-55.7	20.9**	-2.47**	.542*
Plant Height, cm	9	79.5	50.0-116.8	78.2	54.1-108.7	1.3	0.79*	.938**
Date Headed, days from 1/1	9	180.1	173.5-185.6	183.4	171.0-192.4	- 3.3*	1.71**	.943**
Date Mature, days from 1/1	2	210.2	207.5-213.0	212.0	206.3-217.7	- 1.8	2.07	.998

\+ Significant differences by paired t-test are starred.
‡ Significant differences from a slope of 1.0 are starred.
§ Significant correlations of paired values are starred.

Variety development: The expression of the high lysine gene is more dependent on the environment than is that of Hiproly.

Until the question of animal performance is resolved we are doing little more with this gene although most of the necessary genetics concerning it are known.

Riso Mutants

Present status: Hiproly and high-amylose Glacier were exciting discoveries in the search for improved nutritive quality in barley. However, these cultivars were completely overshadowed by the discovery of the Bomi mutant, Bomi/Riso 1508 (15). According to Ingversen et al. (15) the mutant 1508 was found in 1970 in the Danish two-rowed spring variety Bomi treated with ethyleneimine. The lysine content of the protein varied from 5.18 to 5.42% in four replications of 1508 compared to 3.64 to 3.82% in Bomi. A 36% increase in threonine was also found along with a considerable increase in histidine, argine, aspartic acid, glycine and alanine. Glutamic acid and proline and to a lesser extent cystine and phenylalanine were decreased compared to the parent Bomi. Albumins/globulins increased from 27% in Bomi to 46% in 1508 while prolamines decreased from 29% to 9%, respectively. Glutelin was not altered. The lysine content of the prolamines and of the glutelins were 192 and 36% above the respective value for the parent while the amino acid composition of the mutant albumin/globulin fraction is similar to that of Bomi. The mutant is further characterized by a 10% reduction in seed size and a reduction yield per unit area of 28% (9).

In addition to Bomi/Riso 1508, twelve mutants have been discovered from mutagenically treated material (7). These have 10 to 40% more lysine in the seed protein than the parent varieties. The 40% increase in 1508 is due to a single recessive gene (7), (8). The productivity and seed composition of mutant 1508 and the parent variety Bomi are shown in Table 16, taken from Doll and Køie (9).

TABLE 16. PRODUCTIVITY AND SEED COMPOSITION OF MUTANT 1508 AND THE PARENT VARIETY BOMI[a]

	Bomi	Mutant 1508	Relative value of mutant
Lysine content, g/16 g N	3.75	5.20	139
Lysine yield, g/plot	10.4	13.7	132
Protein content, 6.25 x N%	9.7	11.0	113
Protein yield, g/plot	278	263	95
Seed size, mg.	40.8	37.5	92
Number of seeds/plot x 10^{-3}	70.7	63.4	90
Grain yield, kg/plot	2.88	2.38	83

[a] Doll, H. and B. Køie. (9)

Carlsberg II was treated with ethyl methanesulphonate to produce two high-lysine mutants, Carlsberg/Riso 29 and Carlsberg/Riso 86 (6). These cultivars showed an increase of 15% in lysine and a 13 to 19% decrease in yield, respectively. A third Carlsberg/Riso mutant was produced through gamma radiation that possessed a 20% increase in lysine but suffered a 23% decrease in yield.

Nutritional evaluation: Five mutants of Bomi and two mutants of Carlsberg II and Hiproly, Hiproly Normal, Betzes and Betzes segl segl barleys were compared chemically and in rat growth trials. Biological value (BV) and true protein digestibility (TPD) were measured in a metabolism trial on Hiproly Normal, Bomi and the Bomi mutants.

Average Kjeldahl protein of the five Bomi mutants was essentially the same as the parent barley Bomi (13.5 vs 13.4%) on a dry matter basis. The Carlsberg II mutants were higher in Kjeldahl protein than Carlsberg II (12.9 vs 11.4%). The percentage of protein in the Hiproly and Hiproly Normal pair was consistent with previous determinations (20.6 and 17.9%, respectively).

All of the Riso mutants showed a significant increase (12 to 56%) in percentage lysine over the parent Bomi and Carlsberg II with the exception of Bomi Riso 9. A rise in percentage lysine was accompanied by a decrease in the percentage of glutamic acid and proline in the barley proteins. Arginine, threonine and the two nonessential amino acids, alanine and aspartic acid appeared to be increased in the mutant barley proteins. The Bomi/Riso 1508 mutant evidenced the greatest percentage increase (55.6%) of lysine over the parent followed by the Bomi Riso 8 (32.5%) and Bomi Riso 13 (30.5%) mutants.

Fractionation of the protein from Bomi/Riso 1508 indicated variance due to location (Table 17).

TABLE 17. CORRELATION OF ANIMAL PERFORMANCE WITH LYSINE AND PROTEIN FRACTION OF BOMI AND BOMI/RISO 1508

Location	Cultivar	PER	BV	% Lys	% Al/Glo	% Glu	% Hord	% Res
Huntley	Bomi	1.82	65.0	3.62	36	32	20	11
	Bomi/Riso 1508	2.19	78.8	4.41	45	25	15	16
Bozeman 1	Bomi	1.95	70.7	3.53	39	28	21	13
	Bomi/Riso 1508	2.15	72.8	4.45	49	19	15	18
Bozeman 2	Bomi	2.13	75.1	3.61	37	24	25	13
	Bomi/Riso 1508	2.48	91.7	5.60	55	24	5	15

The percentage of hordeins in two samples were identical (15%) and had similar levels of the salt soluble albumin/globulin fractions (45 and 49%). The third sample of Bomi/Riso 1508 had only 5% hordein and showed an increase in the albumin/globulin fraction (55%). The Bomis did not vary in this respect but contained considerable more hordein and less of the albumin/globulin fraction than did the Bomi/Riso 1508 mutants. Animal performance appeared to be influenced to a greater extent by the total percentage of lysine in the seed protein than by the separate Osborn fractions. However, there was a negative correlation ($P<.05$) between the percentage of hordein and the percentage of lysine in the seed protein ($r = .79$).

The Riso mutants 7, 8 and 13 showed a trend similar to the Bomi/Riso mutant 1508 in respect to percentage of hordein and albumin/globulin fractions. The Carlsberg/Riso mutants exhibited a decrease in hordein and increase in albumin/globulin compared to the parent Carlsberg II. There was less difference in the fractions between Hiproly and Hiproly Normal as compared to the Riso mutants, however, the Hiproly did possess the greater percentage of albumins/globulin and less percentage of hordeins as compared to Hiproly Normal. Hiproly contained more hordein and less albumins/globulin than did either Bomi or Carlsberg II.

Rats fed Bomi/Riso 1508 and Carlsberg II/Riso 56 gained equally as well and produced PER values that were not significantly different from the PER of rats fed casein (Table 18).

TABLE 18. GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY RATIOS OF RATS FED ISONITROGENOUS BARLEY AND CASEIN DIETS FOR 21 DAYS, TRIAL 2

Cultivar	Avg gain	Avg feed	Avg feed/gain	Avg PER
Hiproly	71.8^{bcde}	365.9^{ab}	5.12^{bcd}	2.06^{def}
Hiproly Normal	65.4^{cdef}	360.4^{abc}	5.58^{ab}	1.80^{g}
Bomi	68.4^{bcdef}	337.8^{bcd}	5.09^{bcd}	2.13^{de}
Bomi/Riso 7	74.0^{abcd}	323.6^{bcd}	4.44^{ef}	2.38^{de}
Bomi/Riso 8	77.0^{abc}	361.0^{abc}	4.71^{def}	2.25^{bcd}
Bomi/Riso 9	59.6^{ef}	331.2^{bcd}	5.74^{a}	1.83^{fg}
Bomi/Riso 13	74.3^{abcd}	393.6^{a}	5.36^{abc}	1.99^{efg}
Bomi/Riso 1508	84.5^{a}	359.1^{abc}	4.32^{ef}	2.48^{ab}
Carlsberg II	66.7^{cdef}	321.3^{cd}	3.86^{cde}	2.20^{cde}
Carlsberg Riso 29	80.1^{ab}	353.0^{abcd}	4.40^{ef}	2.37^{abc}
Carlsberg Riso 56	84.8^{a}	360.0^{abc}	4.29^{f}	2.44^{ab}
Betzes	63.1^{def}	315.8^{d}	5.09^{bcd}	2.05^{def}
Betzes segl	60.7^{ef}	317.2^{cd}	5.36^{abc}	2.00^{efg}
Casein	80.0^{ab}	338.1^{bed}	4.30^{f}	2.50^{a}

abcdefg Means in the same column with different superscript letters are significantly different ($P<.05$).

Bomi mutants 7 and 1508 produced PER values significantly higher than Bomi. The Bomi mutant which had the lowest percentage of lysine produced the slowest gain, poorest feed efficiency and lowest PER of the Bomi barleys. Although rats fed the Carlsberg II mutants gained faster ($P<.05$) than rats fed the parent Carlsberg II, only Carlsberg II/Riso 56 produced a PER significantly ($P<.05$) higher than the parent.

TABLE 19. BIOLOGICAL VALUE AND TRUE PROTEIN DIGESTIBILITY OF HIPROLY ISOTYPES, BOMI AND BOMI MUTANTS.

Cultivar	PER+	Biological ‡ Value %	True Protein ‡ Digestibility %
Hiproly	2.06	75.82	75.97
Hiproly Normal	1.80	73.50	86.82
Bomi	2.13	75.14	75.39
Bomi Riso 7	2.38	84.36	74.03
Bomi Riso 8	2.25	91.76	69.28
Bomi Riso 9	1.83	82.37	77.69
Bomi Riso 13	1.99	87.06	65.88
Bomi Riso 1508	2.48	91.65	69.15

\+ Obtained in growth trial
‡ Each mean is an average of 4 values.

Biological value and TPD of the Bomi mutants, Bomi and Hiprolys were determined (Table 19). Biological value of the barley proteins was correlated with the percentage of lysine ($r = .86$). A negative correlation was found between TPD and percent lysine ($r = -.84$) and between BV and TPD ($r = -.80$). The Bomi/Riso mutants 1508 and 8 had the highest BV of the barleys compared. The parent barley of the mutants, Bomi, was equivalent to Hiproly in BV in addition to comparable performance in the growth trial.

Variety development: The discovery of the existence of high-lysine Riso mutants with shrunken endosperm expressing xenia was most exciting. The xenia mutants are noted in Table 20. These are presently being allele tested to see how many genes we are actually dealing with. We are also in the process of confirming the presence of high lysine in those kernels that are shrunken on F_1 plants from crosses with low lysine lines.

TABLE 20. HIGH-LYSINE BARLEY AND THE SHRUNKEN ENDOSPERM CHARACTER RELATIONSHIP.

Mutant	% Lys. in Barley CF Normal +	% Lys. in Protein CF Normal +	Inheritance ‡
Bomi Riso #7	+	+	SE
Bomi Riso #8	+	+	SE
Bomi Riso #9	+	+	SE
Bomi Riso #13	+	+	SEX
Bomi Riso #1508	+	+	SEX
CII Riso #29	+	+	SEX
CII Riso #56	+	+	SE
CII Riso #86	+	+	SEX
High Amylose Glacier	+	+	SE
Hiproly lys1 lys1	+	+	SE
Betzes seg1 seg1	+	+	SE
Compana seg3 seg3	+	=	SE
Compana seg4 seg4	+	=	SE
Glacier seg5 seg5	=	-	SE
Ingrid seg6 seg6	=	+	SE
Ingrid seg7 seg7	=	=	SE
Compana sex1 sex1	+	+	SEX

\+ + = more, = = same, - = lower
‡ SE = shrunken endosperm but not readily observable as xenia.
SEX = shrunken endosperm is readily observed among the F_2 seed on F_1 plants.

This expression of xenia allows for a more easily monitored and rapid incorporation of high lysine into other lines and populations including high lysine lines that do not express xenia.

The xenia expressing character may also be employed in a search for suppressor genes to shrunken endosperm. In Table 21 are reported the ratio of plump to shrunken endosperm seed in crosses involving Bomi/Riso Mutant #1508. The Chi square data indicate that for a number of crosses a 3:1, plump:shrunken, ratio probably does not explain the observed ratio. One explanation for the failure to obtain a 3:1 ratio might be the presence of suppressors or modifiers to the shrunken endosperm character. This postulation could easily be verified by finding high lysine plump progeny, especially if the high lysine shrunken plus high lysine plump plants approximate one fourth of the total progency. Several explanations could apply including incomplete penetrance or expressivity.

TABLE 21. THE EXPRESSION OF XENIA FOR SHRUNKEN ENDOSPERM BY BOMI RISO MUTANT #1508, F_2 SEED FROM F_1 PLANTS

Cross	No. Plump Kernels	No. Shrunken Kernels	Total No. Kernels	X^2 for Goodness of Fit for 3:1 Ratio	P-Level
Compana x #1508	613	182	795	1.88	.100-.250
Carlsberg II x #1508	523	147	670	3.34	.050-.100
Betzes x #1508	503	137	640	4.41	.025-.050*
Heines Hanna x #1508	445	103	548	11.25	<.005 *
CI 4961 x $1508	404	104	508	5.55	.010-.025*
Hector x #1508	575	101	676	36.48	<.005 *
Steptoe x #1508	401	101	502	6.38	.010-.025*
Unitan x #1508	276	104	380	1.14	.250-.500
Piroline x #1508	310	101	411	0.0397	.750-.900

* Reject the 3:1 hypothesis

Notch-1 and Notch-2 Mutants

Present status: Two barley mutants were developed by ethyl methanesulphonate treatment of population of the variety NP-113 (3). They were identified as Notch-1 and Notch-2 due to the uniform depression on the dorsal side of the seed. This condition is often referred to as shrunken endosperm. In the case of Notch-1 and -2, the shrunken character appeared to be associated with a chlorophyll deficiency (3). Preliminary analysis showed that these mutants possessed increased protein and lysine contents of about 40% and 20% for Notch-1 and -2 respectively over the parent (4). This was confirmed in a recent paper by Balaravi et al. (1). The two mutants of NP-113 were chemically characterized and compared with Hiproly. The increased level of lysine and other essential amino acids in the mutants were a consequence of a substantial reduction in the prolamin fraction with a simultaneous increase in the salt-soluble fraction. Increased embryo/endosperm ratio in the mutants also contributed towards changes in amino acid composition. The Notch mutants were superior to both NP-113 and Hiproly and regard to chemical score for lysine and biological value. The lysine in g/16g N was comparable to that of Hiproly, 4.0 and 3.96 with a BV of 86.4 and 87.6 for Notch-1 and -2, respectively. The BV of the parent NP-113 was 75.8 and contained 3.38% lysine in g/16g N. With the increased protein and lysine there was a concurrent decrease in seed kernel weight of 16 and 18.5% for Notch-1 and -2, respectively (1).

CI 7115

Present status: Toft-Viuf (30) used the negative relationship of crude protein and the amide content of barley protein to locate a high-lysine cultivar, CI 7115. This barley displayed an increased lysine content of about 15% compared to normal barleys. Lysine (g/16g N) is rather constant

Table 22. Protein and lysine contents of barley and protein of several shrunken endosperm mutants grown at Bozeman, Mt. (except as noted) over a period of years.

Year grown	% protein			% lysine in barley			% lysine in protein		
	Compana seg3 seg3	Compana Seg3 Seg3	difference	Compana seg3 seg3	Compana Seg3 Seg3	difference	Compana seg3 seg3	Compana Seg3 Seg3	difference
1970	17.9	15.7	2.2	.392	.335	.057	2.20	2.14	.06
1971	16.0	14.8	1.2	.370	.325	.045	2.31	2.19	.12
1972	16.2	15.1	1.1	.390	.320	.070	2.41	2.12	.29
1974 +	13.1	-	-	.342	-	-	2.61	-	-
avg.	16.7	15.2	1.5	.384	.327	.057*	2.31	2.15	.16
	Compana seg4 seg4	Compana Seg4 Seg4	difference	Compana seg4 seg4	Compana Seg4 Seg4	difference	Compana seg4 seg4	Compana Seg4 Seg4	difference
1970	20.6	15.7	4.9	.370	.335	.035	1.80	2.14	-.34
1971	20.7	14.8	5.9	.355	.325	.030	1.71	2.19	-.48
1972	15.8	15.1	0.7	.370	.320	.050	2.34	2.12	.22
1974 +	16.7	-	-	.340	-	-	2.04	-	-
avg.	19.0	15.2	3.8	.365	.327	.038*	1.95	2.15	-.20
	Compana sex1 sex1	Compana Sex1 Sex1	difference	Compana sex1 sex1	Compana Sex1 Sex1	difference	Compana sex1 sex1	Compana Sex1 Sex1	difference
1967	18.1	15.3	2.8	.425	.310	.115	2.35	2.03	.32
1970	17.8	15.4	2.4	.470	.312	.158	2.64	2.05	.59
1970	18.0	15.7	2.3	.420	.335	.085	2.33	2.14	.19
1971	21.2	14.8	6.4	.530	.325	.205	2.50	2.19	.31
1972	17.3	14.2	3.1	.405	.315	.090	2.34	2.22	.12
1974 +	12.1	-	-	.344	-	-	2.84	-	-
avg.	18.5	15.1*	3.4	.450	.319	.131**	2.43	2.13	.30*

Table 22 (Cont.)

	% protein			% lysine in barley			% lysine in protein		
	Glacier seg5 seg5	Glacier Seg5 Seg5	difference	Glacier seg5 seg5	Glacier Seg5 Seg5	difference	Glacier seg5 seg5	Glacier Seg5 Seg5	difference
1970	18.1	13.2	4.9	.380	.310	.070	2.10	2.35	-.25
1971	20.7	13.5	7.2	.460	.325	.135	2.22	2.41	-.19
1972	18.1	12.9	5.2	.380	.310	.070	2.10	2.40	-.30
avg.	19.0	13.2	5.8*	.406	.315	.091	2.14	2.39	-.25*
	Ingrid seg6 seg6	Ingrid Seg6 Seg6	difference	Ingrid seg6 seg6	Ingrid Seg6 Seg6	difference	Ingrid seg6 seg6	Ingrid Seg6 Seg6	difference
1972	15.2	13.2	2.0	.420	.330	.090	2.76	2.50	.26
1974 ‡	12.0	13.4	-1.4	.335	.325	.010	2.79	2.42	.37
1975	15.8	14.1	1.7	.500	.390	.110	3.16	2.77	.39
avg.	14.3	13.6	0.7	.418	.348	.070	2.90	2.56	.34*
	Ingrid seg7 seg7	Ingrid Seg7 Seg7	difference	Ingrid seg7 seg7	Ingrid Seg7 Seg7	difference	Ingrid seg7 seg7	Ingrid Seg7 Seg7	difference
1972	16.1	13.2	2.9	.355	.330	.025	2.20	2.50	-.30
1974 ‡	12.3	13.4	-1.1	.325	.325	.000	2.64	2.42	.22
1975	16.2	14.1	2.1	.445	.390	.055	2.75	2.77	-.02
avg.	14.9	13.6	1.3	.375	.348	.027	2.53	2.56	-.03

\+ Arizona grown, not included in averages.
‡ Arizona grown.

TABLE 23. GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY RATIOS OF RATS FED ISO-NITROGENOUS TWO-ROWED BARLEYS AND CASEIN DIETS

Variety	CI No.	Diet No.	Gain *	Feed consumed*	Feed/gain ratio*	Adjusted PER*
Hanna	906	1	58.2^{ab}	288.5^{a}	5.06^{cd}	1.96^{bc}
Bargiers	3383	2	46.4^{abcde}	258.5^{a}	5.65^{abc}	1.77^{cdef}
--	6400	3	45.8^{bcde}	250.1^{a}	5.67^{abc}	1.78^{cdef}
D. Heil Franke	6407	4	47.2^{abcd}	256.1^{a}	2.65^{abc}	1.85^{bcdef}
Bolder	7131	5	53.1^{abcde}	278.6^{a}	5.28^{bcd}	1.92^{bcd}
Lenta	7622	6	48.6^{abcde}	253.0^{a}	5.37^{bcd}	1.87^{bcde}
--	8192	7	41.0^{de}	235.1^{a}	5.86^{abc}	1.72^{def}
--	10236	8	49.2^{abcde}	253.7^{a}	5.55^{abc}	1.82^{cdef}
Italian	10328	9	47.4^{abcde}	256.7^{a}	5.55^{abc}	1.78^{cdef}
--	10375	10	54.9^{abc}	275.7^{a}	5.12^{cd}	1.94^{bcd}
Wiebull's 5573	11201	11	50.2^{abcde}	265.4^{a}	5.46^{bcd}	1.83^{cdef}
Bonus	11308	12	44.4^{cde}	248.5^{a}	5.78^{abc}	1.76^{cdef}
Brage	11310	13	51.4^{abcde}	265.7^{a}	5.41^{bcd}	1.88^{bcde}
Primus II	11315	14	45.3^{bcde}	244.4^{a}	5.52^{abc}	1.80^{cdef}
--	12099	15	53.8^{abcd}	275.6^{a}	5.27^{bcd}	1.91^{bcd}
Imperial	12147	16	47.2^{abcde}	263.6^{a}	5.80^{abc}	1.67^{def}
--	12171	17	40.0^{e}	233.6^{a}	5.99^{ab}	1.68^{def}
Hiproly	3947	18	59.4^{a}	274.8^{a}	4.67^{de}	2.09^{b}
--	12103	19	52.1^{abcde}	280.5^{a}	5.33^{bcd}	1.85^{bcdef}
Compana	5438	20	39.3^{e}	243.4^{a}	6.31^{a}	1.59^{f}
Waxy Compana	294318 **	21	40.9^{de}	254.4^{a}	6.07^{ab}	1.64^{ef}
Unitan	10421	22	49.4^{abcde}	276.0^{a}	5.62^{abc}	1.77^{cdef}
Shabet	13827	23	48.0^{abcde}	272.7^{a}	5.85^{abc}	1.71^{cdef}
	Casein	24	59.7^{a}	246.2^{a}	4.17^{e}	2.41^{a}

* Means in the same column followed by different superscript letters, differ significantly ($P<.05$).

**Montana CI No.

in CI 7115 at various levels of nitrogen fertilizer (30) and resembles Hiproly in this respect. Ingversen and Køie (14) have studied the composition of the salt-soluble proteins of this cultivar. We have not evaluated CI 7115 in our laboratory.

Shrunken Endosperm Mutants

We have shrunken endosperm mutants under investigation for some time, Jarvi and Eslick (16) and these have been analyzed for the presence of high lysine. Compana seg3 seg3 and sex1 sex1, Ingrid seg6 seg6 and Betzes seg1 seg1 (Table 22) have higher lysine than the parental variety. Shrunken endosperm 5 (seg5 seg5) in Glacier is significantly lower in lysine than Glacier.

It would appear that a sieve can be an effective means of selection of additional high lysine mutants, if such are desired.

"Minor" Genes for High-Lysine

Present status: Those who have worked on improved malting barley varieties know that such intangible characteristics as extract, diastatic power, protein solubility, etc. are heritable. No one has identified specific genes for these traits.

Most workers concerned with high lysine barley breeding have noted differences in lysine content of the non-mutant parental varieties. Although single specific genes may be much easier to follow in a barley breeding program we are not aware of investigators who have pursued varietal differences without resorting to spontaneous or induce mutations.

2-Rowed Barleys Selected for High-Lysine

Nutritional evaluation: Nineteen 2-rowed barleys selected from the world collection of high-lysine potential and four commercial varieties were chemically analyzed and each fed to ten weanling rats in isonitrogenous, 10% protein diets. Rats fed the casein control diet tended to gain faster and more efficiently and had a higher ($P<.05$) PER than rats fed any of the barleys (Table 23). Five of the barleys produced rat gains that were not statistically different from those on the casein control diet. These were Hiproly, Hanna CI 10375, CI 12099 and Balder. These diets also produced the highest PER and lowest feed/gain ratios of the barleys in the test. The Hiproly diet was the best in terms of gain, feed efficiency and PER, but all differences were not statistically significant. The casein diet was more efficiently utilized ($P<.05$) when compared to 22 of the barleys but was not significantly different in this regard from the Hiproly diet. There were numerical differences in feed consumption but the large variation in this parameter between rats within diets made these differences non-significant.

The commercial variety, Compana, produced the slowest rate of gain. Rats fed Compana also tended to be the least efficient and had the lowest PER. Waxy endosperm Compana, an isoline of Compana, was similar in all

parameters to the latter barley. The other commercial varieties, Unitan, and Shabet, appeared to be intermediate in comparison to the other barleys. Barleys producing low gains comparable to Compana and waxy Compana were CI 12171, CI 8192 and Bonus. With two exceptions, these three barleys and the Compana isolines also produced the lowest PER and the highest feed/gain ratio. Rats fed the variety Imperial, gained somewhat more than the five low PER barleys, but produced a low PER. Shabet produced a gain of 48.0 g with a PER and feed/gain ratio of 1.71 and 5.85, respectively.

The percentage of lysine in the barley proteins was highly correlated to gain, the feed/gain ratio and PER (r = .73, -.82 and .84, respectively) (P<.01), but was less correlated with feed consumption (r = .46). The PER value appeared to be the best indicator in the growth trial of the percentage of lysine in the barley protein since the latter was responsible for 53% of the variation in PER.

TABLE 24. BIOLGICAL VALUE AND TRUE PROTEIN DIGESTIBILITY OF HIGH AND LOW PER TWO-ROWED BARLEYS

Variety	CI No.	Diet No.	PER[a]	Biological Value	True protein digestibility
Hanna	906	1	1.96	66.0[bc]	74.5[c]
Balder	7131	5	1.92	68.5[bc]	74.9[c]
--	10375	10	1.94	67.9[bc]	75.4[c]
Brage	11310	13	1.88	67.1[bc]	80.0[c]
Hiproly	3947	18	2.09	72.6[b]	74.1[c]
Imperial	12147	16	1.67	59.1[c]	78.2[bc]
--	12171	17	1.68	63.8[bc]	74.9[c]
Compana	5438	20	1.59	65.6[bc]	82.0[b]
Waxy Compana	294318	21	1.64	57.0[c]	81.2[bc]
Shabet	13827	23	1.71	61.6[bc]	80.1[bc]

[a] PER obtained in growth trial.
[bc]Means in the same column with different superscript letters differ significantly, (P<.05).

Biological value and TPD of the five high and five low PER barleys were determined (Table 24). Hiproly had the highest BV but was only significantly greater (P<.05) than two other varieties in this respect, Imperial and waxy endosperm Compana. The barleys with the high PER values tended to be higher in BV but lowest in TPD. The TPD of Hiproly barley was lowest of the barleys compared and was significantly less (P<.05) than the TPD of Compana. The latter barley had the highest TPD of the ten barleys compared.

The BV and TPD of the combined high and combined low PER barleys were different (Table 25) (P<.01). The high PER barleys had the greater BV when compared to the five low PER barleys as a group, however, they were lower in TPD.

TABLE 25. COMPOSITE MEANS OF BIOLOGICAL VALUE AND TRUE PROTEIN DIGESTIBILITY OF HIGH AND LOW PER TWO-ROWED BARLEYS

Barleys	Biological value	True protein digestibility
High PER[a]	68.3[c]	75.7[c]
Low PER[b]	61.4[d]	79.3[d]

[a]Five barleys selected on the basis of highest PER performance.
[b]Five barleys selected on the basis of lowest PER performance.
[cd]Means in same column with different superscript letters differ significantly ($P<.01$).

Protein efficiency ratios obtained in the growth trial were positively correlated ($P<.01$) with BV ($r = .81$) but were negatively correlated ($P<.01$) with TPD ($r = .73$). The percentage of lysine was positively correlated ($r = .71$) ($P<.05$) with BV but was negatively correlated ($r = -.60$) ($P<.05$) with TPD. Tyrosine showed the highest correlation with TPD ($r = .77$) ($P<.05$) and was negatively correlated to BV ($r = .60$) ($P<.05$). Each amino acid tested exhibited the opposite relationship with BV and TPD, as with lysine and tyrosine, although most r values were not statistically significant.

Stepwise multiple regression analyses were computed with the percentage of amino acids (g/16g N) as the independent variable (x) and rate of gain, feed intake, feed efficiency and PER as the dependent variables (y). Lysine was the single most important amino acid for all parameters: R^2 for gain, intake, efficiency and PER were .508, .156, .648 and .679, respectively. The next two most important amino acids were tryptophan and isoleucine in that order for gain, intake and efficiency and in reverse order for PER. Arginine, threonine and histidine followed in order of importance for gain and intake but histidine was reversed with threonine in the case of efficiency. After lysine, isoleucine and tryptophan for PER, tyrosine, glycine and threonine followed in order of importance. These data indicate that the three most important amino acids in this group of barleys for efficient growth of weanling rats were lysine, isoleucine and tryptophan.

Varietal development: We have not attempted to develop even higher lysine types by intercrossing varieties such as Hanna, CI 906 and Balder, CI 7131. Perhaps this approach to breeding for high lysine would also overcome the shrunken endosperm, and yield problem.

CONCLUSIONS

We have presented evidence of high-lysine gene sources in barley derived from spontaneous and induced mutations. In addition we have shown that barleys considered to be "normal" also differ in lysine percentages. Changes in lysine concentrations invariably result in changes in other

amino acids in barley protein. Protein fractions are altered in several mutant barleys and differ also in so called "normal" barleys. The fractions in the normal barleys are probably more dependent upon environmental conditions than in mutant barleys. This is an area that needs thorough study.

Chemical analysis is necessary in the study of protein quality in barley but the ultimate screening procedure must employ animals. The rat has proven to be very effective in our animal evaluation of barleys. The pig is more similar to the human in digestion and metabolism of nutrients than the rat but the pig requires considerably more space, total grain and labor input for a given evaluation. However, there is a salvage value to pigs that is not found with rats.

We have clearly demonstrated with rats and pigs that high-lysine cultivars are superior in nutritive quality in their low-lysine isotypes. We have presented evidence from our laboratory and others that the high-lysine characters are heritable and can be transmitted to superior agronomic genotypes. It is not our wish to maintain or develop a barley solely on the basis of protein quality but to introduce such quality into high yielding, plump, disease resistant adaptable cultivars that can be grown under a wide area of environmental conditions. Our ultimate goal is to improve the health and well-being of people in those areas of the world that depend upon barley for food and feed grains.

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NUTRITIONAL QUALITY RESEARCH IN SORGHUM - PROGRESS REPORT AND CURRENT STATUS

J. D. Axtell

Grain sorghum is an important human food in many parts of the world, especially in Africa and Asia. Unfortunately, the nutritional quality of most grain sorghum varieties being used today is poor relative to that of other major cereals. The purpose of this report is to summarize our research on some of the problems associated with the poor nutritional quality of sorghum, and to review some of our recent research in this area.

We have been studying two major components of nutritional quality in grain sorghum. First, protein availability is apparently limited in some sorghum genotypes by the presence of unidentified polyphenolic compounds located primarily in the testa layer of the grain. These pigmented compounds have not been well characterized chemically and are referred to generically as "tannins." Second, the protein quality of sorghum is limited by the low lysine content of the grain which reflects the high prolamine content of the endosperm. If one looks at the essential amino acid composition of sorghum grain, in comparison with monogastric nutritional requirements, it is obvious that lysine is deficient and that there is great excess of leucine. The methionine content of sorghum is low, but considering the cystine content of 1.5%, the overall sulfur amino acid content approaches the weanling rat requirement. The tryptophan content of sorghum seems to be adequate in contrast to the low tryptophan content in normal maize.

Tannins: Based on the above information one would predict that the biological value of any particular variety of sorghum grain would be directly proportional to its lysine content. This, in fact, is true for low tannin sorghum genotypes. The data in Figure 1, however, illustrate that lysine is not the first limiting component of biological value for a group of high tannin sorghum lines from the world collection. There is an important interaction between tannin content and protein quality in sorghum which is not found in any of the other major cereals. Cummings and Axtell(1) demonstrated this experimentally by feeding whole seed and dehulled high tannin sorghum grain IS 8260 as shown in Figure 2. Rat growth is poor for the whole grain IS 8260 with and without supplemental lysine. In contrast, dehulling IS 8260 grain improves biological value substantially, and also allows a significant rat growth response to the addition of supplemental lysine. A similar technique was used in this study to determine the quantitative effects of tannins on biological value. Using varying proportions of high tannin sorghum grain mixed with its dehulled low tannin counterpart, we were able to provide diets containing a range in tannin content which were essentially isogenic comparisons. Data from this study is shown in Figure 3.

The biological value as measured by rat weight gain is significantly reduced by increasing levels of tannin in the diet. We can conclude from these data that tannins reduce the biological value of grain sorghum for monogastric animals. The cause of this reduction is not

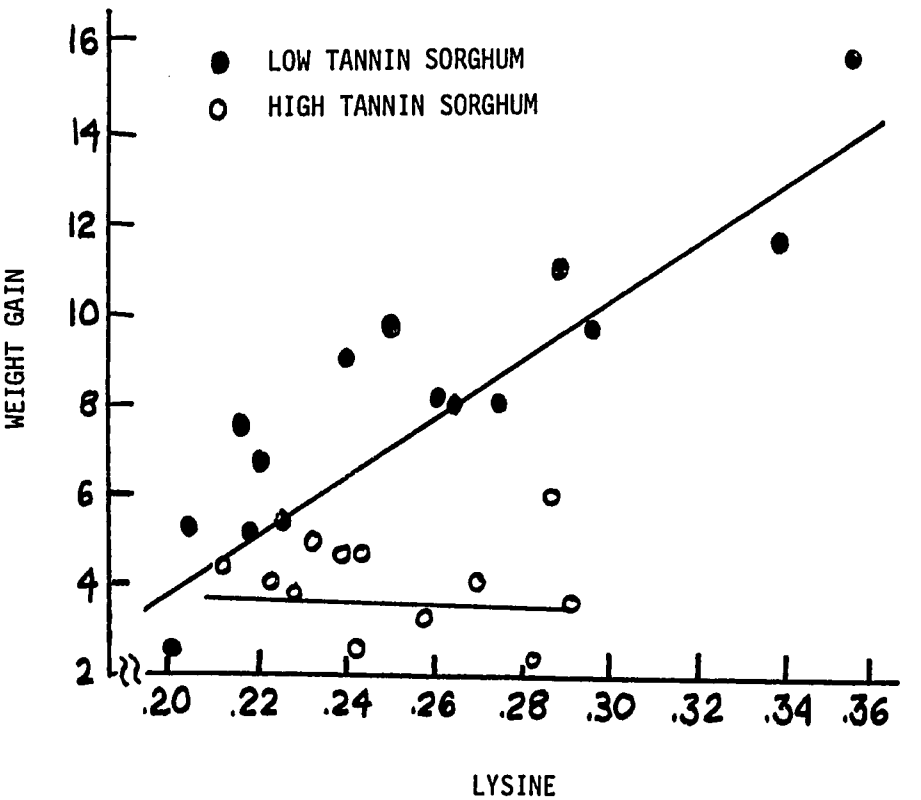

Figure 1. Relationship of biological value to lysine concentration (g/100 g sample) in high and low tannin sorghum lines from the world collection in a 14-day weanling rat feeding experiment.

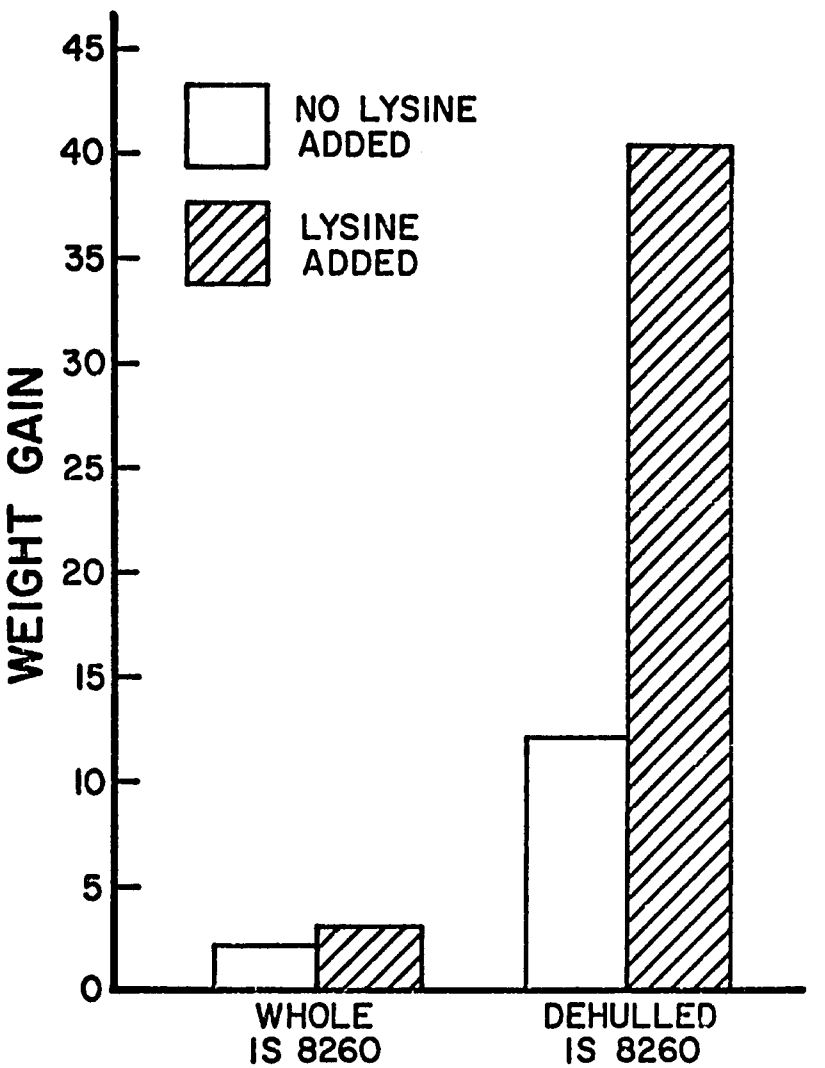

Figure 2. Biological value of whole and dehulled grain of high lysine sorghum line IS 8260 when fed to weanling rats with and without lysine supplementation in a 14-day feeding experiment.

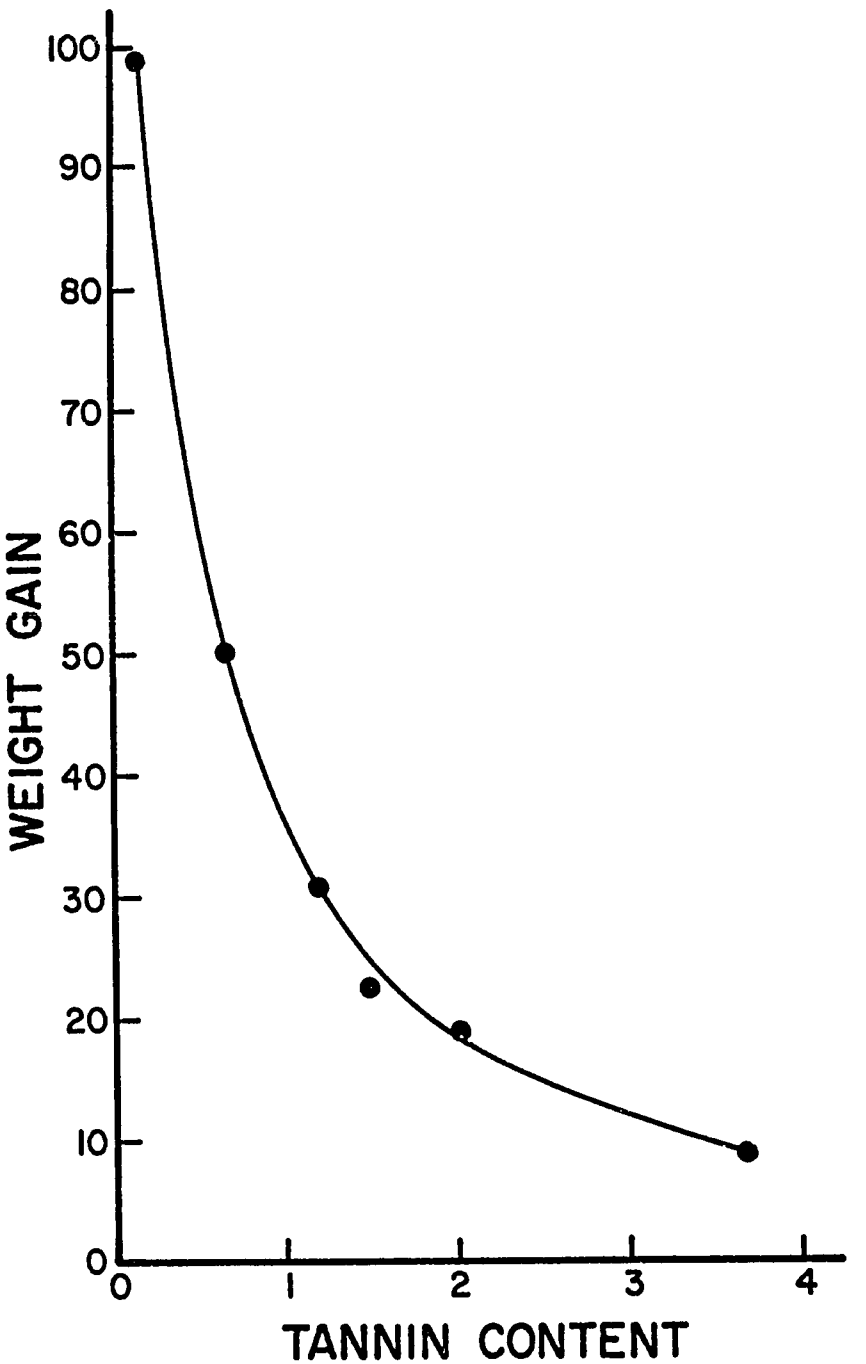

Figure 3. Effect of tannin content (expressed as catechin equivalents) on th biological value of sorghum grain in a 28-day weanling rat feeding experiment.

completely understood. Our present hypothesis is that the seed proteins become complexed or bound with the tannins of the whole grain, and that the complexed proteins are substantially less available for utilization by monogastric animals. It is very important to recognize this tannin interaction with protein quality when assessing the biological value of any grain sorghum variety, since failure to consider the biological effects of tannins has led to substantial confusion in past studies of sorghum nutritional quality.

High Lysine Sorghum - Ethiopia

In 1972, we initiated two approaches which we hoped would lead to the identification of sorghum lines with increased lysine content. The first of these approaches involved cross-sectioning of approximately 9,000 lines of sorghum from the world collection, and screening these sectioned kernels for floury endosperm lines. Using this procedure, we identified 62 floury endosperm lines and evaluated each for lysine and protein content. Most of these floury endosperm lines were normal for lysine content in relation to their protein content. There were two floury endosperm lines, however, which had a high lysine content at relatively high levels of protein. IS 11167 and IS 11758 were both collected from Wollo Province in Ethiopia during the 1960's. The protein and lysine content of these two lines was subsequently verified in Puerto Rico, and the average of these determinations is shown in Table 1 in comparison with the average for several hundred normal sorghum lines from the world collection. The significant point is that lysine content, when expressed as percent of sample, is approximately double that of normal sorghum.

Table 1. Protein and lysine concentration of whole grain samples of high lysine and normal sorghum lines.

Protein Composition	High Lysine Lines IS 11167	IS 11758	Normal
Protein (%)	15.70	17.20	12.70
Lysine (% of protein)	3.33	3.13	2.05
Lysine (% of sample)	0.52	0.54	0.26

The reason for this increase in lysine can be understood by looking at the distribution of endosperm proteins by solubility fractionation using the Landry Moureaux procedure. The distribution of protein in the five solubility fractions for normal sorghum is shown in Table 2, along with the lysine content of each of the five fractions. Over 50% of the endosperm protein in sorghum is present in Fractions II and III, which are both lysine-poor fractions. Fractions II and III are generally referred to as the prolamine fractions. Note that Fractions I and V are relatively high in lysine. The nitrogen distribution in normal and high lysine sorghum endosperm tissue using the same procedure has been obtained by Jambunathan (Table 3, unpublished data).

Data from determinations on five high lysine lines collected from farmers' fields in Ethiopia were averaged and compared to control data from a normal variety grown in the same environment. There is a substantial reduction in the amount of protein in Fractions II and III among the high lysine lines, accompanied by an increase in the proportion of protein in the lysine-rich Fractions I and V. The reduction in prolamine from approximately 55% to 39% largely accounts for the increased lysine content of the Ethiopian lines.

Table 2. Protein and lysine distribution in normal sorghum endosperm.

Solubility Fraction*	Protein (%)	Lysine (% of protein)
I	7.3	5.1
II	16.8	0.3
III	32.4	0.3
IV	7.8	1.2
V	25.3	3.6

*Landry-Moureaux procedure.

Table 3. Protein distribution in normal and Ethiopian high lysine sorghum endosperm.

Solubility Fraction*	Protein (%)	
	Normal	High lysine (hl)
I	6.33	13.58
II	19.16	16.54
III	36.26	23.02
IV	6.65	4.00
V	25.60	33.86

*Landry-Moureaux procedure.

The high lysine content of these Ethiopian lines is controlled by a single gene which has been designated hl. The biological value of the Ethiopian high lysine sorghum grain in comparison with normal has been reported by Singh and Axtell(2). Data obtained by feeding high lysine and normal samples at isonitrogenous levels (10% protein) to weanling rats in a 28-day trial demonstrated that rats fed the high lysine ration increased in weight approximately two to three times more than the rats fed the normal control ration.

The kernel phenotype of the Ethiopian high lysine varieties is partially dented. This suggested the possibility that there may be a block in the bio-synthesis of starch from water soluble polysaccharides similar to that found in shrunken, sugary, or brittle endosperm mutants in maize. The data in Table 4 shows the carbohydrate composition of both high lysine lines and a normal control. There is no change in the quantity of reducing sugars and only a slight increase in the amount of sucrose in the high lysine lines. Water soluble polysaccharides content is similar to that of normal, and there is only a two to three percent reduction in starch which can be accounted for by the slight elevation in the levels of sucrose. We concluded, therefore, that there is no disruption in starch synthesis from water soluble polysaccharides in hl genotypes, and that it may be possible to identify plump seeded high lysine kernels in an appropriate genetic background.

Table 4. Carbohydrate composition of whole kernel samples of Ethiopian high lysine and normal sorghum lines.

	High lysine lines		
Carbohydrate Composition	IS 11167	IS 11758	Normal
Reducing sugars	0.38	0.32	0.34
Sucrose	3.08	2.61	1.03
Total sugars	3.46	2.93	1.34
Water soluble polysaccharides	0.91	1.01	1.11
Starch (% of sample)	58.90	57.80	60.80
Total carbohydrates	63.27	61.74	63.25

We travelled to Wollo Province, Ethiopia, in November of 1973 to see if we could find these same high lysine varieties under cultivation, and if possible to find out how the people were using them. In fact, we first found them growing within one or two kilometers of the site where they were originally collected, thanks to some excellent records of Dr. Jack Harlan. Seeds of the high lysine varieties were collected in cooperation with Dr. Brhane Gebrekidan, who is in charge of the sorghum breeding program at Alemaya, and Mr. Gebisa Ejeta, a graduate assistant from Alemaya, who is now studying at Purdue. The high lysine types were always found growing in mixtures with normal sorghum varieties -- a standard practice for most of the sorghum cultivated in Ethiopia. The local farmers could recognize and identify the high lysine varieties and reported to us that it was used primarily for roasting in the late dough stage. They also recognized that the yield of this variety was lower than that of their normal sorghum varieties, but they included it in the mixture because they liked the taste of the roasted grain.

High Lysine Sorghum - Induced Mutation

The second approach initiated in 1972 was the use of chemical mutagen treatments, specifically diethyl sulfate, to try to induce a mutant which would increase the lysine content of the sorghum grain. D. P. Mohan screened 23,000 M_3 heads during his Ph.D. thesis research at Purdue for the occurrence of opaque kernel mutants. All heads from treated seeds were bagged each generation to prevent outcrossing. Seeds from each M_2 head were placed over a light box and examined for segregating opaque kernels. Mohan identified approximately 475 heads segregating for opaque kernels, and both classes of seed from each segregating head were analyzed for protein and lysine content. The percent increase in lysine of these 475 putative opaque mutants is shown in Table 5. Most of the opaque mutants were normal in lysine content, but there were 33 with at least a 50% increase in lysine. The mutant from this group that appears to have the most promise at this time is P721. The average protein and lysine content of P721 opaque seed and normal sib seed is shown in Table 6. There is an approximately 60% increase in lysine in the P721 mutant grain.

Table 5. Distribution of lysine increases (% of sample) of 475 putative opaque endosperm sorghum mutants derived from diethyl sulfate treatments.

Percent increase in lysine	Number of putative opaque mutants
0-9	135
10-19	114
20-29	85
30-39	53
40-49	25
50	33

Table 6. Protein and lysine composition of high lysine opaque mutant P721 and normal sib grain.

	Protein (%)	Lysine (% of protein)	Lysine (% of sample)
P721 opaque	13.9	3.09	0.432
Normal sib	12.9	2.09	0.270

The inheritance of this opaque mutant is not yet clearly understood, but it appears to be controlled by a single gene which expresses partial dominance in the triploid sorghum endosperm. In some cases, F_2 segregation ratios fit a 1:1 ratio, but in others there is a devi-

ation in favor of more opaque and fewer vitreous kernels. This ratio would be expected if two or three doses of the opaque gene produce opaque kernel phenotypes, whereas one dose of the opaque gene in the triploid endosperm is recessive to normal vitreous expression. We expect that this ratio will vary with genetic background.

A comparison of the biological value of the Ethiopian high lysine sorghum with the newly recovered mutant P721 is presented in Table 7. These data were obtained from two different feeding trials using a standard ANRC casein diet as a check which allows comparison between trials. RS 610 was used as a normal sorghum control in the IS 11758 trial, whereas normal sib seed of mutant P721 was used as a normal control in the P721 evaluation. All rations were isonitrogenous (10% protein) and were fed to weanling rats for 28 days. Feed efficiency ratios and protein efficiency ratios of both high lysine mutants appear to be similar, and we conclude from this preliminary experiment that the protein quality of both sources of high lysine sorghum germplasm is essentially the same. That is, both sources of high lysine germplasm give an approximately two-fold increase in protein quality in comparison with the casein control.

Table 7. Biological value of isonitrogenous (10% protein) diets of Ethiopian (IS 11758) and P721 high lysine sorghum and their respective normal checks, RS 610 and normal sib grain in a 28-day weanling rat feeding experiment.

	Weight gain (g) (28 days)	PER	PER	% of Casein
IS 11758	40	5.8	1.7	58
RS 610	16	10.5	0.9	32
P 721	33	5.7	1.8	64
Normal sib	11	10.6	0.9	32

Since mutant P721 was recovered in a photoperiod insensitive, three-dwarf line with relatively broad agronomic adaptability, this mutant may be most readily useful in temperate sorghum growing regions of the world. The increased protein content of the Ethiopian high lysine varieties, on the other hand, may provide useful sorghum germplasm for use as a protein supplement or weanling food for young children which is needed in many parts of Africa and Asia.

Future Research

The prolamine contents of the major cereals fall into three rather distinct groups, as shown in Figure 4. Since the protein quality of cereals is inversely related to their prolamine content, these cereal groups increase in protein quality as they decrease in prolamine content. (This is an oversimplification which the nutritionists would

justifiably dispute. There are numerous other parameters affecting nutritional quality in cereals. Nevertheless, the improvement in biological value associated with "high lysine" mutations in maize, barley, and sorghum can be largely attributed to a reduction in the proportion of prolamine in the endosperm, together with concomitant enrichment in the proportions of albumins, globulins, and glutelins.) This figure also shows the changes in prolamine content associated with high lysine mutants in three of these cereals. These changes appear to occur in two steps from higher to lower prolamine content. Dr. Oliver Nelson has suggested that this may be coincidence or it may mean that the regulation of prolamine synthesis is controlled by two major genes in these cereals. If the latter interpretation proves to be correct, it should be possible to find a "second-step" mutation in sorghum (or corn) which will reduce the prolamine content to approximately 10%, with further concomitant increases in protein quality. This would provide us with a cereal grain which combines the photosynthetic efficiency and stress tolerance of sorghum with the protein and nutritional quality of oats or rice.

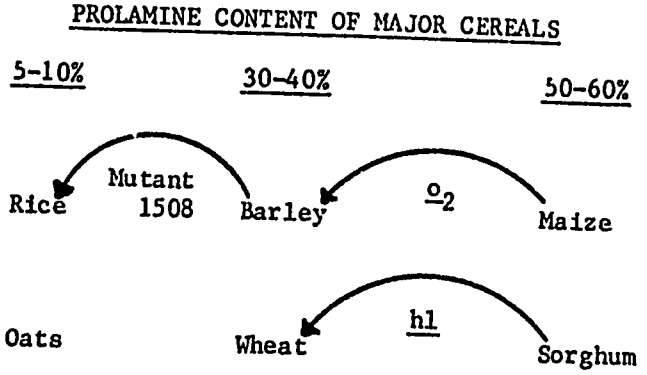

Fig. 4 Effect of high lysine gene mutations in maize (o_2), sorghum (hl) and barley (1508) on prolamine content.

We can speculate still further into the future in our search for a more "perfect cereal" and ask the following question. Will it be easier to breed for increased photosynthetic efficiency in rice or oats, or to take the alternative approach and breed for nutritional quality in sorghum and corn which already have the genetic machinery for a more efficient photosynthetic pathway? The answer will depend on which biochemical process is easiest to manipulate genetically, which in turn can only be decided when we understand the genetic control of these important biochemical pathways in cereal grain.

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WORK ON PEARL MILLET AND GRAIN SORGHUM QUALITY AT KANSAS STATE UNIVERSITY

R. C. Hoseney* and G. M. Paulsen**

Work on pearl millet at Kansas State University was started by A. J. Casady, USDA agronomist. Two open-pollinated pearl millet varieties from Uganda were grown in isolation and seed was harvested from a number of the most agronomically desirable plants. Composited seed from each head was grown interspersed with Tift 239 (dwarf forage type from the U.S.D.A. program). F_1 populations were grown in isolation and allowed to random mate. F_2 populations were grown in isolation and selfed seed from bagged dwarf plants and open pollinated seed from dwarf plants were harvested. F_3 plants from the selfed seed were all of combine height; their grain was used for preliminary nutrition and utilization studies. The open pollinated seed from dwarf plants were composited and grown in isolation and allowed to mate randomly. Seed was harvested from the most agronomically desirable dwarf plants in each isolation. Their seed was composited and grown and about 2000 dwarf plants were bagged for selfing. Four-hundred selfed heads from plants with good seed set, good head exsertion, large seed size, and good stalk strength were harvested. Grain protein content and seed weights were determined on those lines in the Kansas State University Crop Physiology Laboratory.

Two-hundred lines, representing the best 100 each in protein content and grain weight, were entered in replicated S_1 yield trials during 1973. Also during 1973, introduced lines were examined for stalk strength and topcross hybirds were developed for yield trials. Remnant seed of the top 20 yielding S_1 lines in the 1973 trials was grown in Puerto Rico in 1973-74 for recombination by random mating. The population from combining the top 20 lines was released to plant breeders as RMP1(S)C1. This is apparently the only pearl millet released for grain purposes in the U.S. to date. Seed from the random mating in Puerto Rico was grown in 1974, and 200 selfed heads were selected. Replicated S_1 yield trials were conducted in 1975, and remnant seed of the top 20 yielding lines was grown in Puerto Rico in 1975-76 for recombination by random mating. Also, in 1975 an introduced Senegal population was grown, and 200 selfed heads were elected for replicated S_1 yield trials in 1976.

Selection and breeding of pearl millet at the Fort Hays Experiment Station under the direction of W. D. Stegmeier started in 1971. Work to date has centered on selection of desirable material from a large number of introduced lines and populations. Desirable materials are being converted to dwarf, early-maturing forms. Some of the progenies will be maintained as inbreds and tested in F_1 hybrid combinations but the majority will be screened for various traits and then placed in genetically broad-based random-mating populations, modified ear-to-row selections systems, or other recurrent selection programs. Improved lines will be extracted and tested as parents in F_1 hybrid combinations.

Yield trials (unpublished data of G. M. Paulsen) comparing a pearl millet line with maize and sorghum hybrids were conducted at four Kansas locations during 1973 (Table 1).

Table 1. Pearl Millet, Maize and Grain Sorghum Performance in Kansas during 1973

Crop	Yield Kg/ha	Test wt. Kg/hl	Protein %
Pearl millet	4204	71.2	14.8
Maize	3481	71.6	11.3
Sorghum	7278	73.5	12.6

The hybrid sorghum gave the highest yield but the millet line had a much higher protein content than either sorghum or maize. A nitrogen fertility trial with pearl millet showed responses for both yield and protein content (Table 2). In physiology studies, pearl millet was shown to have the same efficient photosynthesis system of maize and sorghum.

Table 2. Pearl Millet Performance at Different Nitrogen Levels During 1973 (Irrigated).

N level Kg/ha	Yield Kg/ha	Test wt. Kg/hl	Protein %
0	3384	72.1	12.3
80	4328	71.8	13.1
160	4412	72.8	14.2
240	4315	73.4	16.6

Table 3. Average Amino Acid Contents of Pearl Millet (Tift 239 X Severe 17, F_3) Grain Produced by A. J. Casady, U.S.D.A. at Manhattan, Kansas.

Amino Acid	% of Protein
Lysine	3.58
Aspartic Acid	8.31
Glutamic Acid	18.63
Valine	5.28
Leucine	9.79

The protein content of pearl millet lines has been 3-4 percent higher than sorghum grown under similar conditions. The amino acid distribution has also been much better in pearl millet than in sorghum (Table 3). Lysine, the major limiting amino acid in cereals, averaged 3.6 percent of the protein in pearl millet compared with about 2.0 in corn and sorghum. The lysine content of pearl millet is comparable to that of high-lysine corn. Pearl millet lines obtained from other sources have ranges as low as 1.9 percent lysine, indicating considerable genetic variability in that characteristic.

Scanning electron photomicrographs of pearl millet endosperm show both the hard and soft endosperm types similar to those found in corn and sorghum. The soft endosperm (Figure 1) is characterized by nearly round starch granules with a thin protein matrix and a large amount of intergranular air spaces. In contrast, the hard endosperm (Figure 2) is tightly packed with no intergranular air spaces. The starch is polygonal in shape and covered with a layer of matrix protein embedded with a large number of spherical protein bodies.

Starch isolated from pearl millet had the same polygonal shape as starch from corn and sorghum. The granule size was about 12 μ diameter compared to 25 μ for corn and sorghum starch. The gelatinization temperature of pearl millet starch ranged from 61 to 71°C, or slightly lower than that of corn and sorghum starch. Pasting characteristics (amylograph) of pearl millet starch are similar to sorghum starch except for a rise in viscosity while holding at 95°C. That may be the result of a lower amylose/amylopectin ratio. Pearl millet starch contains about 12 percent amylose compared to 22 percent for sorghum starch. Malted pearl millet has been relatively high in α-amylase activity (equivalent to barley), but contains essentially no β-amylase activity.

A composite flour (90% wheat, 10% millet) gave bread with excellent loaf volume, crumb grain, and texture. A remarkable feature of the composite flour was that bread characteristics were also excellent when sugar and

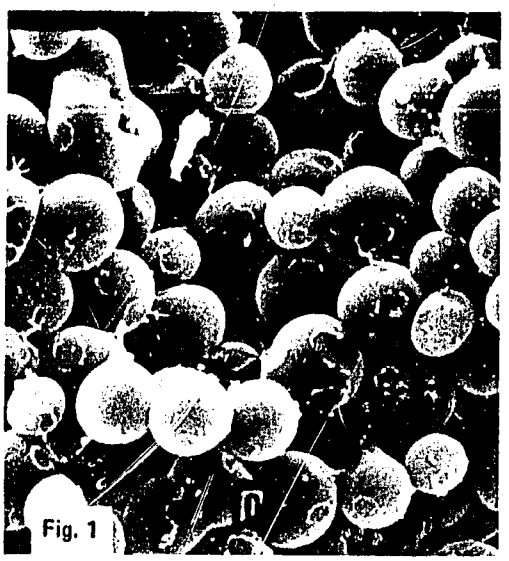


Fig. 1

Fig. 2

Fig. 3

SEE LEGENDS OF FIGURES, Page 203

malted wheat flour were removed from the formula (Figure 3). This is presumably because of high α-amylase activity of the pearl millet flour. The bread has excellent flavor and is nutritionally superior to a 100% wheat flour loaf. This finding should be of particular importance in LDCs where bread is a traditional food but wheat flour and malted wheat flour are imported and sugar is imported or is an important export item.

Procedures have been developed to produce cookies from pearl millet and sorghum flours. The procedure involved hydrating the flour for 3 hours with water followed by air drying and supplementing with soy oil. The cookies have spread and top grain characteristics equal to wheat flour cookies (Figure 4). The cookies are more fragile than those from wheat flour and are slightly more gritty. Mixtures of wheat flour and pearl millet flours (1:1) gave cookies that were not significantly different from 100% wheat flour cookies in eating quality or appearance as judged by a taste panel.

In rat feeding trials, growth was faster on an unsupplemented diet of pearl millet than of corn or sorghum (Nutrition Rpts. Intern. 5 (2): 93 (1972). The rate of chick gain and efficiency of utilization of feed was better for pearl millet than for sorghum as a source of energy and protein for broiler-strain chicks (Sanford, P., et al., 62nd Annual Meeting, Poultry Sci. Assn., 1973). Feeding trials with swine showed millet and maize were equally acceptable and efficient (Table 4).

Table 4. Growth of 18-Kg Pigs During a 28-day Feeding Trial with Corn and Pearl Millet Grain.

Diet	Gain Kg/day	Intake Kg/day	I/G
Corn + Soy	0.70	1.41	2.01
Corn + Millet (1:1) + Soy	0.77	1.55	2.01
Millet + Soy	0.71	1.46	2.06
Millet (Isolysine)	0.67	1.44	2.15

a/ Unpublished data of G. L. Allee and G. M. Paulson

Work With Grain Sorghum

Grain sorghum is composed of both soft and hard endosperm. The soft endosperm (Figure 5) is characterized by large intergranular air spaces and relatively spherical starch granules. The hard endosperm (Figure 6) has no air spaces and polygonal shaped starch granules. In addition, the hard endosperm is characterized by a large number of protein bodies. The protein bodies have been shown to be prolamines. The prolamine proteins are known to be low in lysine. Therefore, while examining a number of sorghum lines from Sudan, our interest was stirred when we noted a sample which appeared to have only few protein bodies (Figure 7). The sample was identified as Dwarf White Milo (DWM), and since has been

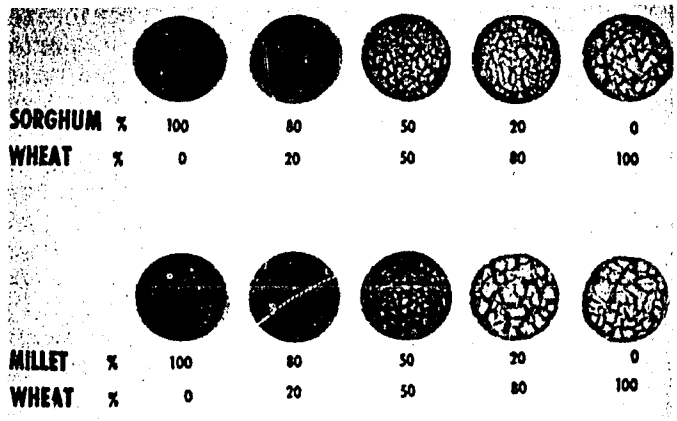
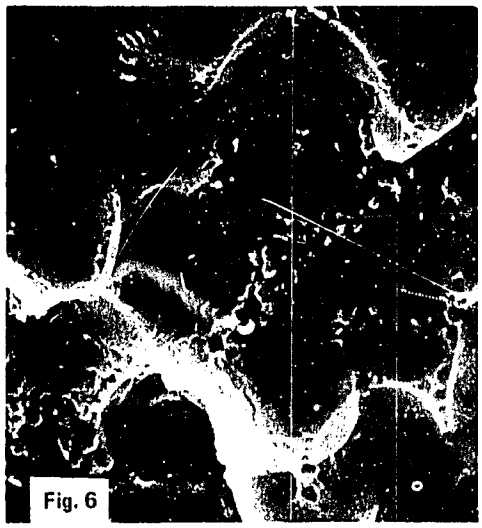


Fig. 4

Fig. 5



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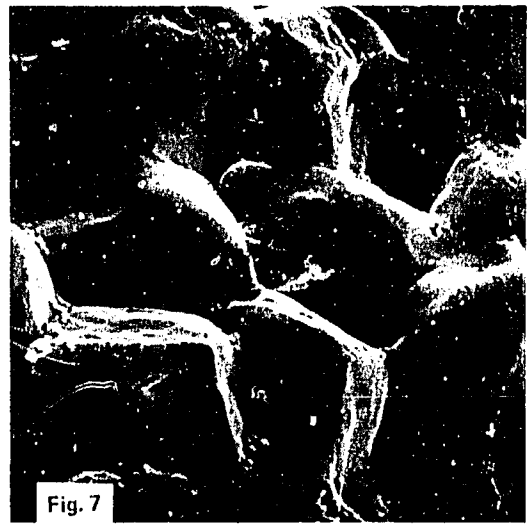

Fig. 7

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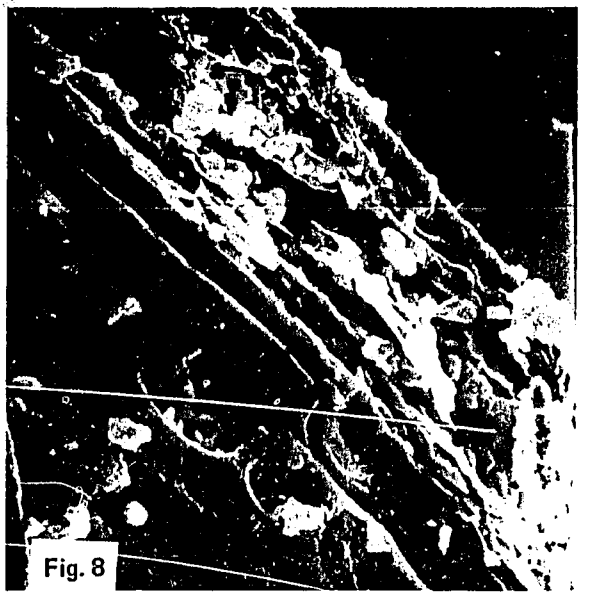
assigned PI 400226. Amino acid analysis showed the sample to contain 3.1 g. lysine per 100 g. protein, a high value for grain sorghum. The sample was increased in the greenhouse and grown in a number of locations in Kansas. The percent of lysine decreased as protein content increased, as is normal in cereals, however, in each instance the DWM was approximately 0.5% lysine higher than were normal grain sorghums at the same protein content.

In 1974 the DWM was crossed with three standard varieties of grain sorghum (Combine Kafir, Plainsman and Redlan). F_1 plants were grown in the greenhouse. In 1975 2740 F_2 plants were selfed and harvested. Data were taken on plant height, heading date, seed color, 100 seed weight, total head weight, lysine value and lysine value/100 seed weight.

The lysine value was obtained using the ninhydrin test described by Mertz et al. (Cereal Chem. 51: 304 (1974) for screening high-lysine mutants of maize, sorghum, barley and other cereal grains. The values were assigned from 1 to 5, with 1 being low in lysine (equal to the standard varieties) and 5 being high in lysine (equal to DWM). The number of samples having a lysine value of 5 varied from 50 to 60% depending on the cross. Correlation of the lysine values with the other characters gave generally low correlation coefficients, indicating that the lysine value was not strongly related to the other factors. In 1976 seed from 135 plants from each cross is being grown. All entries have a lysine value of 5 and were selected for the best combination of other characters.

Certain varieties of grain sorghum are classified as "bird resistant." The "bird resistant" feature has been associated with a testa layer in those varieties (Figure 8). The testa layer is known to be rich in polyphenolic materials. Recent work (A. B. David, Ph.D. dissertation, Kansas State University) has shown the biologically active fraction to be the condensed tannins. A simple, rapid, indirect measure of the condensed tannins was obtained by their inhibition of alpha-amylase (Figure 9). In aqueous systems the condensed tannins were bound by starch and retained their inhibiting properties after being bound (Figure 10).

During maturation, the amount of condensed tannins (expressed as % inhibition) from 8 to 4 week preripe and then depending upon the variety either remains constant or falls rapidly to the ripe samples (Figure 11). The BR-79 sample with relatively high levels of condensed tannis during the 8 to 4-week preripe stage when bird depredation would be a serious problem and yet relative low levels at harvest would appear to be an answer to the question "How to have bird resistance and palatable grain?" Elution profiles (Sephadex LH-20) of condensed tannins extracted from grain sorghum 6 weeks preripe (Figure 12) and ripe (Figure 13) show the condensed tannin from sample BR-79 changing dramatically from relatively high molecular weight to low molecular weight material.



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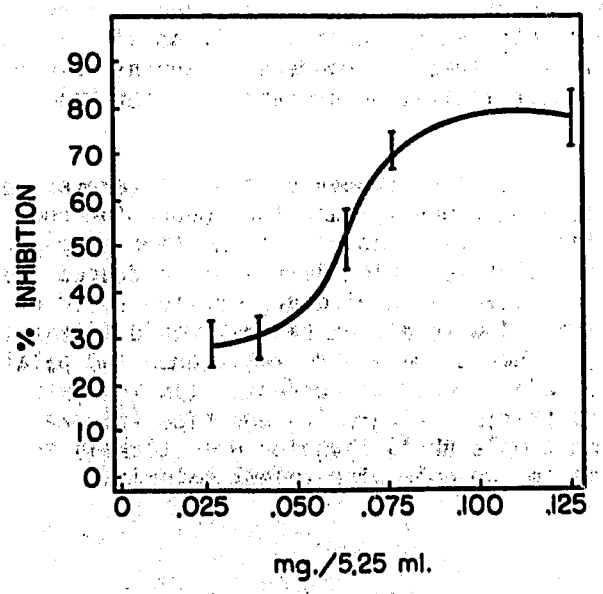

Fig. 9

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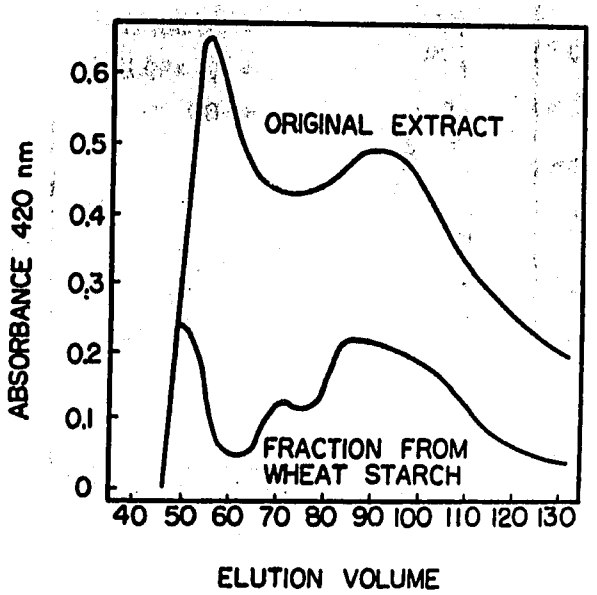

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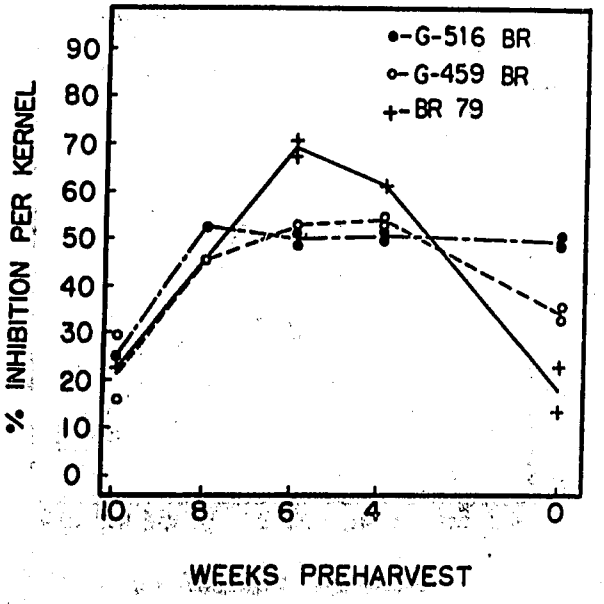

Fig. 11

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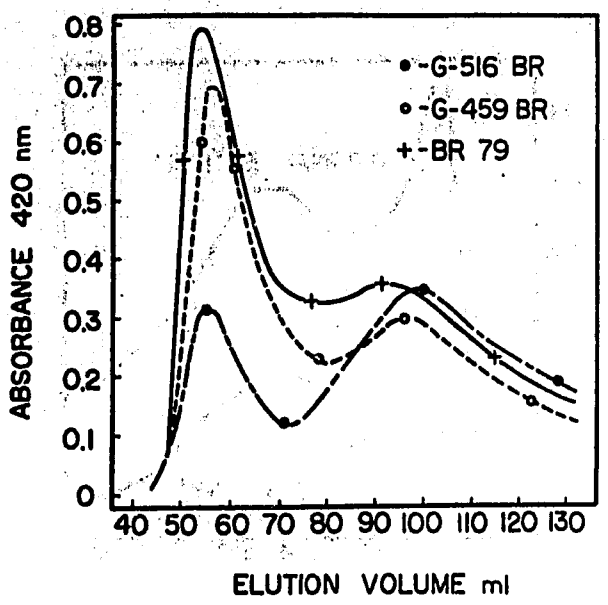

Fig. 12

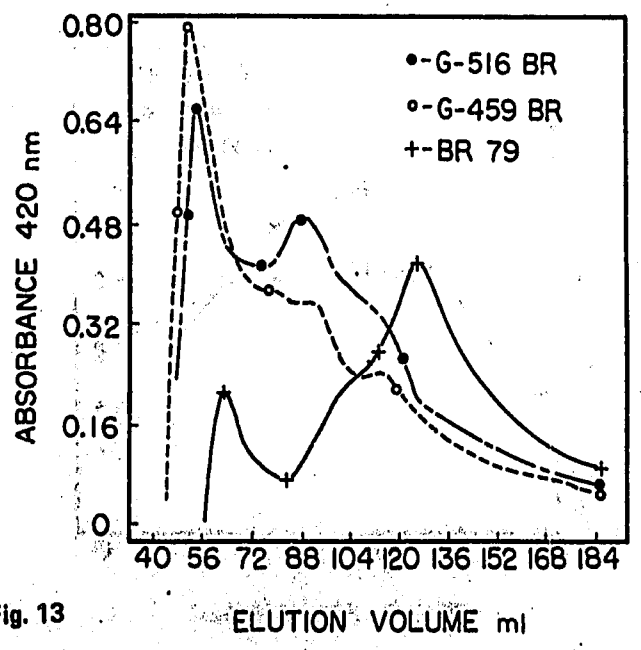

Fig. 13

SEE LEGENDS OF FIGURES, Page 203

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Figure 1. Scanning electron micrograph of pearl millet soft endosperm.

Figure 2. SEM of pearl millet hard endosperm.

Figure 3. Picture of cut loaves of bread (left to right) control formula, wheat flour plus 10% millet flour but with no sugar or malt added, wheat flour with no sugar or malt added.

Figure 4. Cookies made from various mixtures of wheat and treated sorghum flour and wheat and treated millet flour.

Figure 5. SEM of sorghum soft endosperm.

Figure 6. SEM of sorghum hard endosperm.

Figure 7. SEM of hard endosperm from Dwarf White Milo.

Figure 8. SEM of grain sorghum showing a testa layer.

Figure 9. Percent α-amylase inhibition versus concentration of condensed tannins (mg/5.25 ml).

Figure 10. Elution profile of condensed tannins from sephadex LH-20 with 50% acetone for the total extract, and fraction not removed by wheat starch.

Figure 11. Inhibition per kernel (percent inhibition of α-amylase x average kernel weight (sample weight) for condensed tannins extracted from 3 varieties harvested at different stages of maturity.

Figure 12. Elution profile of condensed tannins extracted from 3 varieties of grain sorghum collected 6 weeks preharvest.

Figure 13. Elution profile of condensed tannins extracted from 3 varieties of grain sorghum collected at harvest.

LEGUME BREEDING - AN ASSESSMENT OF PROBLEMS AND POTENTIALS FOR IMPROVEMENT IN THE EDIBLE BEAN

M. W. Adams

Introduction

Nothing in the agronomic world represents more "plant misery" than a bean plant whose root system is squeezed by a compacted soil, starved by insufficient nutrients, suffocated by too much water, consumed by too many soil-borne parasites, and inhibited by a selective herbicide, whose leaves are discolored or deformed by insects or viruses, spotted with lesions of bacterial blights, wilted by dry weather, or made prematurely senescent by air pollution or self-destruction, and whose pods are few, misshapen, or empty on account of ovule abortion.

All is not lost, however; the few remaining seeds might possess as much as 32% protein and 1.5 mgm of available methionine per seed.

In this charade, I have, I hope, created the impression that an average bean plant is sensitive to about everything that happens along, and that it is both a natural and technological miracle that plants survive these rigors to produce a saleable crop. The charade was not meant, of course, to belittle nutritive value per se but to accent my belief that the overcoming of production hazards and the creation of thrifty, high yielding grain legume crops through breeding and management are of highest priority in these species. I would add that, in addition, where possible, their seeds be made nutritionally efficient, and adequate, not necessarily as the sole source of any particular nutritional component, but in a complementary sense in mixed diets.

Problems

The bean plant, more than most other food legumes and other crop species, is sensitive to both physical and biological constituents of its cultural environment. Of course, it is rare that all of the hazards alluded to above affect a given crop simultaneously. But it is not uncommon for the Michigan navy bean to encounter surface crusting to adversely affect emergence, soil compaction, and root rot to suppress extensive root development, prolonged periods of either excess or deficient rainfall, common bacterial blight, excessive flower and pod abortion due to high temperatures, and leaf bronzing or abscission due to ozone in the atmosphere, all of which factors are deleterious to plant growth and seed set. I think these problems are not unique to Michigan nor to the U.S. (16, 18).

The most miserable beans I have ever seen in my life are found in Puerto Rico, where a complex of root and stem rots, leaf rust, golden mosaic virus, bacterial blight, and leafhoppers literally destroy any chances of commercial bean production in most areas.

Because of the almost universal occurrence of plant diseases much of the research on the edible legumes in the U.S., Central and South America, Europe, Asia, Australia, and South Africa has emphasized disease

resistance, to the virtual exclusion, until recently, of work on adaptation, yield physiology, and plant architecture, or upon nutritional components.

But unless the hazards of production can be overcome, and new widely adapted higher yielding types can be developed in the edible legumes, farmers will be less inclined to grow them in competition with other grains or root crops. And the problems of providing a natural balance of nutrients in low-income diets will be intensified.

The experience with Opaque-2 maize in the U.S., and particularly in Colombia, suggests that even a variety superior in nutritive value would not be widely grown unless its yielding ability and ability to withstand hazards were at least equal to standard varieties.

Potential

Let us now look at some of our problems more closely, with the attitude of the true believer.

Yield: I have never seen a dry bean variety ideally constructed for high yield under the growing conditions of temperate zone technological agriculture. Moreover, I do not believe such a variety exists at the present time.

The highest yield known to me is some 5 metric tons/hectare (about 75 bushels/acre). It was obtained on climbing plants, supported on trellises, at moderate density (22 plants/m^2), irrigated, and growing for a period in excess of 100 days (9). Such a yield was not obtained by accident, nor are the reasons for it obscure.

Legume breeders can now identify most of the structural and physiological components that are necessary for high yield. Many of these factors are similar from one grain legume species to another (3, 19). There remain the problems of unproductive interactions among certain components (10) and the job of obtaining genetic recombinants in which the favorable effects and interactions are brought together in one or more varieties. "Ideal" varieties or "ideotypes" in beans have been proposed for particular situations (1, 9, 12); but I shall not describe them here.

I should like to point out, however, that a breeder may evolve one or several ideotypes, according to his perception of the environment(s) and purposes for which he is attempting to breed varieties. The ideotype will include that array of structural and physiological attributes which in the judgment of the investigator best fit the variety to its intended region of use and mode of utilization. Intended goals may include yield *per se*, *high nutritive value*, wide adaptation, freedom or escape from hazards of production, etc.

Nutritive Value: The objective of improved nutritional value is of concern to most legume breeders, and some are actively pursuing it (13, 14). It is appropriate in the present circumstances, I think, to discuss in some detail the Kelly and Bliss paper, since it may

allow me to present somewhat contrasting points of view. I do so with Dr. Bliss's knowledge and permission.

Briefly, they analyzed seeds of individual plants of parental, F_1, F_2, BCP_1, BCP_2, F_3, and F_4 generations of 3 bean crosses for percent protein, percent available methionine, available methionine as percent of protein, and their correlations inter-se and with yield.

Moderate to high values were found for heritability of the quality components in all cases, indicating that selection within genetically variable populations should be effective.

Of particular interest was the finding of a negative correlation (-.30, 63 df) between percent protein and yield. Leleji (15) had also found a negative correlation in beans, and similar findings have been reported in soybeans (17). Kelly and Bliss found a positive correlation of 0.33 between percent protein and percent available methionine. I (1) had previously found a clear negative relationship for these components. Bliss et al (5) have reported relatively high negative genetic correlations in cowpea between yield and percent protein, and yield and methionine as gm/100 gm protein.

The relatively high heritability values for these quality constituents clearly favor ready progress by selection. In populations where large negative correlations exist between yield and percent protein, just as clearly, if progress is made in selection for protein, it may be associated with reduced yield. Bliss has, in fact, encountered this (6).

In populations where the negative correlations are intermediate or low it is usually assumed that there is sufficient independent variation in both protein and yield to permit progress to be made in one characteristic without affecting adversely the other characteristic.

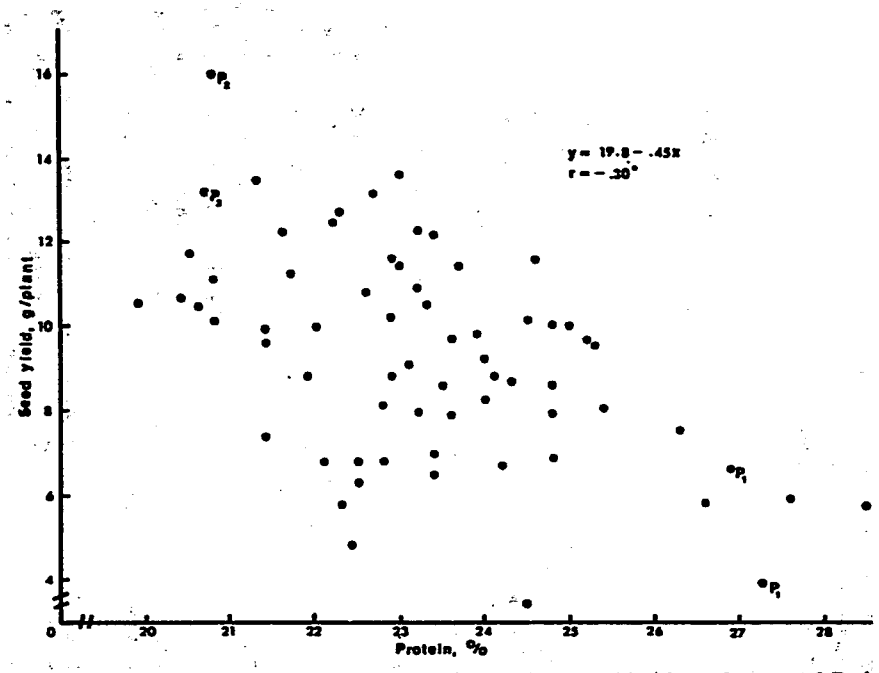
A study of Figure 1 (13) (their figure reproduced here by permission) in which yield and protein values for 65 F_2 plants are plotted with the parents, shows the following:

1. Only four plants produced protein at the level of the high parent (26.5-28.5), and these were among the lowest in yield.

2. Only three plants yielded as high as the high-yielding parent and they had an average of about 22.3% protein, less than the population mean.

3. One plant produced a yield in excess of the best parent. This was intermediate but above the population mean in percent protein (24.5%). In the scatter diagram it was clearly an outlier, separate in yield from the rest of the population.

4. Greatest variance for yield occurred in the 21-23.5% protein range, indicating this is where greatest progress could be expected from selection for yield.



Relationship between seed yield and percentage protein for F_2 plants resulting from the cross of Bush Blue Lake 240 × PI 207227.

(Figure 1 in paper by J. D. Kelly and F. A. Bliss, Crop Sci 15:753-757, 1975; Reproduced by permission of F. A. Bliss)

5. Greatest variance for percent protein occurred in the middle to lower ranges of yield.

The -0.30 correlation is perhaps not as indicative of the potential for simultaneous improvement of yield and protein in this population as is the two-way distribution of families.

Bliss et al (6), in dealing with the negative correlation, suggested selecting first for the high yielding families, followed by selecting within those families for percent protein.

We have already noted, however, in Figure 1 (13) that among the high yielding plants not only is the percent protein at a relatively low level (about 22%) but the variance for protein percentage, upon which depends the potential for selection improvement, is also relatively low. With low variance, and only two parents involved in establishing the initial population, the opportunity for effective recombinations resulting in higher levels of protein is likely also to be low.

Possibly a better strategy in the longer run (3-5 cycles) might be to back off a bit on yield - but retaining yield variance - and recover genetic variance for protein, then practice simultaneous selection for both traits through recurrent cycles.

Referring still to their Figure 1, if straight lines were drawn at the 23% protein level and the 10 gms seed/plant yield level (approximately the respective means) some 12 F_2 plants would lie in the selection quadrant, above the mean in both traits. It would be essential to select another set of 12 plants, similarly chosen, from the F_2 of another cross derived from parents unrelated to those of the first population (4). These plants when self-pollinated will generate 24 F_3 families which should again be analyzed to confirm their superiority in yield and protein. The 24 families would comprise the complete set for the second cycle of intercrosses. With selection through 2 or 3 cycles, either the low negative correlation would become significantly more negative, or several families would appear that would be higher both in yield and protein. Dr. Bliss is not in disagreement with these suggestions and now feels, on the basis of current work incorporating some aspects of these ideas (Personal Communication 1976), quite optimistic that relatively good yielding lines can be obtained which are as high as 27.5% protein or higher.

I personally feel that emphasis should be placed upon screening for lines in which the protein is more highly utilizable, more available, including methionine availability, than in presently used cultivars, provided genetic variability exists for availability. Two questions to be settled are: 1) whether availability of legume protein can be increased by breeding, and 2) whether the methionine-cysteine levels can be increased as protein level itself is increased by genetic means. If this cannot be done, it makes more sense to attempt to select for improved methionine-cysteine levels (perhaps others) at present average levels of protein.

Other properties of legume seeds bearing upon nutritive value, such as trypsin inhibitor, heme-agglutinins, tannins, phytic acid, and their destruction by cooking, and culinary characteristics including hard

seeds and flatulence, are important if one takes a comprehensive view. The extent of genetic variability for most of these factors has not been established and, in some instances, the effect upon availability or biological value is not well known. These questions should be clearly answered before consideration is given to adding them to the list of characteristics to be sought in breeding.

Some Concluding Thoughts

In view of the multi-variate nature of the ideotype, inclusive of nutritional characteristics, the fact that each variate sought may depend for its expression upon a few to numerous genetic loci, that the relevant genes may show linkage, genic interactions, and developmental associations, and that the environment may play an enhancing or masking role with respect to certain variates, it is apparent that the task of assembling a complete array of favorable alleles into a single genotype is formidable indeed. Formidable and yet challenging, for Nature has, in her wending and patient way, assembled and fabricated genotypes for her purposes; why cannot man at least attempt to do so for his purposes? Indeed, he already has, as the continuing success of maize and wheat and rice and race horses demonstrates.

For this multi-variate task, it seems to me that the multi-variate selection technic of Grafius (11) coupled with a modified single seed descent system (7) would provide a logical and strategic starting point. Recurrent cycling processes and efficient screening systems are implicit for success.

I believe essentially the same architectural, physiological, and breeding principles are appropriate for most grain legumes (2). I believe also that in the long run, legume breeders will need recourse to wide crosses and non-conventional methods of genetic engineering in order to realize their ideals. I am persuaded by the activities in progress at Cornell University; Michigan State University; University of Wisconsin; University of Nebraska; by the USDA/ARS group at Prosser, Washington; the University of Florida; Oregon State University; Mayaguez; Puerto Rico; ICTA in Guatemala; CIAT in Colombia; IITA in Nigeria; ICRISAT in India; and the Universities at Cambridge, Reading, and Durham in England, that many of the "miseries" affecting the edible legumes can ultimately be cured, and that new levels of yield and quality are within reach.

In summary, my basic view is that an all-out effort should be put forth to overcome production hazards and to build the kinds of plants which, architecturally and physiologically, are capable of higher yields than those currently being obtained. Coupled with these goals, I would join the goals of higher availability, higher quality of protein, protein quantity, and elimination of anti-nutritional or culinary factors, essentially in the order stated.

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POTENTIAL OF SOYBEANS AS A SOURCE OF PROTEIN FOR CEREAL FORTIFICATION FOR THE TROPICS

D. K. Whigham

Soybean protein has been used more extensively than any other supplement to fortify cereals and other crops [15]. Table 1 shows the total composition of soybean seed [11].

Table 1. Soybean seed composition (percent of dry weight).

Protein	40
Lipid	20
Cellulose and hemicellulose	17
Sugars	7
Ash	6
Crude fiber	5
Pentosans	5
Total	100

from: Soybeans: Improvement, Production, and Uses

Protein content has ranged from 28 percent to 53 percent, but the value commonly used is 40 percent [9, 20]. Since protein and oil are the major constituents in the seed, one must decrease if the other increases appreciably. Therefore, oil content decreases as protein content increases and vice versa [4, 5, 6, 17, 18, 19]. Krober and Cartter [8] reported, that when high protein seed samples were compared to control samples, the high protein samples had one-third less sugar, one-third less oil, and the remaining one-third reduction was holocellulose and pentosans.

Many factors affect the protein level of soybeans. Protein content was not affected by the addition of nitrogen to the soil, if the plants were well nodulated. However, protein content of the seed of nonnodulating isolines appreciably increased with the addition of nitrogen [1, 9, 16]. Early maturity was associated with high protein content of the seeds [3, 17]. Seed size was positively correlated with seed protein [3, 8]

but varietal differences existed [17]. Shattering resistance was also associated with high protein content [5]. Yield was negatively correlated with protein content [4, 5, 18, 19]. Pod removal resulted in increased protein content in the remaining seeds [14]. Pods produced in the top or central section of plants contained more protein than pods produced in the lower portion of the plant [2]. Seed protein decreased with defoliation treatments which resulted in yield reductions [13]. Results from 62 locations in the tropics indicate that minimum temperature and protein content are positively correlated.

Methionine is the only deficient essential amino acid in soybean protein. Soybean germplasm has been screened for variation in amount of this amino acid and the range of methionine reported was from 1.0 to 1.6 percent of the crude protein [20]. Krober and Cartter [9] found that methionine in the protein generally increased when seed protein was increased. In another study Krober [7] found that methionine content of protein varied among varieties. The variety Clark has significantly higher methionine content than either of its parents (Lincoln x Richland), indicating trangressive segregation for the character. There was also a tendency for higher metionine content in the protein when plants were grown at higher temperatures.

According to Luse and Okwuraise [10] "soy ogi," a commercial product for infants marketed in Nigeria, contains three times the protein, 16 times the lysine, and about three times the sulfur amino acids of normal "ogi." They indicate that soybeans have the highest potential for protein production and several legumes are superior to root crops and cereals. Table 2 summarizes their estimates for the protein productivity potential of certain tropical food crops. Protein productivity per day from a hectare of soybeans was twice that of lima beans, three and one-half times that of millet, and more than four and one-half times that of yams. These estimates suggest that more effort should be given to the introduction of soybeans into tropical cropping systems and to soybean food utilization.

Table 2. Protein productivity potential of certain tropical food crops.

Crop	Programmable yield, kg/ha	Protein content, %	Amino acid score, %	Crop duration days	Protein productivity kg/day-ha
Legumes					
(1) Soybean	2,800	38	81	95	9.1
(2) Lima bean	3,200	25	64	115	4.5
(3) Cowpea	1,800	25	65	80	3.3
(4) Pigeon pea	2,000	22	60	80	3.3
(5) Groundnut	1,600	26	79	120	2.7
(6) Winged bean	1,400	31	78	112	3.0
(7) Chick pea	2,500	20	68	125	2.7
(8) Velvet bean	2,500	21	60	160	2.0
(9) Mung bean	900	24	65	75	1.9
Root crops					
(1) Yam	25,000	2.0	74	185	2.0
(2) Sweet potato	20,000	1.3	63	120	1.4
(3) Potato	15,000	2.0	54	125	1.3
(4) Cassava	20,000	1.2	57	220	0.6
Cereals					
(1) Millet	3,000	12.0	62	85	2.6
(2) Rice	5,000	7.5	72	140	1.9
(3) Maize	4,000	9.5	49	120	1.6
(4) Sorghum	3,500	10.1	47	110	1.5

from: Luse and Okwuraiwe 1975

World soybean protein production is currently dominated by three countries, United States, Brazil, and China (Table 3). [12].

Table 3. Leading producers of soybean protein in 1975.

Country	Protein* (1000 metric ton)
United States	16562
Brazil	3887
China	2880
Russia	312
Mexico	240
Indonesia	230
Argentina	194
World Total	25186

*based on 40% protein in soybeans produced

from: Soybean Digest - Blue Book 1976

The United States protein production from soybeans was 16.6 million metric tons in 1975 which is 12 percent more than the estimates for 1976. Brazil has continued to increase production to a high of 4.6 million metric tons of soybean protein in 1976. The 1977 Brazil estimates are for an additional 13 percent increase. Production does not appear to be increasing rapidly in China.

Table 4. Tropical soybean protein production in 1975

Country	Protein* (1000 metric ton)
Indonesia	230
Colombia	67
Thailand	54
Vietnam	7
Burma	6
Bolivia	4
Peru	3
Ethiopia, Khmer Rep. and Tanzania	2
Philippines and Uganda	1

*based on 40% protein in soybeans produced

from: Soybean Digest - Blue Book 1976

Table 4 shows the protein produced in the trpics (latitudes less than 20 degrees) by soybeans in 1975 [12]. Indonesia produced the largest amount reported from the tropics and ranks sixth in the world (Table 3). All of the Southeast Asian countries use soybean protein primarily for human food. However, direct consumption of soybean protein has not been readily accepted in most other areas of the world.

Protein yield per hectare of land for soybeans varies considerably between countries because of available germplasm, management techniques, environmental variation, and many other factors. When soybean grain yields were converted to 40 percent protein, tropical Asian countries averaged 325 kg/ha of protein and the African countries of the tropics produced only 250 kg/ha of protein [12]. Whereas, the United States averaged 764 kg/ha of protein from soybeans.

Since 1973, the International Soybean Program (INTSOY) at the University of Illinois has evaluated soybean varieties in 105 countries. The research was supported by the United States Agency for International Development as part of a contract to improve soybean production and utilization in the tropics and subtropics. Results from these experiments indicate that the potential protein production from soybeans in the tropics greatly exceeds current levels of production [18, 19]. Table 5 shows the potential total soybean protein production from three cycles per year based on the 1974 experiment.

Table 5. Potential soybean protein production in the tropics based on 1974 experiments.

Latitude (degrees)	Altitude (m)	Days to Maturity	Grain yield (kg/ha)	Protein (%)	Protein/year (kg/ha)
0 - 10	<500	93	2264	41.4	2812
10 - 20	<500	99	2001	41.2	2473

from: Whigham 1976

Grain yields were higher at low latitudes and low altitudes, but protein content changed very little. At latitudes less than 20 degrees the mean number of days to maturity was less than 100. If water is available, through rainfall or irrigation, and land is available for cultivation, three crops can be produced each year. A total of 37 sites were averaged for grain yield and 31 sites reported protein content from the 0 - 10 degree latitude zone. The potential total protein production of 2812 kg/ha from three cycles per year far exceeds the present production. Even higher yields are possible from individual sites. Unpublished results from the 1975 experiment at Thirunelvely, Sri Lanka report a grain yield of 6054 kg/ha with variety Hardee in 109 days to maturity. The seed has not been analyzed for protein content; however, converting to 40 percent protein, the potential total protein production would be 7265 kg/ha from three crops in one year. At Maracay, Venezuela in 1974 the grain yield of the variety Jupiter was 5465 kg/ha in 109 days to maturity [19]. The protein content was 43.6 percent which converts to a total protein production of 7148 kg/ha from three crops per year. Another example from 1974 was Kilinochchi, Sri Lanka where the variety Davis yielded 5302 kg/ha in 99 days. With 39.0 percent protein the total protein production would be 6309 kg/ha from three crops per year.

Most farmers in the tropics cannot produce the above-mentioned yields on their fields at this time, but good education and research programs can achieve such yields in the futre. INTSOY and other international and national organizations are developing new soybean lines which are

better adapted for maximum production in the tropics. The demand for field space to produce cereal crops will continue to utilize the major portion of tillable land, but higher yields from these crops will free more hectares for legume production. The absence of a viable market for soybeans in most countries of the tropics is a major limiting factor of farm production. The development and marketing of additional low cost foods with soybean protein fortification will greatly expand the market potential of soybeans.

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SUPPLEMENTATION OF CEREALS WITH PROTEIN THE STATE OF THE ART

Paul R. Crowley

From the perspective of this, the second workshop on Cereal Breeding and fortification, it might be appropriate to consider the state-of-the-art of protein supplementation in terms of the major changes which have occurred since the first workshop in 1970. A review of the report on the first workshop (1) shows that by 1970--

-- The concept of protein fortification of cereals as a major nutrition intervention had been well established among nutritionists and many public officials.

-- Laboratory research had demonstrated the effectiveness of protein fortification as a means of improving the protein quantity and quality in cereal products.

-- Technologies for adding amino acids and protein concentrates to cereal products had been developed and were available for implementation.

-- Several evaluation projects were planned or in progress to show that protein fortification was logistically practical and was an effective nutrition intervention under "real life" conditions.

-- Cereal-based blended foods for children (including CORN-SOY-MILK and WHEAT-SOY-BLEND) which were formulated with soybean flour as a protein source had been distributed widely throughout the world as nutrition supplements for infants and children.

There was a strong note of optimism at the first workshop regarding the future use of protein supplementation and the need to move forward, not only with additional research and evaluation activities, but to implement protein supplementation on a broad scale. For example, members of the workshop urged that "in view of the nutrition benefits possible through lysine fortification of flour and wheat, that AID require fortification of all international wheat and flour shipments sent to such countries as India and Pakistan under PL 480, Title I." The participants also suggested that "joint efforts should be increased to make greater use of oilseeds (soy, peanut, cotton, etc.) as a protein source for humans." It was clear that at the time of the first workshop the art and science of protein supplementation was reasonably well developed, or under study, and that in the views of the participants, only the efforts to practice the technology were lagging.

State-of-the-Art

Other speakers on this program have addressed the current state-of-the-art as to the effectiveness of protein supplementation as a nutrition

intervention. During my presentation and those of Fred Barrett and Bill Hoover which will follow, an update of the state-of-the-art of the technology and its practice will be discussed. In particular, I would like to outline briefly the progress which has been made in the use of protein fortification in the PL 480, Title II program during the past 5-6 years.

PL 480, Title II

The Agricultural Trade Development and Assistance Act of 1954, as amended, which is referred to as PL 480, provides for the sale under concessional terms (Title I) and donation (Title II) of U.S. food commodities overseas. Title II foods are used for disaster relief and work projects and form a large part of the food supplies for nutrition-oriented feeding programs around the world.

Since its inception, PL 480, Title II has provided over 80 billion pounds of food commodities valued at more than $7 billion. Last year over 2 billion pounds of Title II foods were distributed to 41 million recipients, including 13 million school children, 15 million preschool children and mothers, 4 million disaster victims and refugees, and 9 million work project recipients.

In the early years of Title II, the food commodities distributed through the program were generally whole grains and ordinary milled products, such as corn meal and wheat flour. However, by 1970, it had become the policy of the USG to include in its donation programs the most highly nutritious foods consistent with the needs of the beneficiaries, budget limitations, cost/benefit ratios, and other policies of the government. Nutritious blended cereal products with protein supplementation like CSM and WSB had been distributed extensively for use in child feeding programs. However in 1970, the Title II program still did not include any protein fortified food commodities. (In this context, fortified foods are considered to be those foods to which supplementary nutrients are added to improve nutritional quality, but which otherwise are essentially unchanged as compared with ordinary unfortified foods. By this definition, blended foods are not fortified foods.)

Protein Fortified Cereals

Since 1970, protein fortified wheat, corn, sorghum and oat products have all been added to the Title II program as nutritionally improved cereal products. As shown in Table I, the program now makes available soy fortified wheat flour and soy fortified bulgur containing up to 65% more protein than the ordinary unfortified commodities. In addition, soy fortified corn meal, sorghum grits, and rolled oats with 1/3 - 2/3 more protein have also been added to the list of available commodities. Because of the complementary effects of soy and cereal proteins, these new fortified foods have not only more protein but higher quality protein.

Table 1

Soy supplemented PL 480, Title II Commodities

Commodity	Soy flour added	Protein Content: Supplemented product	Protein Content: Base product	Protein Content: Increase
	(%)	(%)	(%)	% of base product
Fortified Commodities				
Soy fortified bread flour (6%)	6	14	11	27
Soy fortified bread flour (12%)	12	16.2	11	47
Soy fortified bulgur	15	17.3	10.5	65
Soy fortified sorghum	15	15	9	67
Soy fortified rolled oats	15	20	15	33
Soy fortified corn meal	15	13	(8)[a]	(63)[a]
Blended Foods:				
Wheat soy blend	20	20	--	--
Corn soy blend	22	16.7	--	--
Corn soy milk	24.2	19	--	--
Whey-soy-drink-mix	36.5[b]	19	--	--

[a] Estimated, not specified by USDA.
[b] Full fat soy flour.

The new fortified cereals together with the cereal-based blended foods and other soy protein products in the Title II program (including whey-soy-drink-mix and soybean flours) have found a growing demand in nutrition programs since their inception. Last year 50% of the 2.3 million pounds of Title II commodities were either protein supplemented blended foods or soy fortified commodities; as shown in Table II this was an increase from 24% of the foods which had been protein supplemented or fortified in 1973.

Table II

PL 480, Title II Commodities Distributed

	Amount (million lbs.)		Amount containing soy flour (million lbs.)		Amount soy flour used (million lbs.)	
	FY 1974	FY 1976	FY 1974	FY 1976	FY 1974	FY 1976
Wheat	397	292	---	---	---	---
Rolled wheat	0.4	0	---	---	---	---
Bulgur	247	77	---	---	---	---
Soy fortified bulgur	135	462	135	462	20	69
Bread flour	508	134	---	---	---	---
Soy fortified bread flour	71	174	71	174	7	17
Wheat soy blend	120	133	120	133	24	27
Corn	157	102	---	---	---	---
Corn meal	72	70	---	---	---	---
Soy fortified corn meal	31	40	31	40	5	6
Corn soy blend	121	34	121	34	27	8
Corn soy milk	155	184	155	184	38	44
Sorghum	632	60	---	---	---	---
Soy fortified sorghum	15	187	15	187	2	28
Rolled oats	10	44	---	---	---	---
Soy fortified rolled oats	11	20	11	20	2	3
Whey soy drink mix	1.5	12	1.5	12	0.5	4
Soy flour	3	10	3	10	3	10
Milk powder	---	76	---	---	---	---
Dry beans	---	15	---	---	---	---
Oil	118	130	---	---	---	---
TOTAL	2,806	2,253	664	1,256	129	216
PERCENT OF TOTAL	(100)	(100)	(23.7)	(55.7)	(4.6)	(9.6)

The soy flour and grits used as fortificants last year accounted for nearly 10% of all the food shipped overseas under Title II, double the amount used in 1973. These figures demonstrate not only the extent to which protein supplementation is used in Title II programs, but the very rapid expansion which has taken place. Clearly the attitudes that prevailed at the time of the first breeding and fortification workshop have had a significant impact on the Title II program and on the expanded use of protein fortified cereal products.

Summary

In summary, it's clear that there have been changes in the state-of-the-art since the first workshop on breeding and fortification in 1970, and one of the significant changes has been the implementation of protein fortification of cereal products as a practical, applied nutrition intervention. This represents, in effect, a change in status from the "theory" or "idea" phase to the "reduced-to-practice" phase. A transition of this sort is a giant step in the development process, one which has come as the result of a great deal of hard work by many, including the participants in the first workshop. I hope that we can see as much progress five years hence, when we look back on the changes in the state-of-the-art since this workshop.

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FORTIFICATION WITH PROTEIN SUPPLEMENTS

F. Barrett

In the past decade or two there has been an increase in the consumption of wheat based foods around the world, and markedly so in developing countries that, unfortunately, must import wheat. This increased use of wheat and the problems associated with its importation place a strain on foreign exchange which in many instances has led to serious consideration for extending wheat flour by adding to it starchy or non-wheat materials. This method of extending wheat flours is called composite flour technology. In addition to the serious economic considerations associated with the increased use of wheat-based foods, comes the nutritional considerations as breadstuffs comprise a bigger share of the diet. Primarily, the concern has centered on improving the quantity and quality of the protein contained in the wheat food, or in cases where composite flour technology is practiced using starch extenders, at least adding sufficient protein supplement to retain the original protein level that was in the wheat flour. Thus composite flour technology and fortification by protein supplementation have evolved over this period of time.

Essentially all early attempts to prepare satisfactory bread and other baked products from the use of even relatively small amounts of indigenous starchy crops or high protein materials such as soy, peanut, bean, or cottonseed flour have produced results of significantly altered texture, appearance, and acceptance of the bread.

As early as the 1940's researchers were studying the properties of soy flour and other protein materials for their utility as an ingredient in bread production, so it is not new. The results were always the same, as the inclusion of even small amounts of the test material would induce adverse effects on the dough properties and resulting bread quality. Over the years improvement has been made in the quality of the protein materials, especially soy flour, and modifications made in the bread-making process. These changes allowed the production of bread which, though improved, was still below acceptable standards. Even the use of soy flour in standardized bread in the U.S. has been limited to 3 percent for this reason rather than of nutritional considerations, and because of this there has been, until recent years, only limited study and development of protein supplementation. However, in several laboratories around the world such as the Institute of Cereals, Flour, and Bread in the Netherlands, the Tropical Products Institute in the United Kingdom, INCAP in Guatemala, and Kansas State University in Manhattan, Kansas, active programs in composite flour technology and protein fortification technology have been carried on, in some instances since 1967. The results have been variable and perhaps less productive in the area of composite flour per se than in the protein supplementation. It is the latter category I wish to speak about today.

Protein supplementation research has been carried out in laboratories around the world on non-wheat protein materials from practical, yes, and even impractical sources, in an attempt to find ways of improving the nutritive value of a wheat food while still retaining the desirable eating characteristics of that food. In addition to those materials I mentioned

earlier, sesamee, sunflower, chick pea, rape seed, and buffalo gourd to name just a few, may be added. The prevailing problem through all of the testing was that the addition of even small amounts of these protein materials displays deleterious effects on the quality of the resulting wheat food.

Possibly the most promising and useful development in the field of protein supplementation has come from Manhattan, Kansas, both from Kansas State University through AID sponsored research and through the research of USDA Hard Winter Wheat Quality Laboratory. The literature contains many articles authored by Finney, Hoover, Shogren, Tsen, Hoseney, Pomeranz, and others describing their research and development in the area. During the period 1967-1971 concurrent research was conducted by both groups on the addition of protein rich materials to a dough to produce protein enriched bread. The goal was to produce a protein supplemented bread without changing the functional properties of the dough or the structural properties of the bread. Various surfactant materials, such as dough conditioners and many glycolipids, were studied for their ability to restore satisfactory product quality to bread containing high levels of protein rich material in the formula, such as up to 12 percent soy flour. The results of these studies showed that several dough conditioners and lipid materials are capable of restoring the bread making properties to wheat flour containing high levels of added non-wheat protein material. As time progressed, the USDA laboratory centered its attention on the glycolipid materials and their functionality with many proteins from a research standpoint. The workers in the Department of Grain Science concentrated on working with soy flour and one of the several dough conditioners, sodium stearoyl-2 lactylate (SSL), in an effort to develop a practical method of producing protein rich baked goods to help fight protein malnutrition.

The KSU team concentrated on soy flour as the protein material since it appeared to have the greatest potential for practical and widespread application and because of its price, uniformity of quality, and ready availability. Also, its high protein level plus high lysine content make it a valuable and reasonable protein additive for bread. From these studies evolved the technology of adding a high protein non-wheat material plus a commercially available dough conditioner for the production of a high protein wheat-based food, primarily bread. The use of 12 parts of soy flour plus one-half part of SSL for each 100 parts of wheat flour was found to produce a leavened bread with texture, color, and volume almost indistinguishable from regular bread.

The proving techniques for the development of this technology were laboratory and pilot plant-scale and there was need to prove the technology in commercial application. Success in commercial use would be the quickest way to implement this technique to improve the nutrition of as many people as possible. In 1972, soy fortified bread flour, as this combination was called, was approved for use in the Food for Peace program after trials were carried out in India and the Philippines using the flour in child feeding programs. Adjustments in formulae and procedures were necessary in order to produce acceptable baked goods. Since 1974 the Philippines have continued to utilize soy fortified flour for their School Nutrition Program, by using it to replace non-fat dry milk in the production of a high nutrition bun called NUTRIBUN. I understand that the

NUTRIBUNS are made in both school and small commercial bakery shops for the controlled feeding program but are not for sale to the general public. Also in India, soy fortified flour has been used in the production of pan bread with success.

The composition of soy fortified flour as procured for Food for Peace is based on 12 parts of soy flour plus 88 parts of wheat flour to make 100 parts of "flour" to be used in producing baked products. This is really 13.6 percent soy flour based on wheat flour, which is referred to as bakers percent. The original KSU work was carried out with 12 parts soy flour plus 100 parts wheat flour.

As mentioned by Crowley, the high protein foods were developed to help overcome dietary deficiencies in LDC's. Corn soy milk (CSM), wheat soy blend (WSB), soy fortified sorghum grits (SFSG), soy fortified cornmeal (SFCM) and the latest addition, whey soy drink mix (WSDM), to name a few, were designed for use with certain target groups, such as children or pregnant and lactating women, as gruel or beverage. They were not designed to be used in the production of baked foods or pasta or products where texture, volume, and appearance of the final product are so important to their acceptance. Thus, it was very important that a technology be developed, as described above, for the protein fortification of wheat flour which would produce acceptable baked products. The increased use of bread products and its important role in the diet make fortified bread a valuable vehicle for helping combat malnutrition in a large segment of the population in many LDC's.

For this new technology of protein supplementation of bread flour to be used in filling the gap in protein malnutrition, it should be implemented through the commercial production of high protein bread. Such implementation in LDC's has been slow in coming. The Philippines' experience with NUTRIBUN is a good example of what can be done. By design or by chance, most applications of soy fortified flour have been in school lunch or captive feeding programs reaching a small segment of a population. A concerted effort to implement protein supplementation utilizing soy fortification technology is needed. This effort should be directed to developing countries with a low-cost, nutritionally improved, food produced and distributed through commercial channels or in nationwide programs to benefit segments of the population. Presently there are several projects underway in countries around the world concerned with the introduction of soy fortified baked products that are mass-produced by the baking industry and distributed through commercial channels. These products are sold at no increase in cost compared to the unfortified product and are economically viable. This last point of no increase in cost to manufacture, and thus no increase in selling price, is extremely important and may be one of the most important considerations in the acceptance and implementation of protein supplementation technology.

Of course not all protein supplementation of cereals is directed to bread or to the "engineered" foods. High protein pasta products have been developed and produced in several countries with varying degrees

of success, depending on the composition and use of the pasta. High protein macaroni has been produced in the U.S. for use in school lunch programs. It utilizes soy flour alone or in combination with wheat gluten or other protein materials. The product is characterized by a dark (light brown) color which places it at a disadvantage for acceptance by the consumer. A modification of this product has the gluten replaced with casein or egg albumin. The latter product is pale yellow in color and highly acceptable.

While the development of a high protein product may be successful, its market introduction and consumer acceptance may not be. An example is a high protein macaroni developed by a U.S. company embodying all of the factors required in a nutritional intervention vehicle to be used in an LDC. It was introduced into a Latin American country with manufacturing rights granted to a local company. From the best information I have, the product has not done well in the marketplace and has not been well received by the people. I do not know the exact reasons for lack of acceptance.

Another area of protein supplementation which has been researched modestly is in producing high protein cookies. Although cookies are not considered a staple food in the same sense as bread or pasta, they are easily fortified, reach a large segment of most populations, and usually are well accepted by the consumer. Protein fortified cookies offer long shelf life which lends itself to wide distribution to reach a great portion of a given population. Fortified cookies have been shown to be readily accepted by children who are usually a target group in the alleviation of protein malnutrition. Soy fortified cookies have been tested with success in schools and school feeding programs in the U.S., Philippines, and Sri Lanka. The levels of protein supplementation in cookies have been from 12 up to 24 percent, although in practice application a 12 percent soy fortified flour is recommended.

Cottonseed flour has been used in the U.S. in cookies and dark product production for years. However, when using soy flour at these levels for cookie production, the technology of using a dough conditioner in conjunction with the protein rich material is practiced. The combination of these two materials retains the physical characteristics and eating qualities that were present in the nonfortified cookie.

Perhaps a word should be said about the level of protein rich materials, and especially soy flour. Nutrition studies were carried out in conjunction with the development work on protein supplementation and it was found that for a meaningful nutritional impact to be made, a minimum of 6 percent soy is needed and that 12 percent was more desirable. As stated earlier, although the developing work performed at the level of 12 parts soy flour for each 100 parts wheat flour was termed 12 percent soy flour levels, later development and adjustment for the Food for Peace program resulted in a blend of 12 parts soy flour and 88 parts of wheat flour being termed officially 12 percent soy fortified flour. The nutritional benefit derived from soy fortified flour is an increase in the protein content of the "flour" by approximately 50 percent and about a three-fold increase in the Protein Efficiency Ratio (PER) in the product resulting from use of the flour. These are meaningful nutritional increases.

The figures apply to soy fortified wheat flour. The figures would be different for the combination of other protein rich materials with wheat flour.

I have centered this presentation around the use of soy flour as the protein supplementation material and only briefly mentioned applications of other protein rich materials because soy flour dominates the area of protein supplementation of cereals, and especially wheat. Use of even low levels of certain commercially available cottonseed materials produces severe color problems in anything but dark or highly colored products, but the newer development of the liquid cyclone process affords the possibility of producing products with much broader satisfactory applications. Peanut flour carries both color and flavor problems into many applications, but newer processes result in products which are desirable in many applications where colorless products are desired. Of course, price and lack of continued availability have deterred the use of these and other higher protein materials as protein supplements in producing high protein foods. Two final examples of high protein foods are from India where cookies containing 25 percent protein are produced from peanut and wheat flour, and a high protein (42 percent) multipurpose supplement comprised of peanut flour and chick pea flour which can be added to other foods in their preparation. I'm sure there are many more examples where some form of oilseed or legume supplementation has been tried or is being used in a special limited feeding program, but is not necessarily available to all the people commercially.

Despite the number of articles written about protein supplementation and high protein baked products, there has been relatively little advancement in the implementation of these concepts around the world with perhaps the exception of India and the Philippines. Perhaps comments in a recent Composite Flour Newsletter can be paraphrased to apply to protein supplementation implementation also. In essence it said that despite much research in technology and economics, there is still remarkably little extension of composite flour (protein supplementation) technology applied to practical levels. Or to put it more bluntly, in few places are the village (or local bakeries) making bread from composite (fortified) flour. Just as some composite flour programs have been inappropriate, according to the economists, so it may be that protein supplementation of breadstuff, while a highly demonstratable technology in the laboratory, may be impractical for implementation around the world. Experience seems to be teaching us that governments or local organizations in developing countries are not waiting with open arms for acceptable products with superior nutrition. Nutrition and the technology to provide it must be "sold."

A CASE STUDY: ECUADOR

William J. Hoover

I will continue the theme of the first two speakers and not bore you by repeating the technological development sequence of soy fortified wheat flour, because they documented it very well. It should be understood that they covered about 10 years of work in 15 minutes. We were challenged following the technological development, and following the introduction of the soy fortified bread flour into the PL 480 Title II programs, to work toward introduction of soy fortified flour as a self-supporting and self-generating commodity in the commercial markets of the wheat consuming countries. Thus, the KSU AID project was extended to undertake feasibility or implementation programs the last two years of the project.

This year, more soy fortified wheat flour is being purchased than regular wheat flour in the Title II programs, which, I think shows a rather significant interest in the product, and records indicate that some 30 countries are using this product or have used it at sometime in the recent past. Thus, it is a real product which has been very well accepted by millions of consumers abroad and has been given some commercial experience, although with some differences in commerical formulations in this country. These domestic commercial products have not been resounding successes, although they have not failed because of lack of acceptability. The lack-luster results with high protein bread in the United States is probably due to a lack of need in the consumer's mind right now that they need a high-powered, fortified bread, and it may be also due to some questionable marketing or promotional practices. Our challenge is to find a way to introduce into these countries, commercially viable systems or government encouraged systems for wide-spread use and not just for selected markets or for institutional feeding programs.

We began by evaluating a number of factors that we felt needed to be studied to select countries which provided the best opportunity to implement a program of this type. Some 40 countries were selected for us, primarily by AID, and we evaluated each of these countries on the basis of the secondary data and information that we could get on these factors. We looked at FAO and USDA statistics and we received direct information from the field, especially from commodity marketing organizations. In the program we selected 9 countries which we thought, on paper, were best bets and those countries were, in no particular order: Brazil, Peru, Ecuador, Columbia, El Salvador, Morocco, Turkey, Iran, and Korea. Next, we started on a series of adventures to these countries that lasted one to three weeks duration each. We tried to document the factors that we found from secondary data, and to add on any other information we could get from firsthand observation or conversation. You might think that one to three weeks is a relatively short time to do a feasibility study, and indeed it is, so we called these prefeasibility studies and reported the results as "impressions." However, if you have good assistance from the AID missions as we did, and you know the questions you want to ask, and you are able to find the right people rapidly, you can get an enormous amount of information in a short time.

After these visits, we came to the conclusion that every one of these countries had the potential to enrich or fortify their breadstuffs if they chose to do so, but that there were negative factors in each and everyone of them that might make the task rather difficult. Not that they couldn't be overcome, but certain key factors had to be solved in each one of the countries and for our purposes, we wanted to choose the best bets. As you have already heard, the USDA had initiated some effort in Korea, so we did not go there. We selected, as a result of the countries that we visited, Ecuador and El Salvador as probably being the best bets, all things considered, to get demonstration programs going that would result in the enrichment or fortification of all the bread wheat flour in the country. Figure 1 is an old picture, but shows us the technology the other two speakers were talking about.

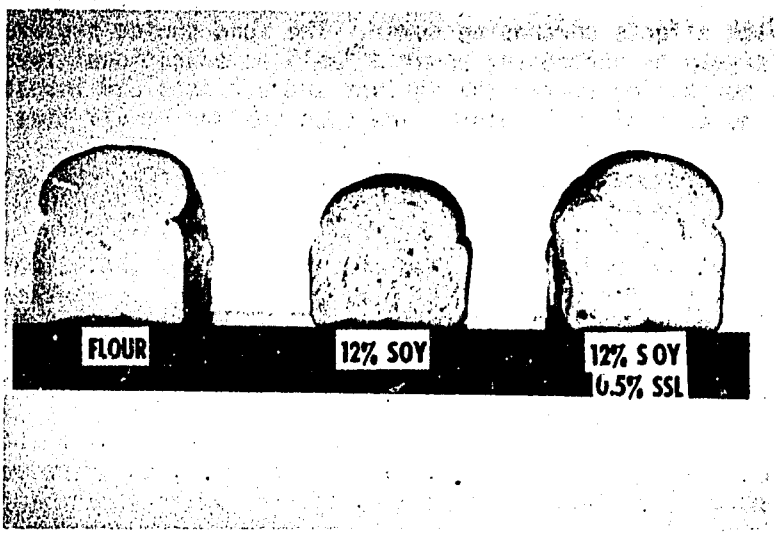


FIGURE 1

When soy flour or any other non-wheat protein material is added to wheat flour, it generally results in a lower loaf volume, coarser texture, a darker color and some off flavor. But with the proper dough conditioner, the bread quality returns to the point where consumers can't tell the difference between fortified and unfortified bread. This statement is made with a great deal of confidence because the fortified bread has been consumed now by millions of people, and very rarely do we find an individual who will claim that he can tell a difference. That usually happens with some cereal chemist when we tell him which one is which, and then he says, "of course I can tell."

My favorite slide (Figure 2) comes from the July-September, 1971 issue of Nutrition Newsletter of FAO in an article authored by G. Donose and J. P. Greaves. This figure depicts the system which exists in any city, village, country or region. The food system is finely balanced. If any element in the system is altered or adjusted, it wil usually result in several adjustments or reactions in other parts of the system. Thus, we need to evaluate each component in the system to determine how it will be affected by the fortification of wheat flour with soy flour.

We as agriculturists and nutritionists know that the top half of this nutritional web is the food chain. It depicts agricultural inputs, production, the commodity marketing to a food processor, and a food marketing system, all leading to the target circle of consumption levels of particular foods.

Now, going into that straight line system if you will, are non-conventional foodstuffs, food additives such as vitamins and minerals, and imports, with exports going out. Now the bottom half of the web represents the people part that is so cause and effect interrelated. Of course, we know that general health is affected by the food consumption levels. Health affects the population rate. The population rate of increase affects the age distribution, birth and death rates, the educational level and the labor supply, which affects purchasing power. The food choice preference is dictated largely by purchasing power as well as educational levels. Now, of course, purchasing power, the savings and earnings and investment influence the agricultural inputs, and thus the system all ties together.

Nutritional improvement, the subject of this conference, can enter into this system in two or three ways.

1. The breeders would enter into the system by giving better agricultural input seeds for particular purposes, whether it's increased yield or increased or improved protein. And the seeds they select are going to affect the acceptability to the food processor, the storability of the product, and finally whether or not it changes the acceptability of the food by the consumer. So even that entry into the system will have technical effects and certainly may have an effect on the cost and price relationships of the finished foods as well.

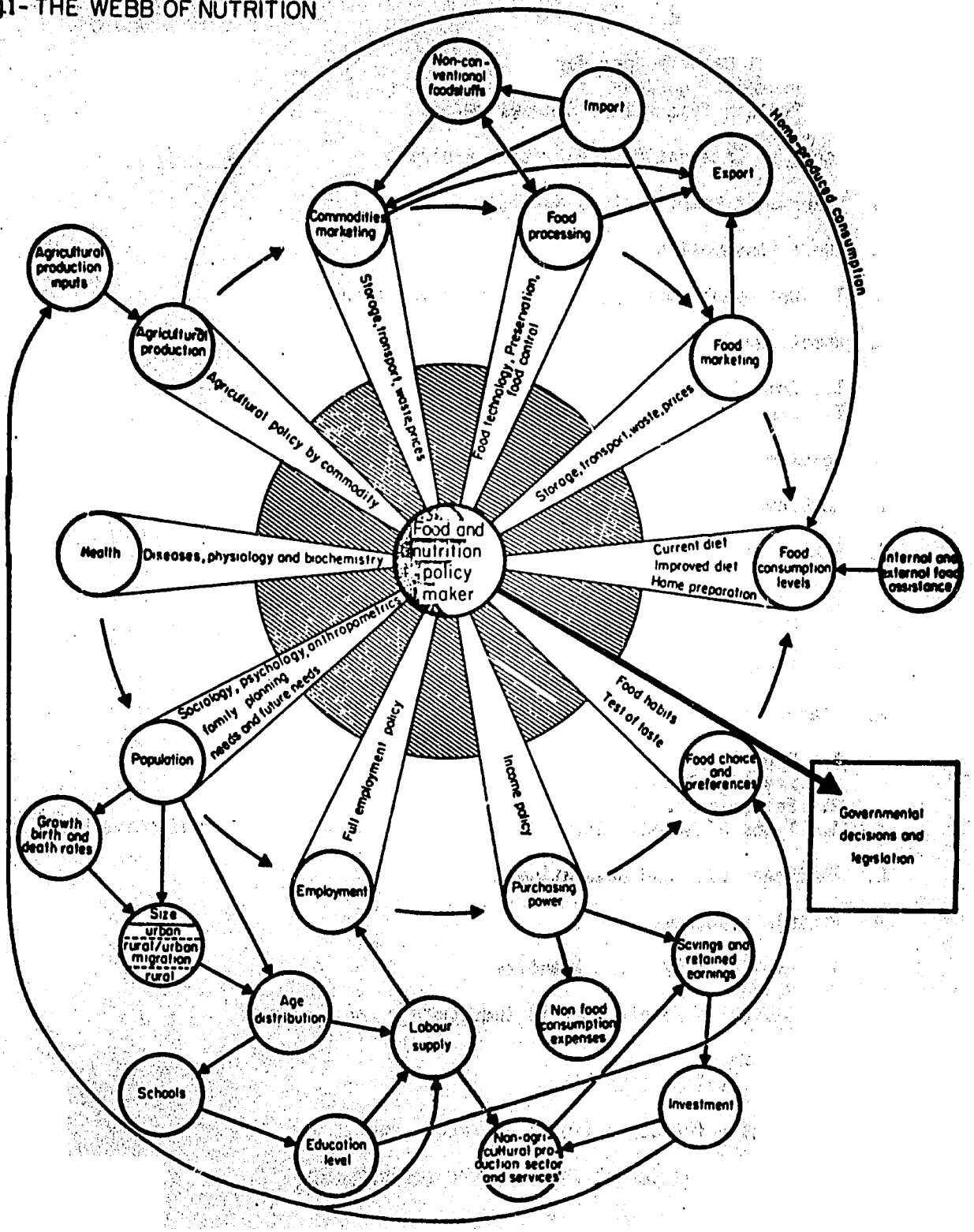
2. At the other extreme, the Title II programs come into the system in two ways. Either as import of commodities such as wheat, corn or grain sorghum where it usually enters into the processing system, or the external food in the form of the blended food or the fortified foods enter at a point close to the consumer, usually distributed and served in an institutional program.

The following is an outline of the factors we studied:

A. Population

1. Total
2. Percent Rural
3. Percent under 15
4. Growth Rate

Fig.1- THE WEBB OF NUTRITION



B. Economic

1. Per capita Gross National Product

C. Nutritional Need

1. Total Calories per day
2. Protein - gms/day
3. Protein from Animal Sources gm/day

D. Local or International Nutrition Programs

E. Government Commitment to Nutrition

F. Wheat Consumption

1. Net wheat consumption per capita and trend

G. Wheat Supply

1. Domestic - 1,000 M.T.
2. Import - 1,000 M.T.

H. Government Controls on Suppl

I. Government Agricultural, Trade and Price Policies

J. Status of Milling Industry

1. Capacity
2. Mill Location
3. Level of Technology
4. Economic Status

K. Status of Baking Industry

1. Capacity
2. Level of Technology
3. Economic Status
4. Types of Breads, Biscuits, and Pasta Products . . . Productive Level

L. Pertinent Laws and Regulations

M. Protein Resources

1. Availability . . . quantity
2. Price
3. Status of Edible Protein Industr

a. Capacity
b. Level of Technology

N. Institution for Composite Flour Technology

O. Availability of Cereal Chemists and Laboratories

In each of these countries we reviewed, we went through these factors and as this is a case study I'm going to indicate our condensed impressions of Ecuador. We evaluated each country for the same factors and produced summary reports of our impressions. The population is about 7 million people. It's growing at a rate of 3% a year. About 60% of the population is under 20 years of age. Half of the population is employed in agriculture. Economic growth is pretty good now because they have oil resources. Per capita gross national product is $350 a year and there is a wide range. Because of the oil exports, only about 1/4 of the GNP now is from agricultural production and that's been growing very slowly in recent years. Wheat consumption is increasing rapidly. They had about 110,000 metric tons in 1960. Now it's well over 250,000 metric tons. Average wheat consumption is higher in the urban areas than in the rural areas. Per capita consumption now is a little over 60 pounds per capita and we estimate in the cities it's at least 100 pounds per capita, which puts it at the same level of consumption in cities as in the U.S. Seventy-five percent of wheat flour is consumed as bread, 5% as cookies and 20% as noodles and other pasta products. Because of changing eating habits, consumption of wheat is increasing because people like it and also because the government subsidizes wheat and wheat products are now the cheapest source of food inspite of the fact that the government has a severe balance of payment problem accentuated by this subsidy for largely imported wheat. In the 60's they were producing 40% of their own wheat. They are now producing 22% of their wheat. Last year and this year, the mills are only getting about 10% of their grist blend from nationally produced wheat. It is not economically profitable to produce wheat in Ecuador compared to other crops a farmer might grow.

We tried to determine a nutritional need. I don't know what all you have said the last couple of days, but I'll assure you that's the most difficult information to get because it is essentially non-existent. There are guesses, there are village studies of clinical status, and there are estimates of food consumption. It is tough to get good data and most data cannot be relied upon anyway. In Ecuador, we found that it appears on an average, that there is about an 85% supply of calories per person and probably about 90% of the protein of the FAO recommended levels. Now I don't know what that means either because there is great disparity from season to season, from location to location, from person to person and from family to family and I came to the conclusion in Ecuador, as I did many places, that it really doesn't make a whole lot of difference whether we emphasize proteins or total calories. You can do both. The reason it doesn't make much difference in Ecuador is that the poorest of the poor are eating wheat and ultimately, we find that we can enrich it or add to it protein without affecting the cost. Ultimately protein is more expensive than the calorie sources they have and, therefore, protein fortifying bread is a good thing to do.

We also know that some of the most severely malnourished children are eating breads. you find this by studies, or you can ride a bus or walk down the street, and bread is what the mothers stick in the babies mouths. Bread is mans' first and still most widely used convenience food.

The PL 480 Title II school lunch program is phasing out in Ecuador. The World Food Program is phasing out its nutrition support. There is a mother-child program developing and the government of Ecuador showing interest to the point of putting their money into it. So Ecuador is a little unusual. At least we have fairly strong indications that as most international food programs are phasing out, the government of Ecuador is putting enough interest in these to start to fund them themselves and that is certainly not true of most developing countries. The commitment to nutrition appears to be pretty good, not excellent, but pretty good. They have a fairly nutritionally oriented Ministry of Health. They have a Nutrition Institute. There is a realization that nutrition needs to have a high priority in that country. There is a central planning agency with a little core, the nutrition oriented group which is making input into governmental policy decisions.

The wheat supply being imported is all hard winter wheat of 11-12% protein from the United States. The government controls the supply of wheat by authorization of quantity of imports by the mills. The government subsidizes the price of the wheat to millers and sets the price that flour must be sold at so the millers are in a sense "toll" processing within a government price system. Mill feed price is not controlled. The government policy definitely is to make the price of bread inexpensive. Wheat and flour are the only major food commodities being subsidized at this time. It has been proposed to remove this subsidy at the end of 1976. Other governmental policies are peripheral to our time here but must be considered in any study that you make of this type.

The milling industry, to meet the increasing demand, is undergoing some expansion at the present time at practically every mill, some of them by 50% to 100%. There are two large mills in the port of Guayaquil that will be capable by next year of processing 60 to 65 percent of the total production in the country and 22 other smaller mills in the Sierras that are together as an association to combat the competition from the coastal mills. The level of technology is good. There are modern, well-managed, well-designed mills. The economic status is not good, operating under the government control price situation. They are like most millers operating with high level of volume and a low per-unit mark-up.

The baking industry has one large modern mechanized bakery and about 10 middle-sized bakeries and many, many small shops producing bread only under partly mechanized or manual method. Their economic status appears excellent. There are many new bakeries. Existing ones are expanding. They have no controls on their product prices and they are getting the raw material flour at subsidized prices.

The type of bread -- all types, the most popular is a little round bun, sweet bun -- almost like our hamburger buns. People like it.

Current laws and regulations will not prohibit fortification or addition of additives. There are some restrictive import duties that must be changed to lower the cost of fortification.

Regarding the protein resources, there is one point I want to make, referring back to the web of nutrition. Unless we utilize a protein resource for bread fortification that is not being utilized currently in the country, we are not really adding significantly to the nutritional status of the population. For example, if a bean is grown and consumed locally, the use of a protein concentrate from that bean for fortification would merely shift the form of consumption unless the new use spurred an increase in production and yields of the bean crop. Ideally, the protein fortificant should be a source not now being consumed as a food or whose use will cause an increased production of the source locally.

In Ecuador, cottonseed, sesame, quinoa, and soybeans represent potential sources of protein. Unfortunately, degossypolized cottonseed, sesame or quinoa are not available in quantity and this the soybean becomes the source of choice. AID is assisting the Government of Ecuador in increasing the production of soybean in the Guayas River basin. Small quantities, to be sure, are being produced now, but it is increasing geometrically. The price of any protein material or even starchy materials is higher than wheat flour in Ecuador.

The status of the protein processing industry is essentially good. There is a shortage of vegetable oil. There are 4 extraction plants processing and refining vegetable oil from the doemstic and imported oilseeds. The solvent extraction plants are very large and quite modern with two of them constructed just within the last year. The protein capacity is far in excess of the level needed for an edible protein source for bread fortification.

There is an institution where modern composite flour technology is known. There are also cereal chemists available and good laboratories in the agricultural research organization.

Following the prefeasibility study in Ecuador, we recommended that further studies be done. Things looked reasonably favorable. We also knew that an action plan had to be developed with the aid of the key persons in the government of Ecuador. It was important that this become their program and not Kansas State's or AID's. We also needed to define the key places where we could provide technical assistance to help the program along when needed.

The prefeasibility study was done in December of '74. In March of '75, we went back for a more detailed study of the same factors in much greater depth and there was also a difference. Appropriate government people in the Ministry of Health, the Ministry of Agriculture, and in the central planning agency had read the report and were working cooperatively with the AID mission, had designated individuals from the Ministry of Agriculture and the Ministry of Planning to help us to do the more in-depth study. There was a shift of emphasis here. It wasn't us any more taking the lead, we were helping them and this is an important factor. We did do a much more detailed study. At this time we tried to estimate the cost factors and effects on each segment of the interrelated food production, processing and marketing chain. What would be the effect upon the government? How

would it affect their agricultural policies, their availability of food supplies, their balance of payments? How would it affect the millers? What would be the profit or loss situation to them? How would it affect the bakers? How would it affect the soy or the oilseed industries, and how would it affect the consumers? Those were the thrusts. As a result, we found in this study that, essentially, there was not going to be an increase in cost of bread as a result of fortification to the ultimate consumer or if there was, it would be so slight that it would not be of any real significance. An active plan involving 28 steps probably covering about a year and a half of work was outlined and we started working on those steps.

The soy processing industry appeared to be a key to the success of the program. I mentioned it as an oil processing industry and that is what it has been. The government is encouraging, through and AID grant, a very much increased level of production of soy and other oilseeds. To encourage the farmers to produce soybeans, the government set the price of soybeans at double the world market price and the farmers began to grow them. The government did not subsidize this. The consumers covered the high bean cost in the price of oil. Now everything looked fine and it didn't affect the price of oil too much, but a problem came up - what to do with the residual protein from the soy. They found that it was priced higher than the cottonseed meal and other protein meals that were being processed and marketed by the same processing plants. They couldn't export it to their neighbors because the price was higher than from other sources.

For this reason, the Ministry of Agriculture became interested in our reports and saw that maybe we had an answer to their problem. There was an edible use of soy protein material not in competition with protein from cottonseed. From that point on we had a leader within the government who wanted to push this bread fortification, not only for nutritional reasons, but particularly for the benefits it would offer to soybean farmers and the soy processing industry. Well, the story became rather exciting from that point on. We had a soy process engineer from the U.S. go down and examine the processing plants and found out that they were capable of using soy flour with certain modifications. It meant maybe a million dollar modification at each plant, but they could do it.

Again, we went back to Ecuador, because in August of 1975, the government people themselves had a modified action plan and proposal with their own reports, and we set out a more effective action plan. We went back in December and reviewed with each of the affected industries; the millers; soybean processors; and the bakers, what effect this fortification program would have on them.

In February, 1976, a detailed plan of technical assistance was developed to be completed by September. Flour, typical of that produced in Ecuador was shipped to KSU and tested with a soy flour, SSL, vitamin and mineral premix. The resulting breads were of high quality. Arrangements were made for a shipment of a significant quantity of premix to Ecuador. The premix was blended at a local flour mill and delivered to a large, a medium, and a small sized bakery. The soy fortified flour was baked and sold in the market to determine acceptability. The product was highly acceptable.

A soy process engineer from the U.S. studied in detail the soy plants and listed detailed specifications and costs of equipment and alterations necessary for converting the plants to production of edible soy flour. A team of two economists studied the marketing and facility requirements for orderly marketing of Ecuadorian soybeans from farmer to processor.

Finally, in July and August, a final feasibility study was carried out by a team composed of myself, an economist, a milling engineer, a cereal chemist specializing in bakery production, and a linguist. A document for discussion has been prepared to assist the leadership in the Government of Ecuador to make a decision as to whether to undertake the fortification of all bread wheat flour. This document provided the history of the development of soy fortified flour, general information, present nutritional situation, the wheat situation, soybean situation, soy processing facilities, estimation of the cost of soy fortified wheat flour in Ecuador, estimation of the cost of bread using soy fortified wheat flour, estimate of the net impact on the balance of trade in raw materials for flour production as a result of fortification, condition of the milling industry, and the condition of the bread baking industry.

In addition, the report gave the details of the proposed Soy Fortified Bread Wheat Flour Program including: enabling law or decree; operational plan; quality assurance program; duties and tariffs; public education; soy flour supply; soy processing capacity; and soy fortified bread wheat flour product specifications.

The effects of this program were explained to millers, bakers, and the economic effects on consumers were evaluated. The effect on the profitability of the millers should be negligible. One advantage is that the capacity of a given plant could be increased about 15% with a minimal expense for blending equipment, whereas a 15% increase in wheat milling capacity would require a rather major expenditure.

The baker is mainly concerned about what might affect his volume of sale. If the quality is not affected, he is only concerned about raw materials costs. Studies indicate the price of fortified flour might increase from 1 to 4% depending upon the source of soybeans. This does not greatly concern the baker because he will have an increase of 6 to 8% in net dough yield, and he will be able to use less shortening than he is now using, and that's his most expensive ingredient. The price of bread should be virtually unaffected. In Ecuador only about 24% of the cost of bread is flour. Therefore, the cost of bread would increase by 1% and in some instances, down to .2% under other conditions. This is not counting the increased yield or shortening savings and it is doubtful whether one can really measure such a small increase because the general inflation rate is much higher than these projected increases. So it looks good. What about the effect on tha balance of payments by the society or the government. The evaluation involves the direct offset in cost of imports of wheat, the cost of imported soybeans or the cost of imported inputs to aid soybean production in Ecuador. We determined 12% soy fortified flour using imported beans for production of the soy flour would add a net cost of $10 per ton of fortified wheat flour -- that's on a base of about $210. If you switch to Ecuadorian beans, there would be a net gain of $34/ton so the government

sees this as a valuable savings and a great incentive. In fact I think it will do a lot toward encouraging the wheat producers into the production of soybeans in certain areas of the country.

I want to tell you one more thing that happened that was quite important. After we completed the studies and visited individually with industry and government groups, a meeting at the Ministry of Agriculture with representatives of the affected government agencies, the representatives of all the industry segments, and press were told the purposes and results of our studies and the proposal that was being considered to fortify the flour. There was about an hour and a half very frank question and answer session. This was followed by a reception at which a range of bakery products prepared with soy fortified wheat flour were served. This resulted in a very positive reaction in the press the next few days. The AID mission director said that as far as he knew that this was the first time in the history of Ecuador when the affected industries and government had gotten together to discuss openly and freely the effect of some government policy while it was under consideration. I'm happy to have been involved in that sort of experience.

The program now is for the government ideally to take a decision this fall on the proposal. Once enabling legislation is passed, there will be more requirements for technical assistance. Technical assistance will be needed in the next year. The hope is that the program would become operational in December '77 or January of '78. Before that time, we must test out the operational plan. There is a great deal of work to be done on the specifics of the quality assurance program. We have to develop a nutritional education program that would let the people know that this bread is a good source of protein, vitamins and minerals. The soybean processing plants need help at the time of start-up, certainly, the flour millers need some detailed help on installation of equipment, and short courses or seminars must be conducted for bakers.

This is the Ecuador case study. It hasn't been approved, yet. It looks pretty good. I hope it does because it will provide a pattern for any country wanting to consider the practicalities of food enrichment and fortification.

There are some keys which I have learned from this. First, a very thorough feasibility study is needed, and not only technical feasibility. Economic evaluations taken in the social and political constraints must be evaluated and those economics have to be favorable. I don't believe that nutrition needs will cause any government into taking this policy of fortification, if the economic factors are not favorable or if another problem can't be solved as it will be in this instance. We need to understand, and I'm repeating because it is so important, how each segment of the system will be affected. Identify your allies and try to neutralize the opponents. Identify the key factor or government or national policy which will be the beneficiary. In this case it was the probelm they are facing in their oilseed production and marketing program. Identify those proponents in government and work with them as it must be their project. Underlying all of this you have to have favorable mission support and AID-Washington has to supply a climate permitting freedom of action and rapid technical assistance capability which so far, we have enjoyed on this project.

CEREAL FORTIFICATION: BARRIERS TO IMPLEMENTATION[1/]

James E. Austin*
and
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The Barrier Spectrum

Cereal fortification, like all nutrition interventions, faces a series of barriers which it must overcome before it can be effectively implemented. Identifying, understanding, and evaluating each of these obstacles is essential to determine the feasibility and desirability of implementing a fortification program.

These implementation barriers fall into the following five general categories.

(1) nutritional need,
(2) technology,
(3) commodity system structure,
(4) consumer acceptability, and
(5) intervent[illegible]ics.

Nutritional Need

When the deficiency to be addressed is protein inadequacy, the laws of nutritional metabolism dictate a basic guideline for protein fortification: in the face of insufficient calories, do not fortify. Nutrition scientists have come to the same conclusion (7).

The lack of clear positive proof of growth improvement from the fortification field research projects in Tunisia, Thailand and Guatemala tend to reinforce the concept of calorie adequacy as a prerequisite to protein fortification. Protein and calorie deficiencies in the cereal based diets of the developing world are, to a great extent, inseparable, both because cereals remain the principal source of both proteins and calories and because the nutrition jury is still not entirely in on the interaction between protein utilization and calorie levels. Scrimshaw et al (6) have published data which indicate lysine supplementation may be beneficial even in light of inadequate caloric intakes. The study revealed that fortification increased the utilization of wheat protein even at low energy levels and raising calorie intake to adequacy levels increased nitrogen retention even more.

The study, however, was conducted with adult males rather than infants or preschoolers who have increased energy and protein needs due to their growth rates; also caloric inadequacy was based on established requirements rather than actual needs. Hegsted (4) has questioned whether the standards of protein requirements are applicable when actual energy intakes are below recommended levels for prolonged periods, thus necessitating metabolic adaptations to survive.

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The potential impact of fortification on protein supplies is impressive. By removing the constraints of the limiting amino acid, fortification can increase the percent of utilizable protein in the basic cereals dramatically: 66% for white wheat flour, 70% for yellow corn meal, and 65% for milled rice (1). Diets, of course, consist of more than just cereals and it is the total amino acid picture and limitations (along with caloric adequacy levels) which will determine the actual improvement possible via fortification.

Clearly, the volume of food would be self-limiting. Another nutritional advantage of fortification is that the protein increase occurs without increasing the physical quantity of grain consumed. This is particularly relevant for interventions aimed at the nutritionally vulnerable target groups of infants and preschoolers whose nutrient intake is limited by their capacity to consume large quantities of food. For example an 18 kg. boy using rice as the sole source of dietary protein would require approximately 27 cups of rice per day to supply the needed amounts of protein. For maize 338 grams daily would be needed to maintain a positive nitrogen balance in a 10 kg. child, and this quantity is the consumption of an adult male in Guatemala. If the rice were fortified, about one half as much would be needed to meet the protein requirements. Even with fortification the bulk constraint still exists to a certain extent, (3) thus indicating the need (and the reality) among infants for protein sourcing from various foods other than just cereals.

The final factor suggesting the need for nutrition planners to consider cereal fortification is its potential role in addressing micronutrient deficiencies in addition to or separate from protein deficiencies. Vitamin and mineral deficiencies need addressing as much as protein calorie malnutrition. Such deficiencies, in many cases, are even more widespread and severe than the macronutrient shortfalls (2). The broad consumption of cereals makes them attractive potential vehicles for correcting vitamin and mineral inadequacies.

Technology

Technical problems, to the extent they exist, seem to relate primarily to the fortifying process: putting the fortificant in a form which is compatible with the vehicle chosen and the processing of that vehicle. The cereal fortification projects in Guatemala, Thailand, and Tunisia encountered certain initial technical problems but all were solved suc that few technical difficulties have arisen in the central, large mill operations (Tunisia) or in the village, small mill operations (Thailan and Guatemala).

In general, it appears that technological barriers to not represent a serious impediment to cereal fortification.

Commodity System Structure

Assuming that the technical dimension of the intervention can be handled satisfactorily, one must next examine the flow of the commodity from the production points through the processing points to the consumption points. This commodity system analysis bears on fortification feasibility in four ways.

First, it is essential to know how much of the commodity passes through the mills and, therefore, can be reached by a fortification program. In rural areas the feasibility of cereal fortification has been questioned because it was believed that many families, especially the poorer semi-subsistance farmers, milled their own grain in their homes by hand.

Mechanical processing saves considerable time and physical effort; these economy and convenience factors are very important to consumers, even low income ones. Thus, even though poor rural families may not market all of their grain and consume most of what they produce, they generally still enter the commodity system by purchasing milling services and, thus, are reachable via fortification. This situation could vary among countries and regions and should be verified as part of a country specific feasibility study.

The second reason for examining the flows of the commodity is to assess its likelihood of reaching the nutritionally vulnerable groups which at the first level of targeting are low income families. Cereals are particularly effective vehicles in this regard because, in general, the pooer the family, the greater the dependence on cereals. However, frequently preferences within and among cereal types vary along income lines.

A third aspect of the commodity system which bears on the feasibility of a fortification intervention is the structure of the processing segment. Conventional wisdom is that a prerequisite to fortification must be centralized processing of the carrier. The rationale underlying this assertion is quite sensible and pragmatic: the fewer the processing points, the easier to control the operation and the more economical to manage. Despite its logical appeal, this bit of nutritional conventional wisdom should be questioned.

The negative side of centralized processing is that a few mills supply most of the population which includes both those who are not nutritionally needy as well as those who are deficient. Consumption of the fortified product by the non-needy group represents excess coverage and an undesirable expenditure in terms of achieving nutritional improvement goals. Conversely a decentralized milling structure, as is common for most basic cereals produced locally allows regional and even village level targeting. What has been viewed as a severe disadvantage might in fact be a significant economic advantage. This can dramatically reduce the costs of excess coverage especially where deficient groups tend to be concentrated in selected areas. Such costs are particularly burdensome for protein fortification but not insignificant for micronutrients.

The cost of the excess coverage under a centralized system would have to be weighed against the added control costs under a decentralized system. We shall illustrate such a comparison in the subsequent section on intervention economics.

The final need to examine the commodity systems flows is to determine the volume of grain consumed by the target groups which is an input to determining the quantity of fortificant to be added to the cereal. Consumption implies acceptability, which moves us to our next barrier.

Consumer Acceptability

A commonly cited advantage of fortification is that it does not affect the carrier vehicle's appearance or organoleptic characteristics and, therefore, avoids the problems of consumer resistance to new foods.

More specific than cultural factors are the particular characteristics of the fortified product itself. Our research aimed to test the "invisibility hypothesis" by way of blind taste tests. INCAP personnel administered 720 taste disctinction and preference tests to persons in four towns and one village in the four main regions of Guatemala. Three different types of fortified tortillas (using soyflour, amino acids, and soybeans) and one nonfortified tortilla were used. Each person tasting the unidentified tortillas was asked about the taste, texture, and color differences and general likability of each tortilla. The respondents seemed able to distinguish among the types of tortillas; they were correc 56% of the time as against the 33% which would arise by chance. However, there was no clear preference for the unfortified tortilla over the fortified, nor did any of the three types of fortified tortillas emerge as a clear favorite. This approach and findings are significant to intervention design because first, it provides a green light to pass the organoleptic barrier and, second, it reveals that the planner still has options in terms of which fortificant to use. Other criteria, e.g. cost, local availability, etc. could then be applied to the fortificant section process.

The organoleptic characteristics are not the only relevant paramaters to acceptability. Preparation and cooking characteristics should also be examined. For the tortillas and malleability of the fortified and nonfortified maize proved to be indistinguishable to the tortilla makers preparing the samples for the taste tests. Storability did surface as a limitation of the soy fortified tortillas in the village (Sta María Cauqué) where INCAP's fortification project had been going on for several years. The fortified tortillas tended to decompose more quickly and, therefore, limited their storability which led some groups within the village to refuse to have their corn fortified. Even if the final fortified product is indistinguishable, the process of fortification and, therefore, the intervention, at the village level is highly visible. Centralized processing serving urban or distant rural consumers can be "invisible." Not so at the village level where the consumers bring their own grain to the mill for grinding. Without their acceptance no fortification will take place, except if it were legally mandatory and even the avoidance would occur if the consumers are not willing. Consequently, it is important to identify the key people influencing the fortification decision.

Intervention Economics

The final, and in some respects the most basic, barrier to fortification feasibility is the economic one. For protein fortification the dominant cost is the fortificant. The protein fortificant alternatives are either synthetic amino acids or natural proteins (which also increase the absolute quantity of protein and calories of the carrier).

The economics of these protein supplements are largely determined on the demand side by the animal feed industry (primarily swine and poultry). Currently, human food uses are minimal.

The fortificant in protein fortification interventions constitutes over 80% of the total operating cost. In determining costs there are two necessary steps and a third optional one. Step (1) consists of a listing of the inputs required to execute a particular program design. Step (2) involves the costing of these inputs in a common currency (either the local one or an internationally used currency, usually U.S. dollars). This results in what might be called the nominal cost of the program. Step (3) would constitute the conversion of some of these nominal cost items, as appropriate, into adjusted social cost terms. The social cost principle implies (1) that all inputs which have alternative uses anywhere in the society (and only such inputs) should be included in the costing and (2) that all such inputs included should be valued at the benefits forgone in their best alternative use. Frequently, nominal prices are accepted as a sufficiently accurate reflection of social cost. However, when there are significant price distortions involving inputs which constitute a significant share of program costs, then price adjustment, or shadow pricing, is required. The two kinds of inputs which are commonly shadow-priced are unskilled labor (which is sometimes overvalued by nominal prices) and internationally tradable commodities (which are sometimes undervalued).

Tables 1 and 2 illustrate the first two steps in the costing procedure for two possible programs in Tunisia. The country's market for wheat, which is the carrier selected for experimental fortification programs there, is a dual one. The first program is based on the conventional wisdom that an effective fortification program requires a distribution system for the fortificant which includes a central processing stage. Some 40-50% of Tunisia's wheat supply meets this requirement, since it passes through 18 large urban mills, 14 of which are located in Tunis. Table 1 itemizes the estimated capital cost (U.S. $283,000) and annual operating cost (U.S. $6.8 million) of fortifying this part of the wheat supply with lysine and vitamins. Table 2 costs out a possible alternative or additional program which would fortify the remining 50-60% of the wheat supply which passes through some 3,200 village mills. This program, which involves use of a tablet-form fortificant and requires greater outlays for training, supervision and persuasion, turns out to cost about U.S. $1.9 million in capital cost and U.S. $11.6 million in annual operating cost. Both tables state cost in nominal terms. These operating costs in terms of flour amount to .7¢ per pound for the centralized mills and 1.2¢ for the village mill system.

Anyone with programming experience will appreciate that program-costing cannot be done with anything like the precision that a naive reading of Tables 1 and 2 would suggest. Nearly all the figures cited, both quantities and prices, should really be thought of as ranges. Since all this possible variation creates an unmanageably large number of permutations, the usual tendency is to cite only the one best estimate in each case. When this is done, however, the programmer should also conduct sensitivity analysis to get some idea of how much effect reasonably likely input price changes would have on the total program budget. (This is worth doing even though the definition of "reasonably likely input price changes" is a subjective matter).

We can illustrate the use of sensitivity analysis by referring to Table 1, where the capital cost of the fortification system is shown as $283,188 and the annual operating cost of $6,814,543. Various cost-increasing contingencies can be imagined. For example, if the start-up data of the program were delayed by one year for some reason, this might raise capital costs by, say, 10% or to roughly $311,500. On the other hand, a 10% rise in imported components of capital cost only would raise total capital costs by only 5.5%, since imports account for 55% of capital costs (assuming that all equipment is imported and that imported materials make up half the cost of construction).

Table 1

Nominal Cost of Fortification System

through 18 Large Mills

(U.S. $)

Capital cost	
A. Fortificant blender (5,000 lb. capacity)	$27,000
- shipping	16,000
- unloading and transport to blending point (Stim)	100
- scales	2,500
- ladders	1,000
B. Feeders and spare parts (20 @ $2,000)	40,000
- shipping	18,000
- unloading	100
- transport to mills (14 in Tunis, 4 regional)	300
C. Training (for blender and feeder operators and supervisors)	2,500
D. Laboratory equipment	30,000
E. Warehousing	
1. Tunis (size: 30.3M x 39.4M)	
- contruction	72,000
- land	4,298

Table 1 (continued)

2. Regional	
a. Sousse (size: 7.5M X 9M)	
- construction	4,050
- land	324
b. Sfax (size: 5.45M X 5.45M)	
- construction	1,800
- land	108
c. Ebba Ksour (size: 5.45M X 54.5M)	
- construction	1,800
- land	108
F. Warehouse equipment	5,000
G. Office equipment	2,000
H. Publicity (newspaper, radio, posters)	30,000
I. Program start-up costs (planning and initiation of program)	
- personnel (2 administrators, 1 accountant, 1 scientist, 1 secretary) for 6 months	13,200
- office expenses	3,000
- travel expenses (Europe, Japan, Tunisia)	8,000
Total capital cost	$283,188

II. Annual operating cost	
A. Fortificants (lysine and vitamins: 2101 tons @ $3,130/ton)	$6,576,130
B. Shipping of fortificants (assume from France to Tunis @ $29/ton)	60,929
C. Liners (used in drums for distribution of premix: 70,300 @ $0.25)	17,575
D. Shipping of liners (4 tons @ $29/ton)	116
E. Additional drums (i.e. additional to vitamin and lysine drums reused for premix): 3,500 @ $5	17,500
F. Cargo landing fee (lysine, vitamins and liners)	5,254
G. Transportation of fortificants and liners from dock to central mill	
- loading onto truck	1,400
- transportation	1,765
- unloading	1,400
H. Transportation of blended fortificant to 17 mills	
- to other 13 Tunis mills	2,688
- to Sousse, Sfax and Ebba Skour	2,151
- loading and unloading	2,800
I. Labor	
- blending supervisors (2 @ $225/month)	5,400
- laborers for Stim warehouse (2 @ $175/month)	4,200
- supervisors of feeders, fortification, quality control 1 each mill (18 @ $300/month)	64,800
- laborers (one each at 3 regional warehouses @ $175/month)	6,300
J. Electricity	
- blender	2,495
- feeders	7,300

Table 1 (continued)

K. Warehouse operation	
1. Telephone, utilities, maintenance	
- Tunis @ $180/month	$2,160
- regional warehouses (3 @ $130/month)	2,500
2. Supplies	
L. Administration and quality control	
- administration (1 @ $500/month)	6,000
- administrative assistant (1 @ $300/month)	3,600
- scientist/quality control supervisor (1 @ $450/month)	5,400
- accountant (1 @ $450/month)	5,400
- secretary (1 @ $300/month)	3,600
M. Office supplies	1,000
Total annual operating cost	$6,814,543

Source: Data supplied by Christopher Mock

Table 2

Nominal Cost o[illegible] System

through Village Mills

(U.S. $)

I. Capital cost	
A. Feeders for fortificant tablets (1 per mill X 3,200 mills X $100)	$320,000
B. Warehouses	
- Tunis	73,267
- 12 regional warehouses	89,131
C. Warehouse equipment	15,000
D. Office equipment	
- Tunis (central) office	2,000
- regional offices in warehouses	6.000
E. Trucks	
- pickup vans for delivery/quality control administrators (56 @ $7,000)	392,000
- jeeps for on-call mechanics/quality control (24 @ $6,000)	144,000
F. Training costs	
1. Training program for village millers (2-day program in each gouvernorat to explain nutritional aspects, train in use of feeders, build support)	
- supplies and miscellaneous	7,800
- per diem for millers @ $20.day	28,000
- transportation for millers @ $25	80,000

Table 2 (continued

2. First training program for regional administrators (8-day program in Tunis covering nutrition, program management, location of millers, relations with millers and community)	
- supplies and miscellaneous	$2,000
- per diem @ $30/day	3,280
- transportation @ $60	720
3. Second training program for regional administrators: 5-day program in Tunis covering distribution system (warehouses) and mechanical system (feeders)	
- supplies and miscellaneous	1,500
- per diem	2,050
- transportation	720
4. Training program for quality control advisors (10-day program in Tunis covering nutrition, feeders, milling simple lab tests)	
- supplies and miscellaneous	4,000
- per diem: 90 @ $30/day	27,000
- transportation @ $50	4,500
5. Training program for local representatives in each gouvernat (to explain program to consumers; would be local inhabitant --school teacher, student or health worker--available to work one month in summer); one representative per 5 mills = 640 rep's; 2-day program for each gouvernat	
- supplies and miscellaneous	7,800
- per diem @ $20/day	25,600
- transportation @ $25	16,000
G. Start-up costs (planning and initiation of system)	
1. Tunis staff (8 months)	
- 1 administrator @ $500/month	4,000
- 2 administrators @ $450	7,200
- 1 assistant @ $300	2,400
- 1 accountant @ $450	3,600
- 1 scientist @ $450	3,600
- 2 secretaries @ $300	3,800
- travel (Europe, Japan, Tunisia)	10,000
2. Regional core staffs (4 months)	
- 13 administrators @ $450/month	23,400
- 80 assistants (future quality control personnel) @ $350	112,000
- travel	20,000
H. Quality control equipment	13,000
I. Publicity	
- media: newspapers	1,000
radio, TV (60 days)	19,000
- billboards: 400 @ $50	20,000
- posters (in each mill) @ $10	32,000
- salary for local representatives: 640 rep's @ $175/month for one month	112,000
- travel for local rep's: $2/day for 20 days	25,600
- supplies: $30/village X 3,200	96,000
Total capital	$1,861,968

Table 2 (continued

I. Annual operating cost	
A. Fortification tablets	
- fortificant (2,813 tons @ $3,130)	$8,804,690
- tableting: 1,125,500,000 tablets (2/kg. of wheat) @ tableting cost of $0.45/1,000	506,475
B. Shipping of tablets from France to Tunis: 2,813 tons @ $29	81,577
C. Cargo landing fee in Tunis	7,033
D. Transfer of tablets from dock to central Tunis warehouse	
- loading	1,563
- truck transport	2,363
- unloading	1,563
E. Transfer of tablets from Tunis warehouse to 12 regional warehouses	
- loading	1,514
- truck transport	25,786
- unloading	1,514
F Vehicle operation	
- 56 vans: fuel	22,624
maintenance and other	25,200
- 24 jeeps: fuel	9,695
maintenance and other	7,200
G. Payments to millers	
- 2/3 of millers receive basic fee: 12 days/month ($28.80)	729,907
- 1/3 of millers receive basic fee plus premium for extra output: average of 20 days/month ($48)	626,688
H. Warehouse operation	
1. Utilities and maintenance	
- Tunis	2,640
- 12 regional warehouses @ $80/month	11,520
2. Supplies and other	
- Tunis	1,800
- 12 regional warehouses	7,200
I Feeder operation	
- fuel (3,200 @ $20)	64,000
- replacement parts (3,200 @ $5)	16,000
J Central administration	
- administrator (1 @ $500/month)	6,000
- administrative assistant (1 @ $300/month)	3,600
- accountants (2 @ $450/month)	10,800
- secretaries (2 @ $300/month)	7,200
K Labor for program administration, fortificant distribution and quality control	
1. Program and warehouse management/Tunis	
- administrator (1 @ $450/month)	5,400
- assistant (1 @ $300/month)	3,600
- accountant (1 @ $450/month)	5,400
- secretary (1 @ $300/month)	3,600
- laborers (3 @ $175/month)	6.300

Table 2 (continued)

2.	12 regional warehouses	
	- 1 administrator/regional director @ $450/month	$64,800
	- 1 accountant/secretary @ $350/month	50,400
	- 1 laborer @ $150/month	21,600
3.	Distribution/quality control teams (56 teams for 13 regions, to visit each mill once every two weeks)	
	- 1 driver/laborer (unloads fortificant) @ $150/month	100,800
	- quality control inspector (inspects operations, advises) @ $350/month	235,200
L.	On-call quality control personnel (24 for 13 regions) @ $350/month	100,800
M.	Publicity: radio (4/day @ $10 X 365)	14,600
	Total annual operating cost	$11,598,652

Source: Data supplied by Christopher Mock.

As already noted, fortificant cost is a major influence on total operating cost. In Table 1 (the centralized system), fortificant cost accounts for 96.5% of the total; in Table 2 (the decentralized system), the comparable figure (including fortificant tableting) is 80.3%, lower but still by far the largest share of total cost. If fortificant cost doubles, as the price of synthetic lysine did in 1972-74, the cost of the centralized system could rise by 96.5% and that of the decentralized system by 80.3%. However, this would occur only if no substitution of lower-cost fortificants was possible. If, on the contrary, we were to assume that an alternative fortificant package was available at 50% higher cost than that programmed in Tables 1 and 2, and that the price of that alternative fortificant did not change (a rather unrealistic assumption, since prices of competing products tend to move in the same direction, although not necessarily at precisely the same rates), then fortificant cost could rise by no more than 50% and total operating cost by a maximum of 48% in the centralized system and 40% in the decentralized system. The other factor affecting fortificant costs in addition to its price is the amount to be added to the carrier. This should be determined by the amino acid deficiency in the total diet of the target group and the percent of that deficiency to be covered via fortification and the amount of the carrier the target group consumes.

The total costs of the centralized and decentralized intervention schemes should only be thought of as the first step in evaluating the two approaches. Their relative effectiveness must also be considered along with their costs. Effectiveness can be defined initially in terms of the ability to reach the target groups with the needed nutrients and subsequently in terms of biological impact on the health status of these groups. We would expect few governments to attach much value to receipt of the fortificant by groups outside the primary target population. This means that deliveries to nontarget populations increase costs without increasing benefits. The burden of this excess coverage is particularly acute for protein fortification which is much more costly than micronutrient fortification. There are thus incentives for designing narrowly spotlighted programs. But there are also costs associated with the focusing

process, with the difficulties of designing a project which will include those who should be included and exclude those who should be excluded. These two types of cost -- one of which increases as the program becomes more narrowly focused and the other increases as it becomes more general -- must be weighed against each other. That is, an optimal degree of focus must be sought.

The target group at which protein fortification programs are principally aimed is certainly going to be less than 20% of the total population, and probably less than 10%, even in countries with the most youth-oriented population structures. This is illustrated in Table 3, which is based on data for a developing country in which the birth rate has been about 40 per thousand and the rate of natural increase nearly 3% for several years prior to the year to which the table applies. In this kind of population structure the size of the target population for a protein fortification program is highly sensitive to the age cut-off point selected. Based on Table 3, a target population of six-month to six-year olds plus pregnant and lactating women would constitute 18.2% of the total population. Dropping the cut-off age to five years reduces it to 9.1%. If only part of the total population in these groups is defined as the target population for the program (e.g. those living in the poorer regions of the country), the target population naturally becomes a smaller proportion still. Thus the arithmetic of population structure is such that the overcoverage involved in an extensive program may multiply the cost per member of the target population served by a factor of five, ten or even more. Clearly, this may make the program prohibitively expensive.

Table 3

Illustrative Data on Share of Target Population in Total

Group	% of Total Population
6 months - 1 year	1.70
1 - 2 years	3.21
2 - 3 years	3.31
3 - 4 years	3.21
4 - 5 years	3.18
5 - 6 years	3.17
Pregnant women (taken as 3/4 of annual births)	0.55
Lactating women (taken as 1/2 of annual births)	0.66

Source: Estimates of Population for West Malaysia (1967) (Kuala Lumpur: Department of Statistics, 1969).

Like all other services supplied by the government to the public, fortification programs must be financed either by a user charge, from general taxation or by a combination of those two means. Which method should be chosen in this case? To deal with this question, we must weigh two major criteria, efficiency and equity.

When the benefits of consuming any good or service accrue solely (or mainly) to the individual consumer (and this includes production benefits which the invidiaul can capture through higher earnings), efficiency is usually served by requiring the consumer to pay the cost. Otherwise, price and more resources will be allocated to that particular line of production than is warranted by society's willingness to pay. Where significant "externalities" are involved, or where the service in question is a "public good" (i.e. where much or all of the benefit is not captured by the individual consumer but accrues to society as a whole), this conclusion must be modified. If individual consumers cannot capture the full benefits, charging them the full cost can only lead to under allocation of resources to the good or service in question. In this case, the service must be subsidized, or even provided free, to ensure an efficient allocation of resources.

On efficiency grounds, therefore, one could argue that consumers of fortified foodstuffs should be charged the full cost of the fortification program. Efficiency-related arguments to the contrary could be based either on the presumed presence of externalities or on the view that individual tastes cannot be allowed to determine what is best for the consumer in this case. From the equity point of view, it is hard to imagine a service for which a user charge would be a less appropriate means of finance. A tax on a basic foodstuff is as regressive as any that can be levied, since both the proportion of income spent on food and the proportion of food expenditures devoted to purchases of the basic food grain bear strong negative relationships to the level of household income. As in many other areas of social policy, therefore, policymakers must ultimately decide whether efficiency or equity is to be the dominant canon of public finance.

A Concluding Note

Our research has neither tried to condemn or promote protein fortification of cereal grains. Rather, the analysis has attempted to present an approach whereby nutrition planners can weigh the desirability and feasibility of protein fortification interventions. It is our belief that the methodology designed and employed in our research is also generally applicable to evaluating micronutrient fortification programs. To the extent that generalizations can be made, our judgement is that protein fortification has a place in the nutrition planner's protfolio, but it should be used only when the caloric deficits are also being addressed. Additionally, serious consideration should be given to combining macro and micro nutrient fortificants to increase overall nutritional impact. Finally, efforts should be made to target the fortification to avoid the costs of overcoverage.

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WIDESPREAD MALNUTRITION -- A PROTEIN PROBLEM, A FOOD PROBLEM OR A POPULATION PROBLEM?

A. E. Harper

In devising programs to alleviate malnutrition and bring food production and population growth into balance, it is important to recognize that the world-wide problem of malnutrition is not the result of a food and population imbalance. The incidence of malnutrition will increase if population growth outstrips food production, but widespread malnutrition was recognized as a critical health problem for several decades before there was evidence that food production might not be keeping pace with population growth.

Malnutrition is responsible for high death rates among young children in poor countries, even though overall death rates in many of them are not much higher than those in rich countries. A high death rate among children leads to the desire by parents for a large number of children and, when death rates begin to fall even slowly, a continuing high birth rate becomes a major factor contributing to rapid population growth. Malnutrition is thus a cause rather than an effect of any food and population imbalance. It is also, however, a symptom -- a symptom of poverty associated with a low literacy rate, an unsanitary environment and a lack of health care, which in turn are associated with an inequitable distribution of wealth and resources and a slow rate of economic development (21, 12). Failure to recognize this can lead to proposals for bringing food production and population growth into balance and for alleviating malnutrition that are directed toward treating symptoms rather than causes.

For many years widespread malnutrition was equated with protein deficiency and emphasis in international programs was placed on theneed for increasing protein supplies (19), despite the fact that Cicely Williams (21) has been emphasizing the complexity of the problem regularly since 1950 (20). Gopalan (6, 7) reported nearly 10 years ago that children in India who developed the two extreme forms of malnutrition that had been identified as energy deficiency and protein deficiency, consumed essentially the same diets. When nutrient intakes of such children were measured, deficits in the consumption of energy sources were almost without exception greater than the deficits in protein consumption. During the last few years, further observations of this type together with reevaluatio of protein requirements (5), have led to a shift in emphasis away from the view that widespread malnutrition is the result primarily of protein deficiency, and toward the view that it is more closely associated with energy deficiency or more accurately, with food deficiency (12, 16, 19).

Despite this shift in emphasis generally, there have been remonstrations that the recent FAO/WHO Expert Committee (5) on protein and energy underestimated protein requirements, with the result that the protein problem has been eliminated only on paper (17). It is important initially, therefore, to assess the validity of the evidence that the current recommendations for safe intakes of protein are too low, and are likely to result in unsound proposals for solving international nutrition problems.

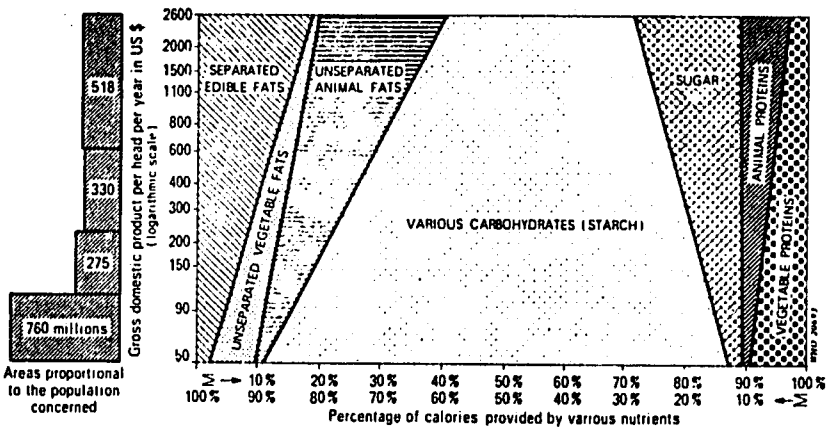
Protein requirements of infants up to one year of age are based on the amount of protein consumed by infants who are ingesting enough milk or formula diet to support a satisfactory rate of growth (5, 22). For children one year old, the average requirement value is 1 gm of protein/kg of body weight/day. This figure is increased by 30% to allow for individual variations in requirements in developing guidelines for safe intakes of high quality protein for public health and nutrition programs. The amount of protein recommended is further increased when lower quality proteins are used to meet protein needs. Requirements for older children have been estimated in large measure from knowledge of the protein requirement for maintenance and the amounts of protein that accumulate in the body during growth. These have been adjusted upward as for infants in order to establish safe intakes. As growth rates and milk intakes can be measured quite accurately, the allowances and safe intakes of protein for young children based on such measurements are considered to be highly reliable (19). They have been generally accepted for many years and appear not to be in dispute (19). In fact, the FAO/WHO recommended safe intake of protein for young children was lower in 1965 when the protein gap was being emphasized than it is now (19).

Interestingly, the basis for the criticism that the proposed protein requirements and allowances are too low is that the maintenance requirements of adults for the highest quality proteins have been underestimated (17). Three groups of investigators (2, 13, 17) have concluded that the FAO/WHO safe intake of high quality protein for adults is 15 to 20% below the amount required to maintain experimental subjects in nitrogen equilibrium when energy (caloric) intake is just at the maintenance level. They have also concluded that, in many of the experiments from which information used for establishing maintenance requirements for protein was obtained, the subjects consumed amounts of energy-yielding nutrients in excess of their needs.

Amino acids can be oxidized by the body to provide energy, and as energy needs have precedence over protein needs, energy intake can greatly influence protein utilization. If energy requirements are not met, amino acids are diverted into energy-yielding reactions and are not available for the synthesis of tissue proteins. A severe energy deficit can lead to protein deficiency in the face of what would otherwise be an adequate intake of protein. From this it follows that if energy intakes of experimental subjects used in nitrogen balance studies are inadequate, their protein requirements will be overestimated. On the other hand, if energy intakes are in excess of energy needs in such studies, retention of nitrogen may be enhanced to the point where protein requirements will be underestimated. The validity of the value obtained for protein requirement thus depends on the validity of the estimate of energy requirement. Since energy expenditure can fluctuate greatly from day to day and from individual to individual, it is difficult to estimate either energy or protein requirements with great precision. It should also be noted that the experiments from which it was concluded that adult protein requirements have been underestimated were done only with the recommended amounts of high quality proteins and not with the higher amounts recommended when requirements are met from mixed diets.

While it is important to extend our knowledge of protein-energy relationships in human nutrition, it is equally important to assess realistically whether underestimation of the adult requirement for high quality protein would have practical implications for nutrition programs. In typical diets, even in poor countries, protein provides 10% or more of the total food energy (calories) (Figure 1) so that adults who are eating enough food to meet their energy needs consume enough protein to meet their protein requirements, even from cereal-based diets.

CALORIES DERIVED FROM FATS, CARBOHYDRATES, PROTEINS AS PERCENTAGE OF TOTAL CALORIES ACCORDING TO THE INCOME OF THE COUNTRIES (1962) *



* Correlation based on 85 countries.
Source: Périssé, J., Sizaret, F. & François, P. (1969) *FAO Nutr. Newsletter*, 7, 3, p. 1.

This is borne out by the fact that protein-energy malnutrition is primarily a disease of the pre-school child and is rare among adults except under conditions of famine or food deprivation. Also, Arroyave (1) has recently reported on a retrospective analysis of experiments done at INCAP on both children and adults in which he used the proposed FAO/WHO scoring pattern as a standard to determine how closely the estimates of protein requirements obtained in those studies would have been predicted and how well they agreed with the recommened safe intakes. The predictions were surprisingly accurate, as shown for children in Table 1, and the values obtained did not exceed the FAO/WHO recommended safe intakes of protein for either children or adults.

Table 1

Average Nitrogen Retention of Children 1-2 Years of Age Fed Cow's Milk or Corn and Beans as Protein Source*

	Range of Protein Intake	Nitrogen Retention
	mg/kg body wt/day	mg/kg body wt/day
Cow's milk	0.5 - 0.7	- 28
	0.7 - 0.9	11
	0.9 - 1.2	33
Corn-bean mixture	0.8 - 1.0	18
	1.0 - 1.33	20
	1.33 - 1.55	52

*Based on Arroyave (1). Nitrogen retention of 20.7 mg/kg of body wt/day is adequate for children at this age. Recommended safe intake of milk protein is 1.27 gm/kg/day and for the corn-bean mixture was calculated to be 1.74 gm/kg/day.

Thus, even if the maintenance requirement of adults for high quality protein (5) may have been somewhat underestimated, and this remains in dispute (11, 19), there is no evidence to support the view that this would be a cause for concern in the planning of programs for the alleviation of malnutrition.

That widespread malnutrition is commonly associated with an inadequate intake of sources of energy has long been recognized (18, 21) and has been reemphasized in recent reviews of this subject (12, 19). In order to assess the relative significance of protein and energy deficits as underlying causes of widespread malnutrition, information about the protein and energy intakes of the most susceptible groups in relation to their needs is required.

Information in Table 2 from Gopalan (7) provides evidence that among poor children in South India, the deficit in consumption of energy sources exceeds the deficit in protein consumption.

Table 2

Protein and Energy Intakes of Poor South Indian Children

Age	Protein			Energy		
	Ave. Requirement*	Ave. Intake	% Deficit	Ave. Requirement*	Ave. Intake	% Deficit
yr.	gm/kg body wt/day			cal/kg body wt/day		
0.5-1.0	1.7	1.9	0	110	82	25
1-2	1.4	1.8	0	103	79	23
2-3	1.3	2.2	0	100	94	6
3-4	1.2	2.0	0	100	86	14
4-5	1.1	1.6	0	99	73	26

(After Gopalan (7). *FAO/WHO (5), assuming efficiency of utilization of dietary protein is 70% of that of high quality proteins.

Waterlow and Payne (19) have recently summarized information from several countries on protein and energy intakes of children 1 to 2 years of age (Table 3) which indicated that this is a fairly general phenomenon.

Table 3

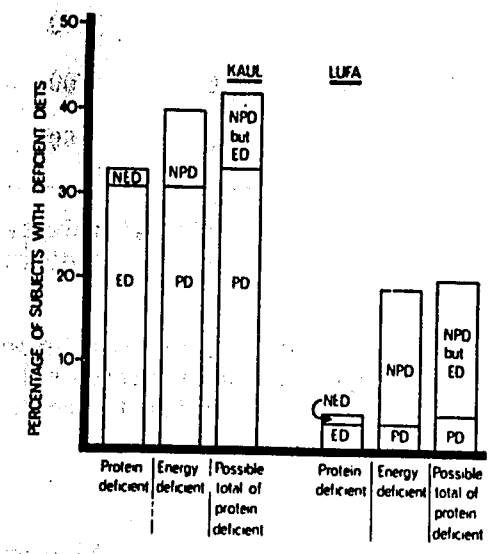
Protein and Energy Intakes of Children Aged 1-2 Years in Various Countries

	Protein*		Energy	
	Intake	Deficit	Intake	Deficit
	gm/kg/day	%	kcal/kg/day	%
Requirement	1.25		110	
Ghana	1.19	5	86	22
Guatemala	1.16	7	77	30
Jamaica	1.47	0	83	25
Polynesia	1.32	0	70	36

Thailand	0.61	51	52	53
Uganda	1.28	0	68	38

[a]Calculated as high quality protein assuming utilization of dietary protein is 60%.
(Adapted from Waterlow and Payne (19)

Results of studies of food intake among children in New Guinea (3) show that even with the low protein diets consumed there, protein deficiency occurs in conjunction with energy deficiency (Figure 2).



Percentages of subjects consuming diets deficient in energy or protein or both calculated by comparing intakes with requirements or safe levels of intake (FAO/WHO, 1973). Deficient: less than 80% of requirement or safe level; ED: energy deficient; NED: not energy deficient; PD: protein deficient; NPD: not protein deficient. (From Ferro-Luzzi et al., Am. J. Clin. Nutr. 28: 1443-1453, 1975)

Habicht and associates (10, 14) have demonstrated that reproductive performance of women consuming poor diets in Guatemala is improved if they are provided with a supplement of only energy as shown in Table 4. Inclusion of protein in the supplement did not result in further improvement. Provision of both energy and protein was required to improve the growth of young children consuming similar diets (10).

Table 4

Adequacy of Protein and Energy Intakes of Pregnant Women in Four Rural Guatemalan Villages

	Protein gm/day	Energy kcal/day
Requirement		
Maintenance	25.5	1273
Tissue synthesis	3.7	41
Activity	-	399
Allowance for 70% utilization	12.5	-
TOTAL	41.7	1713
Intake	44.8	1558
Intake/Requirement	107%	91%

(Adapted from Lechtig *et al*. (14).

Gopalan *et al*. (8) have demonstrated that a low-protein high-energy supplement will improve the growth of children consuming diets in which the deficit of protein is less than that of energy. Graham (9) had earlier shown with children in Peru that consumption of a wheat-noodle supplement was as effective in improving growth as the same supplement fortified with fish protein. Evidence that either amino acid fortification or protein supplements by themselves are of value in alleviating malnutrition is sparse (12). The quality of poor protein can be improved by amino acid fortification but improvement of protein quality does not represent improvement of the protein nutriture of children.

All in all, the accumulated evidence indicates that widespread malnutrition is associated with an inadequate intake of food sources of energy (calories) accompanied by varying degrees of inadequacy in the intake of protein and other nutrients. This is borne out by a recent analysis of reports of efforts to treat the disease with different types of nutrient supplements (15). However, the difficulty in demonstrating responses even to food supplements in field trials in areas where malnutrition is prevalent (10, 12) makesit clear that protein-energy malnutrition is not simply a nutritional deficiency disease. Its occurrence, nature and severity depend upon the pattern of infections of various types to which the child is exposed and, therefore, upon conditions of sanitation and

health care and various other environmental factors (21). The complexity of the underlying causes has been emphasized frequently enough (5, 6, 7, 12, 16, 19, 20, 21) to make clear that nutritional improvement of diets is only one of many measures that are required for its alleviation.

Proposals for food fortification, for the provision of food supplements and for the development of a more nutritious food supply may relieve some suffering if they are implemented wisely. However, unless they are implemented in conjunction with programs for total community and national development, they are unlikely to contribute toward a permanent solution for either malnutrition or the pending food and population imbalance. Even increased production of food, unless it is accompanied by programs that will lead to more equitable distribution of the available resources, will not solve these major problems. They are problems for which there is no simple solution. Although this seems painfully obvious, unless it is reiterated continuously, we run the risk of being distracted from the major problems by programs directed toward solving minor ones and of losing perspective about the place of proposals for the nutritional improvement of the food supply and increased food production in the development of programs to alleviate malnutrition and to bring food production into balance with population growth.

In my view, major emphasis in any attack on these problems should be on programs for agricultural development directed toward improving the food supply and conserving foreign exchange as the starting point for the type of national development that will be required if food and nutrition problems are to be solved. The prime need is for increased food production, so in plant breeding programs I would emphasize development of high-yielding varieties of staple crops with disease resistance and other characteristics required to ensure that the potential yield will be attained. Improvement of protein content or quality, I would consider very much secondary to this, particularly if it cannot be achieved without reducing the potential for total food production. Protein is critical primarily for the young child, so the emphasis I would place on protein production would be directed toward the development of varieties of crops needed for the production of weaning foods of high nutritional quality. If there is a place for amino acid fortification of low quality proteins or the development of protein supplements it is for this purpose and for improving the efficiency of animal production. Only in areas where extremely low protein root crops such as cassava are major staples does it seem to me that great emphasis on protein production for human use can be justified. Development of novel sources of protein such as leaf protein and microbial protein concentrates as commercial products has little merit, in my judgement, except where such products can be used as replacements for cereals and legumes in animal production so the staple crops can be conserved for human food.

Above all, it must be kept clearly in mind, that improvement in the quality of the food supply and even in the quantity of food available, will do little to relieve malnutrition or bring food production and population growth into balance unless such accomplishments are accompanied by, or are components of, broader programs directed toward total community and national development, programs that will improve the standard of living and alleviate poverty and the maldistribution of resources that underlie international food and nutrition problems.

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PRODUCTIVITY AND IMPROVED NUTRITIONAL VALUE IN BASIC FOOD CROPS

Ricardo Bressani

Introduction

During recent years, it has been suggested that low levels of protein and of some essential amino acids in diets are very important contributing causes of poor health and malnutrition in many communities of developing countries. This was particularly true for the most vulnerable groups. such as pregnant women and children. As a solution to such a problem, efforts were made to develop new protein sources to be used as supplements to the diets consumed as well as to improve the protein quality of basic food crops. It was expected that such efforts would result in benefits for the target population in terms of health and performance.

These efforts, however, have not given such expected results. The reasons are not at all clear, and one can only speculate. Among the reasons which can be offered are: 1) they physical environmental constraint; 2) the biological environmental factors; 3) disease and infection; 4) pattern of food intake; 5) physiological adaptation to increase nutrient intake; 6) length of trial, and possibly other still unrecognized factors which decrease the nutritional efficiency of a food as determined in the animal or human nutrition laboratories. When a mechanical instrument is first marketed, many problems develop and performance is not as expected. If such thing happens with a mechanical instrument, why is it expected that the same thing will not be encountered in a field nutrition intervention?

However, the possibility must also be recognized that present intake of foods is low, affecting primarily calorie requirements, and also that poor quality protein may be limiting food intake, decreasing then, both total calorie and protein intake.

Today, however, the general opinion among various nutritionists is that dietary protein or inferior protein quality is not a major cause of malnutrition and suggest that the most important need of the malnourished is for more of their traditional types of food. That is to say, while the patterns of these diets might, in some cases, need modification to correct a deficit of some particular vitamin or mineral, or indeed of their protein/energy balance, traditional diets are likely to provide the most economic and practical basis for meeting protein/energy needs (8) This last statement offers a good start for a review of the problem and for whether or not poor quality protein foods should be supplemented or cereal or legume grain protein quality should be improved.

It has been claimed that the need is for more of their traditional types of foods. The question is, what is considered as the traditional types of foods? Table 1 shows the results of dietary surveys in one town in Guatemala (14)

TABLE 1

FOOD INTAKE OF PRESCHOOL CHILDREN IN RURAL GUATEMALA

Food	Intake g/day	%
Tortilla 2/	103.5	31.9
Bread	19.5	6.0
Rice	16.1	4.9
Beans	47.9	14.7
Sugar	28.9	8.9
Meat broth	26.4	8.2
Beef	4.8	1.5
Egg	7.8	2.4
Vegetables	21.2	6.5
Fruits	29.4	9.1
Potato	4.3	1.3
Bean broth	12.5	3.8
Coffee	2.6	0.8
	324.9	100.0

1/ Archivos Latinoamer. Nutr. 24:221, 1974.
2/ Lime-treated corn.

The interesting points in this Table are that corn accounts the weight of the diet, followed by beans at 14.7%. Likewise, of significance is that the diet contains egg and other types of animal protein. There would not be any argument if it were possible to increase the intake of these traditional types of food, and much better if it were the most economic and practical means for meeting protein/energy needs. However, what would be the result if such small amounts of protective insurance foods were not consumed and the diet consisted only of corn and small amounts of beans? The answer is, a situation worse than at present. In view of present trends in the cost of animal protein this is reason enough to do everything possible in improve the quality of the basic foods: cereal and legume grains.

The Concept of Agricultural Productivity

The increased attention given in very recent years to the intensification of food crops and animal productivity, by governments, financial institutions and scientists, is the result of the analysis of the present and future food situation in the world. It is recognized that world population is rapidly increasing, particularly in developing countries; also that per capita food production is not keeping pace with population growth, the increasing purchasing power of people everywhere, particularly in developed countries, are demanding animal food products fed on grain and population pressures are diverting arable land to urban and industrial uses.

This is partially shown in Table 2.

TABLE 2

RICE CONSUMPTION, POPULATION AND ARABLE LAND, 25 MAJOR RICE CONSUMING AREAS, 1970[1/]

	Rice consump. per capita kg/year	Calories from rice %	Protein from rice %	Populat. (Mill)	Arable land ha/capita
South East Asia	112	57	48	256.3	0.170
East Asia	115	46	32	168.0	0.056
South Asia less India	142	67	55	121.3	0.270
Rice-China	118	57	45	230.8	0.258
Rice-China	108	53	41	491.7	0.157
Other Rice Dependent	113	48	40	15.8	1.027
Average/total	115	55	43	1283.9	0.186
Rest of the World	13	5	4	2439.0	0.482

[1/] Taken from: Brady, 1975.

The data refer to rice consumption, population and arable land in 25 major rice-consuming areas for 1970 (1). In these areas, particularly in the most populated, rice provided more than 2/3 of the calories and more than half of the protein intakes. Furthermore, the area of arable

land is as low as 0.056 ha/capita. However, expressing the significance of rice in such broad terms such as crude calories and total protein, is not enough to understand the full meaning of productivity. In order to meet the challenge of providing sufficient food to people, three major sets of biological processes are being maximized. The efficiency of the plant to utilize energy, carbon dioxide and water to produce desirable products; the more efficient use of soil nutrients and their supply and, finally, biological processes dealing with a more efficient control of disease and pests. These activities altogether will result in an increase in production, which is now called "Productivity."

The term productivity, however, should not be expressed in terms of increased production per unit area, or production or income per farmer, or number of people fed per farmer. The term should also include as shown in Table 3 the efficiency with which the products of agriculture, particularly food crops, can contribute to improving nutrition, and in order to do so, productivity should also be viewed as the efficiency with which the nutrients in the food meet best the needs of the population, particularly of those with greater nutrient requirements. This, in turn, can be translated into a more efficient use of the land and of other inputs in food production. Although it may be too much to ask, a further attribute to be considered in productivity is technological efficiency. This component takes into consideration such things as quality of conservation, acceptable characteristics for the consumer and from industry, as well as other desirable functional properties.

TABLE 3

BASIS FOR SELECTION OF FOOD CROPS

Productivity = Yield (kg/ha) X Nutritive value X Tecnological value

Nutritive value of the food itself or as component of a diet. Expressed as utilizable protein or in terms of percentage of protein-calories of total calories.

Tecnological value (includes all functional characteristics) expressed as percentage of standard.

One example which shows the effect of better land use through a more efficient utilization of the protein in cereal grains, is presented in Figure 1.

PROTEIN INTAKE FROM CORN FOR NITROGEN EQUILIBRIUM
IN HUMAN SUBJECTS

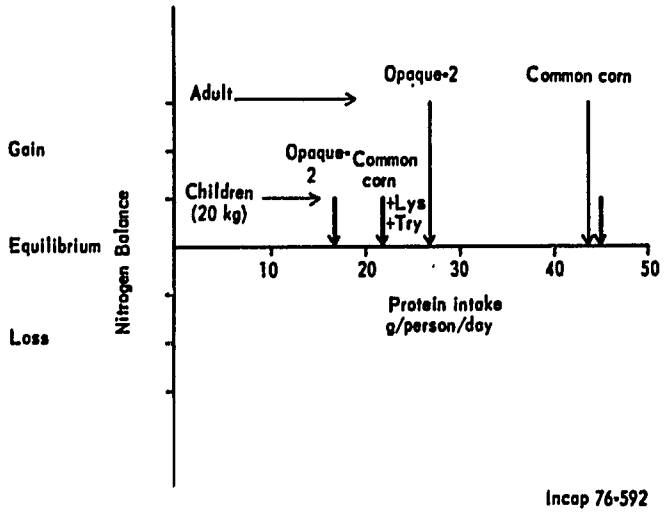

Figure 1

This Figure shows the amount of protein needed by a 20 kg child, and adult humans to be at nitrogen equilibrium when fed Opaque-2 corn, common corn supplemented with lysine and tryptophan, and common corn alone. The figures for children are: 16.9, 22.0 and 45.0 g protein per day. For adult human subjects, the corresponding value for Opaque-2 corn is 27.9 and 43.8 g for common corn. These data come from studies with human subjects (3), and were converted into the equivalent amounts of corn as shown in Table 4.

TABLE 4

AMOUNT OF CORN PROTEIN FOUND EXPERIMENTALLY TO BE NECESSARY FOR NITROGEN EQUILIBRIUM IN CHILDREN AND ADULT SUBJECTS

	Type of Corn		
	Opaque-2 corn	Common corn + Lys + Try	Common corn
		CHILDREN	
g protein/child/day	16.8	22.0	45.0
g corn/child/day	188	244	500
kg corn/child/year	69	89	182
ha/person/year	0.013	0.017	0.035
		ADULTS	
g protein/head/day	27.9	-	43.8
g corn/head/day	250	-	547
kg corn/head/year	91	-	200
ha/person/year	0.018	-	0.040

On the basis of a yield of 5000 kg/ha.

For children the protein needed for equilibrium, that is for no gains or losses, is equivalent to 65, 85 and 173 kg/yr, of Opaque-2 corn, amino acid supplemented corn and common corn, respectively. These amounts in terms of protein are lost through feces and urine since the figures represent the condition at protein equilibrium and are equivalent to 0.013, 0.017 and 0.035 ha per person per year. For adults, the results show the same trend, that is, lower land losses due to a more efficient utilization of the nutrients in Opaque-2 corn than those in common corn. This is also true even if one accepts a 10-15% less yield from the Opaque-2 corn. Therefore, productivity as expressed in production per unit area should also include the efficiency of utilization of the crop produced. Even with the 10-15% lower yield and with a slightly lower technological factor, Opaque-2 corn has a higher productivity than common corn.

TABLE 5

AMINO ACID INTAKE FROM CORN WHEN FED IN AMOUNTS TO MEET FAO/WHO PATTERN IN IDEAL PROTEIN

	Amino Acids in 0.55 g prot/kg of ideal protein	In corn protein		Daily intake of corn protein to satisfy AA req		Amino Acids in a daily protein intake of	
		Opaque-2	Common	g/kg		0.44 g-	0.59
		mg/g		Opaque-2	Common	Opaque-2	Common Corn
Isoleucine	9.9	31	37	0.32	0.27	13.6	21.8
Leucine	13.8	81	125	0.17	0.11	35.6	73.7
Lysine	12.1	42	27	0.29	0.45	18.5	15.9
T.S.A.A.	13.2	30	35	0.44	0.38	13.2	20.6
T.A.A.A.	13.8	80	87	0.17	0.16	35.2	51.3
Threonine	7.2	32	36	0.22	0.20	14.1	21.2
Tryptophan	3.6	12	6.1	0.30	0.59	5.3	3.6
Valine	9.9	48	27	0.21	0.21	21.1	28.3

There are other ways to analyze the problem. Table 5 shows a different approach. In this case, the amount of corn protein needed to meet the essential amino acids in 0.55 g/kg/day of ideal protein was calculated, and also for common corn and Opaque-2 corn (15). This calculation shows that for common corn 0.59 g of protein are needed to meet the tryptophan level of 3.6. For Opaque-2 corn, 0.44 g protein are needed to meet the total sulfur amino acid level of 13.2. With these two figures, calculations were made to determine the intake of all other amino acids. The figures are shown in the last two columns for both types of corn. Subtracting from these figures, the respective values in the ideal protein and adding up all differences, it is found that Opaque-2 corn gives an excess of 73.1 mg while common corn a value of 153 mg, twice as much. This, then, was interpreted to mean the inefficiency with which the protein in common corn is utilized, since all those amino acids would be completely wasted, having taken energy, space, capital investment and time to accumulate them in the kernel. The situation for children is even more dramatic, since from common corn 831 mg of amino acids would be lost, while only 231 mg from Opaque-2 corn. A similar case can be made for rice.

A difference must be made between physiological hunger, or the need to have a full stomach, and nutritional hunger, that is the need to provide balanced nutrition. This last expression indicates that a highly nutritious food intake is the one which is utilized by the animal or human organism with a minimum of waste, providing at the same time adequate amounts of the nutrients needed to meet the demands for the efficient physiological functions of the organism. These data then, indicate that better protein quality is important, not only with respect to the individual consuming such protein, but also in terms of the energy and other activities, including arable land that is used to produce the food crop. It is recognized, however, that total production is the first priority, and also that the situation is not as dramatic if one considers that cereal grains are consumed with other protein sources, such as legume grains, which are protein supplements to cereal grain protein. The effectiveness of this supplementary effect, however, depends on the essential amino acid content of the two foods.

Supplementary Effect Between Cereal and Legume Grains

Legume grain protein is a natural supplement to cereal grain protein, because the amino acids in one protein complement, to a certain degree, the amino acids in the second protein. Thus, maximum protein quality values between rice and beans are obtained when of the total protein, 80% is derived from rice and 20% from beans (6). In the case of corn and beans, maximum values result from mixtures in which each food provides 50% of the protein (7), as shown in Figure 2.

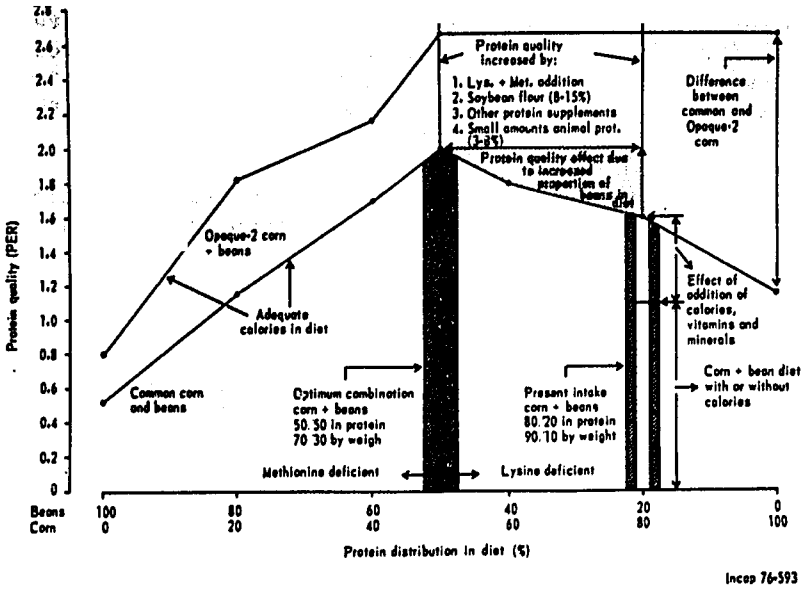

Figure 2

The Figure summarizes a very large number of biological studies using experimental animals, however, a few of the results have been confirmed in children experiments. The Figure shows the maximum protein quality value of combinations of common corn and beans and of Opaque-2 corn and beans. The value is the same for both situations at a 50/50 protein contribution, except that it is higher for Opaque-2 corn. Furthermore, the protein quality line for this corn does not decrease as its concentration increases as for common corn. These results were obtained using adequate amounts of calories in the diet. For common corn and beans, any combination to the left of the maximum point, that is, as more beans are present in the diet, is deficient mainly in methionine. To the right, of the maximum point, that is, as more corn is present in the diet, it becomes more and more deficient in lysine. The efficiency of utilization of the best mixture of corn and beans or of the present mixture, is improved by the kinds of supplements shown in the Figure, but, not by additional calories supplied as fat, at least in experimental animals.

Present intake ratio for adults and children in Guatemala for example is shown in the bar at the right, with a lower protein quality value, which increases to the maximum by having more beans in the diet. This is shown by the hatched areas between and above the two columns. Now, if increased intake of corn were to take place as suggested as a solution to the nutrition problem, and bean intake remains the same, it would mean a shift to the right of the present mixture and such a diet would be more deficient in lysine with a decrease in efficiency of utilization.

This, however, does not take place if intake of Opaque-2 corn increased, provided the grain replaced common corn. It should be indicated that the present ratio of 90/10 corn/beans has not changed for at least the last 20 years when there were less people than today with a greater production/capita. This may suggest that no more of the same two foods can be ingested, as well as that the present ratio represents the optimum caloric intake for the protein ingested.

Increasing common corn intake, besides increasing lysine deficiency, may also reduce the efficiency of utilization as shown in Figure 3.

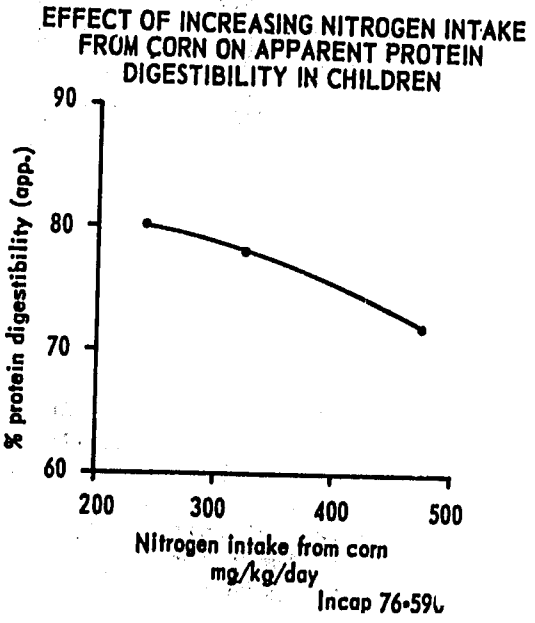

Figure 3

These are preliminary observations from nitrogen balance studies in children fed corn with and without amino acid supplements. The values plotted represent the feeding of corn protein alone. They show that as protein intake increased from 239 to 473 mg nitrogen per kg body weight per day, equivalent to 16 to 33 g corn/kg/day, apparent protein digestibility decreases from 80 to 72% (2).

This implies therefore, increased waste and lower efficiency of utilization. The same result could take place with beans which have different protein digestibilities, inherent or acquired. This will also have nutritional implications as well as an increase in waste, as shown in Table 6. In this case the values used were from nitrogen balance studies in children. The low digestibility in bean protein as compared to the highly digested proteins, implies greater losses of protein through feces which reduce the efficiency of land utilization as well.

TABLE 6

EFFICIENCY OF LAND UTILIZATION IN TERMS OF THE PROTEIN FROM BEANS (*Phaseolus vulgaris*)

	Protein digestibility 64%					Protein digestibility 84%				
Yield of beans/ha, kg	1000					1000				
Yield of protein/ha, kg	230					230				
Protein absorbed/ha, kg	147					193				
Protein waste/ha, kg	83					83				
Waste as beans/ha, kg	360					160				
% land poorly utilized	36					16				
	NI	FN	UN	NA	NR	NI	FN	UN	NA	NR
	mg/kg/day					mg/kg/day				
	227	81	109	146	37	227	36	109	191	82

There are other considerations which support the need for better quality protein in a situation as that described. During dietary surveys, total amounts consumed are measured but very seldom is the frequency of intake indicated. As was indicated, bean protein supplements cereal proteins, but in order to do so, they must be consumed together. Table 7 shows the frequency of bean intake in a village of Guatemala (12).

TABLE 7

FREQUENCY OF BEAN (*Phaseolus vulgaris*) INTAKE PER FAMILY

Frequency days/week	Number of families	Distribution %
2	10	12.7
3	20	25.3
4	36	45.5
5	10	12.7
6	3	3.8

Santa María Cauqué, Guatemala, 1972.
Average of 690 g *Phaseolus* served in 3 meals to a 6-member family.

García, B. INCAP. 1972.

As it can be seen, only a very small number of families consumed it daily. Therefore, the supplementary effect is reduced significantly if common corn is consumed, but not if Opaque-2 corn were consumed because of its superior quality. A situation not appreciated is the need to have the supplement daily, otherwise, results as those shown in Table 8 will be obtained.

TABLE 8

EFFECT OF FREQUENCY OF SUPPLEMENTATION OF A CORN/BEAN DIET*
WITH DIFFERENT LEVELS OF A PROTEIN SUPPLEMENT

Amount given, g	Frequency days	Basic diet consumed, g	Ave. Weight gain, g	PER
0	0	344	52.9 ± 2.3	1.72 ± 0.05
2	5	386	62.5 ± 2.7	1.72 ± 0.08
2	3	373	72.2 ± 3.4	1.94 ± 0.09
2	4	416	88.2 ± 3.7	2.00 ± 0.07
2	daily	435	113.2 ± 6.7	2.07 ± 0.09

*90/10, corn/beans by weight.
DeSouza, Elías, Bressani, 1970.

In this case, a fixed intake of a supplement given at intervals of from 0 to 5 days, resulted in lower weight gains and lower efficiency of protein utilization (11). It is of interest to point out that the supplement increased intake of the basic diet, which does not take place if the diet is deficient and more of it is provided. Although the evidence is not yet available, it has been indicated by people consuming corn, that they are able to consume more corn and beans if meat is also provided in the diet. Quality protein is, therefore, a sure way to increase intake, and such quality is achieved by the consumption of only small amounts of foods of high protein quality.

EFFECT OF ADDING AND REMOVING SUPPLEMENT TO CORN ON NITROGEN BALANCE

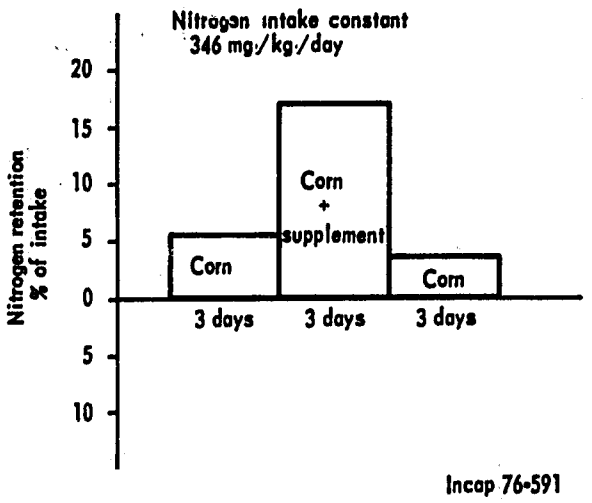

Figure 4

The effect of removing or adding a supplement on nitrogen balance is shown in Figure 4 to show that this is a situation which probably takes place when beans or any other supplement is not ingested together with corn. In the example shown, removal of the supplements lysine and tryptophan, in this case, (although milk, soybean or any other rich source in these amino acids would cause the same effect), reduced nitrogen balance (2). This reduction not only means a loss in efficiency of utilization, but also an increase in the risk of malnutrition, particularly if an infection or any other adverse factor, would intervene at this particular moment. The risk will be less if beans are present daily and much more if another good protein supplement, or improved protein quality cereal grain, is also present. To further indicate that protein quality is more effective than increasing energy intake, some recent results are shown in Table 9.

TABLE 9

EFFECT OF SUPPLEMENTING CORN/BEAN DIETS WITH DEFICIENT AMINO ACIDS OR CALORIES ON YOUNG GROWING SWINE PERFORMANCE

Treatment	Corn/Bean basal diet 87/13 kg	70/30 kg
None	2.35 ± 1.6	10.88 ± 1.9
+ Lys + Tryp + Met	4.25 ± 1.2	13.63 ± 1.9
+ Calories	6.00 ± 2.3	8.63 ± 3.6
A.A. + Cal + Min + Vit	35.00 ± 4.7	37.00 ± 6.5
Control	54.88 ± 3.6	

Experimental period: 8 weeks

In this study young pigs fed individually, had access to the diet all the time so as to simulate high availability of food. The diet in one case consisted in a mixture of 87/13 corn and beans, and in a second case, of 70/30. Each diet was supplemented with the same quantities of lysine, tryptophan and methionine in one treatment with calories in a second, with amino acids, calories, vitamins and minerals in a third. A fifth group of pigs were fed a diet meeting all nutrient requirements of this animal. Comparisons within treatments for the 87/13 diet indicated quality protein to be more effective than calories. Likewise, the 70/30 diet, without supplements, of a better protein quality than the 87/13, gave better performance. The two diets completely supplemented induced relatively good performance but significantly less than the positive control. Our interpretation is that quality protein is more essential than increased calories (10).

The Value of Increased Protein Content

Very little information is available on the possible nutritional benefits to be derived from a higher protein content in basic food grains. The data available suggests that as protein content increases protein quality decreases, however, the decrease in quality may not be as significant in practice, as higher protein content. There are two advantages to a higher protein concentration. One is that with less material, higher intakes of protein are possible, which is of importance if the food tends to be bulky. The second is that if the decrease in quality is not large enough, more total protein is utilized from a higher protein-containing food. To analyze the significance of protein content, some examples will be given. The first for corn in a mixed diet with beans, is shown in Table 10. In this case, rats were fed corn samples with 8.3 and 13.0% protein content, with and without the addition of lysine and tryptophan.

Table 10

EFFECT OF HIGHER PROTEIN CONTENT IN CEREAL GRAIN ON PROTEIN UTILIZATION IN MIXED DIETS*

Change in Basal Diet*	Protein in diet, %	Ave. Wt. gain, g	PER	Utilizable protein, %
Low protein corn (8.3% protein)	9.7	45	1.67	4.3
High protein corn (13.0% protein)	12.4	56	1.52	5.0
Low protein corn (8.3% protein) + Lys + Tryp	10.0	81	2.49	6.7
High protein corn (13.0% protein) + Lys + Tryp	12.6	119	2.45	8.3

*72.4% Corn + 8.1% Beans.

Weight performance was better for the high protein corn since they gained 56 and 119 g in comparison with 45 and 81 g for the low protein-containing corn, with and without amino acids added, respectively. The protein quality, however, was slightly lower for the high protein corn than for the low protein corn. However, utilizable protein, the product of quality times quantity, was superior for the high protein-containing corn with and without addition of amino acids. This shows, therefore, the benefits of high protein.

A second example is shown in Table 11 for rice (9).

TABLE 11

MEAN DAILY NITROGEN BALANCE OF ADULT HUMAN CONSUMING EQUAL AMOUNTS OF HIGH AND LOW PROTEIN RICE

Rice	Nitrogen balance, g/day			
	Intake	Urine	Fecal	Retained
High protein (2.44% N)	12.06	8.00	2.65	1.41
Low protein (1.33% N)	6.72	4.92	1.55	0.24

Amount consumed 480 g of each.

Clark et al., 1971.

In this case, human subjects were fed the same quantity of a high protei rice (BPI) and of low protein rice (BB). Subjects fed 480 g of BPI rice consumed 12.06 g of nitrogen and retained 1.41 g as compared to the same quantity of the BB variety supplying 6.72 g N and retained only 0.24 g. The higher retention from the BPI rice was obviously due to a higher intake of essential amino acids, even though the amounts on a mg/g N

basis were somewhat higher in the BB variety. These results, therefore, show that protein concentration has benefits, since from the same amount of a food, which becomes bulky when cooked, higher nitrogen retention is obtained. However, efficiency is less.

The high (14.3%), intermediate (7.32%) and low protein rice (5.67%) were evaluated using a multiple point protein assay technique using body N analysis. The results are shown in Table 12.

TABLE 12

PROTEIN QUALITY OF RICE WITH DIFFERENT PROTEIN CONTENT

Protein source	Protein content, %	Regression of carcass N and N intake	Utilizable protein, %
INTAN	5.68	Y = 0.91 + 0.49 X	2.8
IR 8	7.32	Y = 0.94 + 0.47 X	3.4
IR 8	9.70	Y = 0.96 + 0.36 X	3.5
Casein	-	Y = 0.89 + 0.53 X	-

Bressani et al., 1971.

It can be seen that the high protein rice had the lowest quality, however, in terms of utilizable protein, the higher protein rice was superior to the other two. This demonstrates the point that the decrease in quality is compensated by total protein (5).

In a recent publication (13) in which high protein wheat was assayed in children, the authors reported equal protein qualities for the high and the low protein-containing wheats, and concluded that the increased protein content of the two protein varieties listed offered significant advantages when equal quantities are consumed.

A more dramatic example is given by the situation which exists among populations subsisting on roots and legume foods. In this case, which is as extensive in terms of numbers of people as those of cereal/legume, both quantity and quality protein, are of high importance. An example of this is shown in Table 13.

TABLE 13

EFFECT OF ADDING METHIONINE ON THE NUTRITIVE VALUE OF VARIOUS CASSAVA/BEANS MIXTURES

Dietary treatment	% protein in diet	Weight changes g/28 days	PER	Cassava/ Bean Ratio
Cassava (100)*	1.9	-16.0	-	-
Cassava (87)* bean (13)*	4.3	-10.0	-	6.7
Cassava (87)* bean (13)* + Methionine**	4.1	- 7.0	-	6.7
Cassava (74)* bean (26)*	6.4	- 6.4	-	2.8
Cassava (74)* bean (26)* + Methionine**	6.7	+29.0	1.78	2.8
Cassava (61)* bean (39)*	8.8	+11.0	0.66	1.56
Cassava (61)* bean (39)* + Methionine**	9.0	+67.0	2.42	1.56

*Figures in parenthesis refer to amounts, in grams, in the diets.
**Amount added: 0.03 g of DL-Methionine per gram of bean protein.

These results show that on cassava alone, and on cassava and small amounts of supplemented or unsupplemented beans, the animal was not able to gain weight. With a high protein quality bean, 26% of this food was needed in combination with 74% of cassava to induce increased weight performance. Even 39% of common beans, of present quality, did not induce a significant weight increment.

The Need for Nutritional Standards and Objectives in Breeding

The evidence available indicates very clearly that nutritionally improved varieties of cereal grains and food legumes will have a significant impact on the nutritional condition of humans independent of whether calories or protein is in greatest need. Furthermore, higher protein quality will be a very valuable asset for future generations in the context of the entire problem of food production, land and people. The difference in opinion between the importance of calories and protein is really a relative one, since both are required and since people really want foods, and those should be the carriers of the nutrients needed. The critical point is whether or not, quality protein can be introduced into food crops as known and consumed today, and without interfering with other attributes, such as yield, appearance and technologically desirable characteristics. In order to do so, it is important to define and establish the desirable attributes and objectives, particularly in terms of nutrient content and technological characteristics together with yield. These attributes have not been defined or established and in this last section, some nutritional standards will be proposed based on the work carried out, indicating that it is essential to establish in the near future, the methodology which can provide better values. Essential amino acid patterns have been proposed as a reference (15). This ideal pattern, however, can be obtained from a single food or from a combination of foods or diet, which have proteins which complement each other. Which approach should be used? Both have advantages and disadvantages; however, the standard on a per food basis, probably the preferred approach, has the disadvantage of requiring greater efforts to achieve and to maintain other desirable characteristics at the same time. On the other hand, the approach on a diet basis is easier to achieve, with lower risks of interfering with other attributes and it also appears to be more practical.

TABLE 14

ESSENTIAL AMINO ACID PATTERNS

Amino Acid	From biological experiments. Mixture giving maximum protein quality*	Provisiona Amino Acid scoring pattern suggested level, mg/g N**
Lysine	335	340
Tryptophan	73	65
Total S.A.A	195	220
Threonine	243	250
Isoleucine	313	250
Leucine	508	440
Phenylalanine	324	380
Valine	337	310
Arginine	428	-
Histidine	162	-
Total E.A.A.	2919	2255

*Calculated from mixture giving maximum protein quality values.
**Source FAO/WHO, 1973.

Table 14 summarizes a reference essential amino acid pattern (4) established from studies which include: a) analytical studies of t indicated nutrient content; b) amino acid and protein supplementati studies in experimental animals and human subjects; c) feeding experiments of mixed diets with and without amino acid or protein supplements; d) feeding experiments of free choice of foods, and e) protein complementation studies. From these studies and the pattern, limiting nutrient levels have been calculated as shown in Table 15.

TABLE 15

PROPOSED NUTRIENT LEVELS IN VARIOUS CEREAL AND LEGUME GRAINS OF COMMON CONSUMPTION IN DEVELOPING COUNTRIES

Basic food	Protein %	Lysine g/16 gN	Tryptophan g/16 g N	Total sulfur amino acids g/16 g N
Maize	13-14	3.6 - 3.8	0.80 - 0.90	3.2 - 3.4
Sorghum	14-15	3.6 - 3.8	0.80 - 0.90	3.2 - 3.4
Rice	8-10	3.5 - 3.6	0.80 - 0.90	3.2 - 3.4
Beans*	28-30	6.3 - 6.4	1.10 - 1.20	2.3 - 2.4
Beans**	28-30	6.3 - 6.4	1.10 - 1.20	3.2 - 3.4
Cowpea*	28-30	6.3 - 6.4	1.10 - 1.20	2.3 - 2.4
Cowpea**	28-30	6.3 - 6.4	1.10 - 1.20	3.2 - 3.4
Pigeon pea*	28-30	6.3 - 6.4	1.10 - 1.20	3.2 - 3.4

*When supplemented to cereal grains.
**When supplemented to cassava - based diet.

As shown, it would be highly desirable to have a higher protein content in both cereal grains and legume foods. Likewise, lysine in cereal grains should, as a first approximation, be not higher than 3.8 g/100 g in cereal grain and about 6.4 g/100 g in legume foods. Tryptophan in cereal grains should be as high as 0.90 g/100 g and 1.20 g/100 g in legume foods. Finally, methionine or total sulfur amino acids should be between 2.4 and 3.4 g/100 g depending on where it is to be used.

As indicated, the values shown were calculated from a variety of feeding studies, using mixed diets. More precise values may be obtained by additional research and biological confirmation in which breeders and biochemist have better contacts. It is hoped that this type of approach to the problem in improving the protein quality of the main basic food crops will stimulate more research in this area and what is more important, applications of the results obtained.

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NUTRITIVE QUALITY IMPROVEMENT OF DIETS BASED ON CORN

R. Bressani[1/], J. J. Urrutia[2/], and L. G. Elias

The importance of cereal grains in the nutrition of millions of people around the world is well recognized. Because of their relatively high intake in developing countries, cereal grains cannot be recognized only as energy sources, since they provide significant amounts of protein as well. It is also well known that cereal grains are low in protein concentration and that the quality of their protein is limited by deficiencies of essential amino acids, mainly lysine. The above statements represent the reasons for the intensive research efforts being carried out to improve the protein quality of the cereal grains by genetic means or agronomic interventions. Extraordinary results have already been obtained, although other desirable attributes of the cereal crop, such as yield and physical characteristics, in some cases should also be improved. In the meantime, the need for increased cereal grain availability is overwhelming. Although increased productivity is being obtained, the quality of the protein remains the same. Because of well defined sociological trends in developing countries, more people will depend in future years on industrialized food products and it would appear, therefore, that a logical and efficient way to improve protein quality would be by supplementation procedures. This approach seems to represent the best method to increase agricultural production and to achieve improved nutrition as well.

The present report deals with the improvement of the protein quality of diets based on corn. The information in this paper has been divided into five sections, dealing first with the nutritional quality of typical diets consumed in some Latin American countries; followed by some basic information on the improvement of the protein quality of corn. The third section deals with the physical and nutritional characteristics of fortified tortillas, including nutritional stability under processing; the fourth section includes systems for the addition of the supplement. The last section summarizes the nutritional evaluation of diets containing the supplemented tortilla and Opaque-2 corn ending with prospects for future work and implementation of the research findings.

I. The Nutritional Quality of Typical Diets Consumed in Some Latin American Countries

The initial place to start the description of the work concerning the improvement of the nutritional quality of corn in the form of the traditional tortilla, consumed in various countries in Latin America, is a consideration of the type of diet ingested by rural people in these countries. Since the preschool children population is more affected by the lack of a balanced and ample nutrient intake, the diet to be discussed is that ordinarily consumed by this population group.

By means of dietary surveys, the data shown in Table 1 were obtained.

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TABLE 1

FOOD INTAKE OF PRESCHOOL CHILDREN IN RURAL GUATEMALA -

Food	Intake as consumed g/day	%
Tortillas	103.5	31.9
Bread	19.5	6.0
Rice	16.1	4.9
Beans	47.9	14.7
Sugar	28.9	8.9
Meat broth	26.4	8.2
Beef	4.8	1.5
Egg	7.8	2.4
Vegetables	21.2	6.5
Fruits	29.4	9.1
Potato	4.3	1.3
Bean broth	12.5	3.8
Coffee	2.6	0.8
Total	324.9	100.0

1/ Arch. Latinoamer. Nutr. 24:221, 1974.

2/ Lime-treated corn.

The data indicate the consumption of thirteen food items with cereal grains making up 42% of the total weight, starchy type foods 19%, beans about 15%, vegetables around 6.5%, and animal food products about 4%. Since the data represent average values from a relatively large group of children, it is obvious that some probably do not get any animal protein source at all, and those who might consume some probably do so at the most two or three times per week. Therefore, the picture for most of the children is quite poor. Of the total intake of 325 g, 32% comes from corn, this food being ingested in the largest amount, followed by beans on an item basis (10).

Food composites of this diet were analyzed with the results shown in Table 2.

TABLE 2

CHEMICAL COMPOSITION AND PROTEIN QUALITY OF RURAL PRESCHOOL CHILDREN DIET 1/

	As consumed g/day	%
Moisture	210.2	64.7
Dry matter	114.7	35.3
Protein	14.0	4.3
Fat	2.9	0.9
Crude fiber	2.9	0.8
Ash	2.6	0.8
Energy (kcal)	455	140 2/
PER	1.58	

1/ Arch. Latinoamer. Nutr. 24:221, 1974
2/ kcal/100 g.

The total intake of 325 g is made up of 210 g of water equivalent to 64.7% and 115 g of dry matter, equivalent to 35% of the total intake. The dry matter provides 14 g of protein, 2.9 g of fat, 2.6 g of minerals and 455 kcal of energy. The protein content of the diet was 4.3% with 0.9% fat and 140 kcal/100 g. On the basis of the protein and calorie content of corn and beans as consumed, calculations indicate that lime-treated corn provides 31% of the total protein and 45% of the energy, while beans supply 24% of the protein and 12% of the energy. Therefore, corn constitutes the main food item in terms of protein and calorie contribution to the whole diet.

For children, less than five years of age, the protein ingested from the diet represents about 82% of the gross protein and 76% of the energy needs, therefore, both nutrients are present in deficient amounts. The protein quality of the diet as determined by the PER method is 1.58, which is equivalent to a biological value of 48%. Therefore, the amount of effective protein is significantly less than the 82% adequacy on a gross protein intake basis. This information permits the conclusion, therefore, that corn is the most important food, that the diet is deficient in total protein content, of a low quality, and that it is also low in energy content.

The nutritional importance of other nutrients in the diet is also of interest. Rather than showing contents in the diet, results are presented in Table 3, showing the biological effect of the addition of groups of nutrients, to a corn-beans diet used for experimental convenience, although it may be argued that other food items may provide some of these nutrients (6).

TABLE 3
LIMITING NUTRITIONAL FACTORS IN BASIC CORN-BEAN PRESCHOOL CHILDREN DIET[1/]

Nutrients added	Average diet intake, g/28 days	Average weight gain, g/28 days	PER
None	271 ± 8.8[2/]	26 ± 2.3[2/]	1.09 ± 0.07[2/]
+ Vitamins	367 ± 18.7	49 ± 4.0	1.52 ± 0.06
+ Minerals	388 ± 15.8	65 ± 4.3	1.91 ± 0.06
+ Calories		23 ± 1.23	0.95 ± 0.05
+ Lys + Tryp	266 ± 14.4	26 ± 2.5	1.10 ± 0.08
+ Vit + Min + Lys + Tryp	484 ± 14.7	107 ± 4.9	2.55 ± 0.06

1/ Basic diet: 72.4% corn + 81% beans
2/ Standard error.
In: Nutritional Improvement of Maize, 1972.

The results shown indicate that vitamin additions, as well as mineral additions alone, tend to improve the quality as indicated by an increasing food intake which, in turn, results in better weight gain and protein quality as determined by PER. Of interest is the fact that the addition of the two amino acids lysine and tryptophan, previously determined as limiting the protein quality of the diet, added without the vitamins and minerals, did not improve food intake, weight gain, or protein quality. Furthermore, the addition of calories as oil did not induce any favorable change with respect to the control. However, when all items were added together, there was a significant improvement in all the measurements made.

This information then, suggests that the diet is deficient in vitamins and minerals, which are needed if the full benefit of improving protein quality is to be obtained. However, amino acid supplementation, although improving quality of protein does not increase protein concentration, which in our opinion is also necessary. In this respect, it is of interest to examine the results in Table 4.

TABLE 4

EFFECT OF INCREASING PROTEIN INTAKE FROM BASIC CORN-BEANS DIET[1/] ON NITROGEN BALANCE

Protein intake	Nitrogen retention g/kg/4 days	Ave. Wt. gain, g/4 days
3	-1.02	-101
4	-0.02	- 7
5	+0.96	13

1/ Diet: 82.6% corn + 13.2% beans + other ingredients.

In: Arch. Latinoamer. Nutr. 24:221, 1974.

In these studies protein intake was increased from 3 to 5 g/kg/day in experimental animals (10). The intake of 3 g/kg/day in the experimental animals used is equivalent to a protein and energy adequacy of 82 and 76% in children, respectively. The effect of increasing protein intake was measured by the nitrogen balance method and weight changes were also obtained. It can be seen that increasing protein intake resulted in an increase in nitrogen retention from a negative value of -1.02 to a positive one, of 0.96. Weight gain also increased. These data indicate that a higher intake of the diet would have a positive effect on growth and nitrogen retention, even with a poor efficiency of utilization. Children, of course, cannot voluntarily increase their protein intake as carried out for experimental animals, because of bulk and because of the amounts of food available at home. As indicated before, an alternative means of achieving higher intake of protein would be to increase protein concentration of the diet.

So far the limitations of the diet in terms of protein content and quality have been discussed as well as of other nutrients. As indicated, calories are also deficient. Some experimental evidence shown in Figure 1 demonstrates the effect of energy addition to the diet. These data were obtained in experimental animals using nitrogen balance techniques (10). The results show that increasing energy intake 25% at two levels of protein intake, of 3 and 4 g/kg/day, without or with lysine and tryptophan added, results in higher nitrogen retention values at either level.

EFFECT OF ADDING LYSINE AND TRYPTOPHAN TO A MAIZE-BLACK BEAN DIET WITH AND WITHOUT ADDITIONAL ENERGY

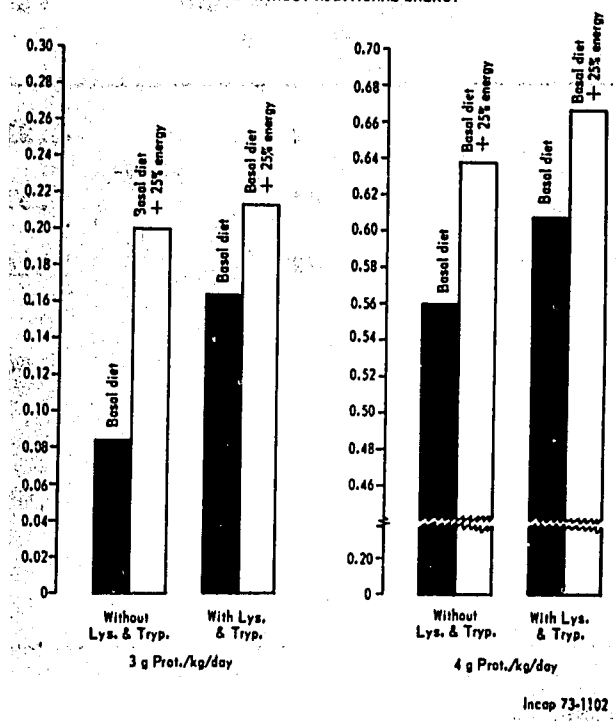


FIGURE 1

This evidence indicates, therefore, that the quality of the diets can also be improved when the energy density of the diet is increased, provided total intake is increased. The problem then is to develop a supplement which would increase total protein concentration, have a better protein quality, additional vitamins and minerals, and increased energy content. All these, of course, should be done without altering significantly the physical or organoleptic quality of the main item in the diet, corn.

II. Basic Nutritional Studies on the Improvement of the Protein Quality of Corn

There are many studies published on the subject of improving the protein quality of corn proteins. And it is well-known that corn protein is deficient in the essential amino acids, lysine and tryptophan. In order to follow our approach to the problem, summarized information will be presented on the limiting factors for improved protein quality of corn, within the context that it represents the main food component of the diet.

A summary of various studies carried out with lime-treated corn in experimental animals are shown in Table 5.

SUPPLEMENTATION OF LIME-TREATED CORN WITH AMINO ACIDS

Amino Acid added, % 1/	Average Weight gain, g	PER
None	32	1.21
Lysine (0.21%)	41	1.51
Tryptophan (0.10%)	22	1.15
Lysine (0.31%) + Tryp (0.05%)	100	2.66
Lys (0.31%) + Tryp (0.05%) + Threo (0.20%) + Ileu (0.20%) + Met	112	2.69

1/ L-Lysine HCl, DL-Tryp, DL-Ileu, DL-Threo, DL-Met.

Arch. Latinoamer. Nutr. 18:123, 1968

The evidence indicates a limited improvement from lysine addition alone, and no effect from the single addition of tryptophan. However, when both amino acids are added together, there is a significant increase in weight gain and protein quality. Other amino acids added together with lysine and tryptophan do not improve performance any further (3). Evidence of the same nature has also been obtained in children (1). Even though the addition of the two amino acids improved the protein quality of corn proteins, total protein concentration remains the same. Since the diet consumed by children, as shown in previous results, is low in total protein, it was felt that a more complete improvement would be one which included in one intervention both higher protein content and higher protein quality. Therefore, studies were carried out to determine the optimum levels in terms of protein quality of the protein supplements added to corn. Some of these results are shown in Table 6.

TABLE 6

RECOMMENDED LEVELS OF PROTEIN CONCENTRATES TO SUPPLEMENT LIME-TREATED CORN

Protein concentrate	Level %	PER
None	–	1.00
Casein	4.0	2.24
Fish protein concentrate	2.5	2.44
Soybean protein isolate	5.0	2.30
Soybean flour	8.0	2.25
Torula yeast	2.5	1.97

J. Agr. Food Chem. 6:517, 1963.

All proteins added increased the protein quality of corn, because they are good sources of the two limiting amino acids in corn proteins (4). Furthermore, the protein content is also increased in the mixture from about 2 to 4 g. By means of this approach, it was felt that the basic corn-bean diet consumed by the child population would be increased in terms of total protein as well as in protein quality.

More recent considerations suggest that besides higher protein content and quality, it would be desirable to increase energy content as well. An approach was attempted, which so far is giving good results. This approach consists in the addition of whole soybeans to corn, which would provide protein content, protein quality, and energy as well because of the oil in soybeans (5). The results of various studies indicated that soybeans could be cooked together with corn by the traditional way in which corn is processed in some Latin American countries. Some representative nutritional results of this approach are shown in Table 7.

TABLE 7

PROTEIN QUALITY OF LIME-TREATED CORN SUPPLEMENTED WITH LIME-COOKED WHOLE SOYBEANS

	PER	Utilizable protein, %
Lime-treated corn 1/,2/	0.95	2.1
85% lime-treated corn + 15% lime-treated whole soybeans 1/	1.98	7.1
92% lime-treated corn + 8% soybean flour 1/	1.98	6.6
85% lime-treated corn + 15% lime-treated whole soybeans 3/	1.98	7.1
Casein 1/	2.60	9.4

1/ 5% refined soybean oil added.
2/ Diet contained: 9.0% protein. All other diets had 12.5% protein.
3/ No oil added.
J. Food Sci. 39:577, 1974.

It can be seen that a 15% addition of whole soybeans is equal in its effects to 8% soybean flour, providing an equal or similar increase in protein content and quality. The data shown in the table also indicate that the additional energy provided by soybeans is of nutritional benefit as indicated by the results in the last line of the table. Therefore, it would appear that by using 15% whole soybeans, with 85% corn, a food is produced which is equal to the traditional tortilla but of much better nutritional value.

The results thus obtained were used to formulate the composition of the supplement to be used to improve the protein quality of corn protein as well as the nutritional value of the whole diet. The formulation is shown in Table 8. Besides the protein source, the complete supplement includes thiamine, riboflavin, niacin, iron, and vitamin A. All of these nutrients are deficient in the diets as consumed (6).

TABLE 8

FORMULATION OF LIME-TREATED CORN SUPPLEMENT 1/

Ingredient	Composition of the supplement	Content of 8% added to maize, g
Soya flour	97.5000	7.800000
L-Lysine HCl	1.5000	0.120000
Thiamine	0.0268	0.002196
Riboflavin	0.0162	0.001296
Niacinamide	0.1930	0.015440
Ferric Orthophosphate	0.6000	0.048000
Vitamin A -250-SD	0.0313	0.002504
Corn starch	0.1327	0.010616
Total	100.0000	8.000000

1/ Nutritional Improvement of Maize, 1972.

Table 9 summarizes the improvement in protein quality of corn supplemented with amino acids or protein and the values are compared to those from Opaque-2 corn. The results show Opaque-2 corn to be somewhat superior in quality to the supplemented corn and definitely superior to common corn.

TABLE 9

PROTEIN QUALITY OF SOYBEAN FLOUR SUPPLEMENTED TORTILLA IN RATS

Food	Protein %	PER	Utilizable protein, %
Lime-treated corn	7.9	1.26	2.7
Lime-treated corn + Lys + Tryp	8.0	2.78	6.0
Lime-treated corn + 8% soybean flour + 0.1% Lys	9.7	2.43	6.3
Casein	9.8	2.80	7.3
Opaque-2 corn	10.1	2.66	7.2

A batch of lime-treated corn supplemented with soybean and lysine was also tested in children using the nitrogen balance methodology (13). The results of the nutritional evaluation is shown in Table 10. At equal nitrogen intake the soybean supplemented corn gave nitrogen retention values expressed as percentage of the intake similar to those resulting from milk protein.

TABLE 10

PROTEIN QUALITY OF SOYBEAN FLOUR SUPPLEMENTED TORTILLA IN CHILDREN

	Nitrogen Balance			Nitrogen	
	Intake	Absorbed	Retained	Absorbed	Retained
	mg/kg/day			%	
Lime-treated corn	192	144	30	75	16
Lime-treated corn + 8% soybean flour + 0.1% Lys	197	154	63	78	32
Milk	195	157	75	80	38
Lime-treated corn (87%) + beans (13%)	207	150	36	72	17

III. Physical and Biological Characteristics of Tortillas with and without Supplements

The nutritional improvement of the supplemented corn is only one of the aspects in the solutions of the protein problem. From the chemical and biological points of view, the supplement increases protein content and also increases the content of limiting amino acids, which results in higher protein quality. Other added nutrients, such as vitamins, increase the overall nutritive value of the food. Physical and organoleptic characteristics of the supplemented food are also very important points to be taken into consideration, from the industrial and the consumer's points of view. The addition of synthetic amino acids is not expected to change those characteristics; however, the use of a protein supplement may have an effect on the rheological and organoleptic properties of the corn dough used in the preparation of tortillas. Unfortunately, only limited research has been carried out on the rheological characteristics of the corn dough itself, mainly because tortilla preparation is a homemade process with only a very small contribution from the industry, at least in some countries. It is expected, however, that as industrialization progresses, convenient corn-based products will have their place in the market of corn-eating countries, and those properties have to be studied. Table 11 summarizes the effect of adding corn masa, soybean flour, with different nitrogen solubility (7).

TABLE 11

IMPROVEMENT OF THE PROTEIN QUALITY OF LIME-TREATED CORN SUPPLEMENTED WITH SOYBEAN FLOURS OF DIFFERENT NITROGEN SOLUBILITY

Supplement	Nitrogen solubility of soybean flour, * %	% Protein in the diet	Weight gain g/28 days	Protein Efficiency Ratio
None	-	6.8	31	1.65
8% soybean flour - 1	99.7	9.6	78	2.20
8% soybean flour - 2	96.7	9.4	73	2.26
8% soybean flour - 3	82.3	9.3	89	2.50
15% whole soybeans**	-	11.7	106	2.46
8% soybean textured protein	-	10.7	96	2.31

* Nitrogen solubility in NaOH 0.02 N.
** Lime-treated with 85% corn.

The results show the improvement obtained in the protein quality of corn masa and a tendency to obtain a better response with the soybean flour with less nitrogen solubility. It is also of interest to note that the best preparation, in terms of weight gain, was presented by the addition of whole soybeans. Acceptability trials using "The Rank Method" at laboratory level were carried out on various food preparations using the type of corn normally consumed in the Central American countries. Although no significant differences were found among the tortillas, those prepared from corn and whole soybeans were consistently ranked first. Water retention capacities of the supplemented and unsupplemented tortillas were also carried out, by weighing the different preparations over a period of three days (5, 7). The results obtained are shown in Figure 2 indicating that over a 72-hour period, at room temperature, water retention was similar for the different preparations. This is an important aspect from the consumers' points of view, since the tortillas consumed at breakfast are those prepared the previous day; in a sense, water holding characteristics are a measure of the texture stability of the product through a definite period of time.

WATER HOLDING CAPACITY OF TORTILLAS MADE FROM CORN AND CORN SUPPLEMENTED WITH VARIOUS TYPES OF SOYBEAN PROTEIN

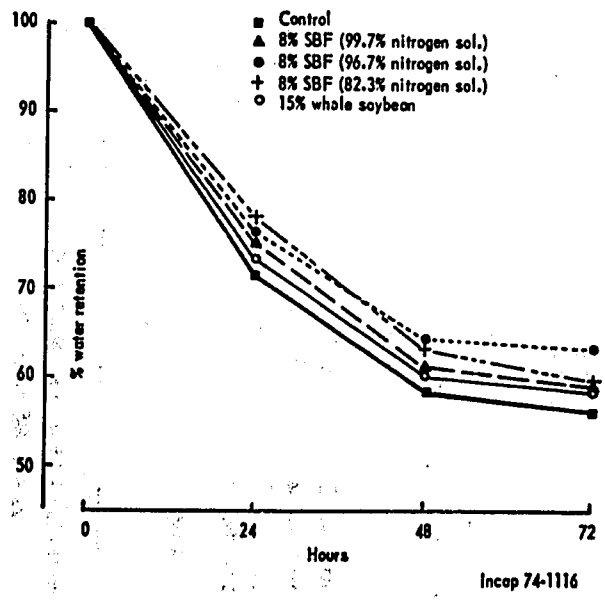


FIGURE 2

Other physical measurements were determined in the corn dough as well as in the corn dough supplemented with soy flours and whole soybeans. Table 12 shows the farinogram values obtained with the different preparations (7).

TABLE 12

FARINOGRAPHIC CHARACTERISTICS OF CORN DOUGH CONTAINING 8% SOYBEAN FLOUR

Supplement	Water absorption	Time required for the development of maximum dough stiffness (B), min	Resistance of the dough to break down (CD), min	pH
Control	136.0	14.0	8.5	7.05
8% soybean flour - 1	135.0	16.0	4.5	6.95
8% soybean flour - 2	136.0	16.5	6.0	6.95
8% soybean flour - 3	134.0	22.5	7.5	6.95
15% whole soybeans	131.0	23.5	stable	8.70

It may be observed that water absorption by the various preparations during mixing is very similar, although there is a slight tendency to decrease with the addition of soy flour, being more pronounced in the case of whole soybeans. Since water absorption is related in some way to dough consistency, the time required for the development of maximum dough stiffness is longer for the supplemented corn dough as compared to the corn dough alone. Again the corn-whole soybean sample took a longer time to reach the maximum stiffness. The data also shows that the stability or the resistance of the dough to break down is higher for unsupplemented corn as compared to the supplemented samples except in the case of the corn-whole soybeans sample. In summary, these results show that the addition of soybean tends to decrease the resistance of the corn dough to break down, probably due to the smaller amount of starch in these mixtures, in which corn proteins were replaced by soy proteins. It is known that soy globulins do not possess the same rheological properties as cereal proteins. The different behavior of the sample with whole soybean is probably due to the mild wet heat treatment given to it in comparison to the heat treatment generally applied in the preparation of some soy flours, which have been shown to have an adverse effect on the bread making potential.

These observations are confirmed on the amilogram values obtained on those samples (7), as shown in Table 13.

TABLE 13

AMILOGRAPHIC CHARACTERISTICS OF CORN DOUGH CONTAINING 8% SOYBEAN FLOUR

Supplement soybean flour 1/	Time for maximum viscosity, min	Temperature for maximum viscosity B.U.	Maximum viscosity B.U.	Viscosity at 94°C	Temperature for 500 B.U.
Control*	44.0	91.0	1030	996	81.2
No. 1	43.0	89.5	760	708	82.0
No. 2	43.0	89.5	860	770	83.5
No. 3	44.0	91.0	880	840	83.5
No. 4*	43.3	89.9	1130	1050	82.0

1/ Lime-treated corn samples contained 8% soybean flour of different nitrogen solubility except No. 4, which contained 15% whole soybeans.

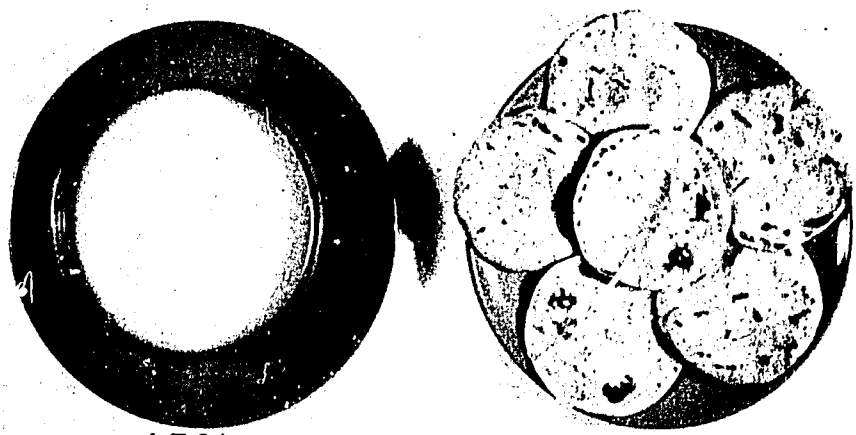
* Were subjected to an additional tension of 125 g.

Again it can be observed that maximum viscosities were obtained with the unsupplemented corn dough and the corn-whole soybeans dough. In the case of the latter sample it is possible that its more alkaline pH has influenced the higher viscosity since it has been demonstrated that there is an effect of pH on the gelatinization and breakdown of corn starch.

From the results obtained it can be said that the use of whole soybeans as a supplement to corn protein has not only nutritional advantages but also maintains the physical characteristics of the dough used in the preparation of tortillas.

Although the results obtained with the amilograph and the farinograph, showed some differences in the physical characteristics of the supplemented dough, it seems that they are not significant from the practical point of view, since the taste panel test, carried out with the tortillas prepared with the supplemented corn, showed no statistically significant differences as compared to the tortillas made with the normal corn. However, it is important to indicate that the data obtained suggest that the addition of higher levels of soy flour to corn flour could affect the consistency of the corn dough used to prepare the tortillas more drastically. With the present level of soy flour additions, it seems that the only possible practical problem is a small increase in the time required to reach the "normal consistency" usually obtained with the normal corn dough. The addition of soybean flour, furthermore, does not change the appearance of the tortilla, or its color, and it can be used to prepare other food preparations as shown in Figures 3 and 4. The first shows the common tortilla, while the second shows taco preparations and fried tortillas (7).

ALIMENTOS NATIVOS
PREPARADOS CON MEZCLA
MAIZ – SOYA



ATOL TORTILLAS

FIGURE 3

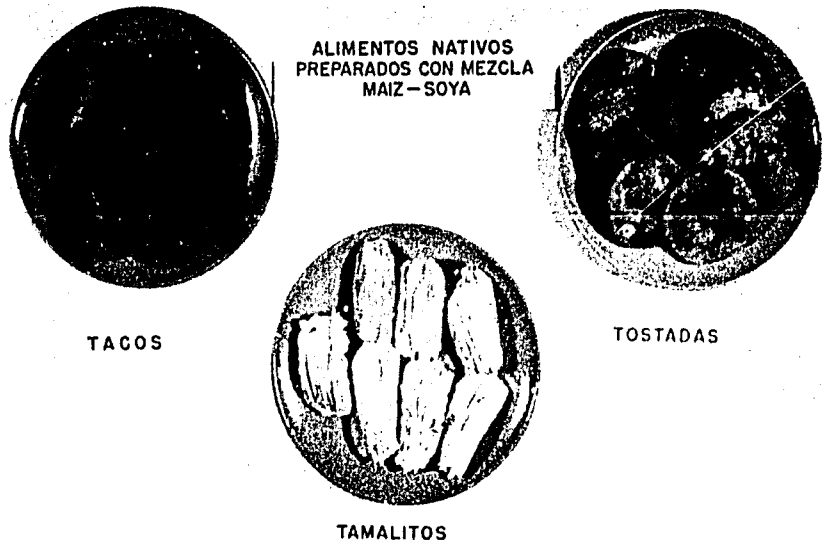


FIGURE 4

Stability trials

The preparation of tortillas uses a process involving wet and dry heat cooking. On the other hand, it is well-known that high temperature cooking decreases protein quality by amino acid inactivation. Since the "supplement" is added to the dried or wet corn flour, just before the dry heat step, it was important to find out the stability of the nutrients added in the final cooked product.

Table 14 shows the effect of processing on the chemical stability of amino acids added to corn dough and to tortillas baked for three and six minutes (6). The percentage of lysine and tryptophan recovery shown in the last column indicates that these amino acids were not significantly affected, even at the maximum time of heating (six minutes).

Table 15 shows the determination of lysine and tryptophan in the tortillas supplemented with Torula as the protein concentrate, and the protein concentrate plus lysine (6). The results again show that the amount of lysine from the Torula yeast, as well as from the Torula yeast plus lysine, were not significantly altered.

TABLE 14

EFFECT OF PROCESSING ON THE STABILITY OF AMINO ACID ADDED TO THE DIFFERENT PREPARATIONS

Preparation	Cooking time min	Content		Recovery	
		g/lys/g N	g /Tryp/ g N	Lys %	Tryp %
Masa	0	0.198	0.053	-	-
Tortilla	3	0.205	0.057	-	-
Tortilla	6	0.199	0.055	-	-
Masa + 0.30% Lys + 0.10% Tryp*	0	0.370	0.080	106.3	95.2
Tortilla + 0.30% Lys + 0.10% Tryp	3	0.359	0.080	103.2	94.0
Tortilla + 0.30% Lys + 0.10% Tryp	6	0.344	0.099	98.8	117.8

* L-Lys HCl; DL-Tryp.

TABLE 15

EFFECT OF PROCESSING ON THE STABILITY OF AMINO ACID ADDED TO THE DIFFERENT PREPARATIONS

Preparation	Cooking time	Content		Recovery	
		g/lys/g N	g/tryp/g N	Lys %	Tryp %
Masa + 3% Torula**	0	0.255	0.056	105.8	94.9
Masa + 3% Torula	3	0.259	0.055	107.5	93.2
Masa + 3% Torula	6	0.247	0.056	102.5	94.9
Masa + 3% Torula + 0.10% Lys*	0	0.302	0.057	96.5	96.6
Tortilla + 3% Torula + 0.10% Lys	3	0.319	0.058	101.9	98.3
Tortilla + 3% Torula + 0.10% Lys	6	0.228	0.064	72.8	108.5

* L-Lys HCl; DL-Tryp.
** 3 g of Torula = 0.118 g Lysine; 0.021 g Tryptophan.

The biological evidence which is summarized in Table 16 indicates that the availability of the nutritional components provided by the supplements added was not affected by the heat treatment applied (6).

TABLE 16

PROTEIN QUALITY OF THE CORN DOUGH AND TORTILLAS WITH VARIOUS SUPPLEMENTS

Diet	Treatment	Ave. Weight gain, g *	Protein Efficiency Ratio
Masa	-	25 ± 2.0**	1.28 ± 0.6**
Tortilla	-	23 ± 3.5	1.25 ± 0.5
Masa	3% Torula	52 ± 3.6	1.86 ± 0.8
Tortilla	3% Torula	47 ± 3.5	1.76 ± 0.6
Masa	3% Torula + 0.15% Lys***	64 ± 3.0	2.29 ± 0.7
Tortilla	3% Torula + 0.15% Lys***	64 ± 4.7	2.17 ± 0.5
Masa	0.30% Lys + 0.10% Tryp	59 ± 4.6	2.42 ± 1.0
Tortilla	0.30% Lys + 0.10% Tryp	61 ± 7.3	2.53 ± 1.0
Casein		101 ± 8.6	3.06 ± 0.8

* Initial weight: 47 g.
** Standard error.
*** L-Lys HCl; DL-Tryp.

This conclusion can be inferred from the weight gain and PER obtained with the different preparations. This is indeed a fortunate finding which is due to the characteristics of the process used to prepare the tortillas as shown in Table 17.

TABLE 17

CHARACTERISTICS OF THE COOKING PROCESS OF CORN MASA FOR THE PREPARATION OF TORTILLAS

Measures	
Temperature of the surface	150 - 200°C
Average time of cooking	3 - 4 min
Average weight of tortilla before the thermic treatment	55 grams
Average weight of tortilla after the thermic treatment	44 grams
Moisture content of tortilla before the thermic treatment	54.6%
Moisture content of tortilla after the thermic treatment	34.6%
Water lose during thermic treatment	20%

The well-known Maillard reaction responsible for the destruction or inactivation of the amino acids during the heat treatment requires nitrogen compounds and reducing groups as well as other factors such as temperature, time, moisture, and adequate pH. In this particular process the temperature is high (150-200°C), the cooking time is short, and the moisture content is high (54.6%). These conditions associated with the low content of free sugars in the corn dough (1.52%) explains in part the absence of this reaction during the preparation of tortillas (6).

IV. Systems for Addition

It is obvious that improvement of the nutritional value of tortillas can be achieved at laboratory level with the basic information already presented. However, the transference of this technology to a large scale is a real problem in part because tortilla preparation is almost entirely a homemade process with a very small but a growing participation from industry (9). It will be helpful to introduce at this point the method of preparing tortillas in Guatemala in order to discuss the possibilities of incorporating the supplement at the industrial and village level. The traditional (2) method is shown in Figure 5.

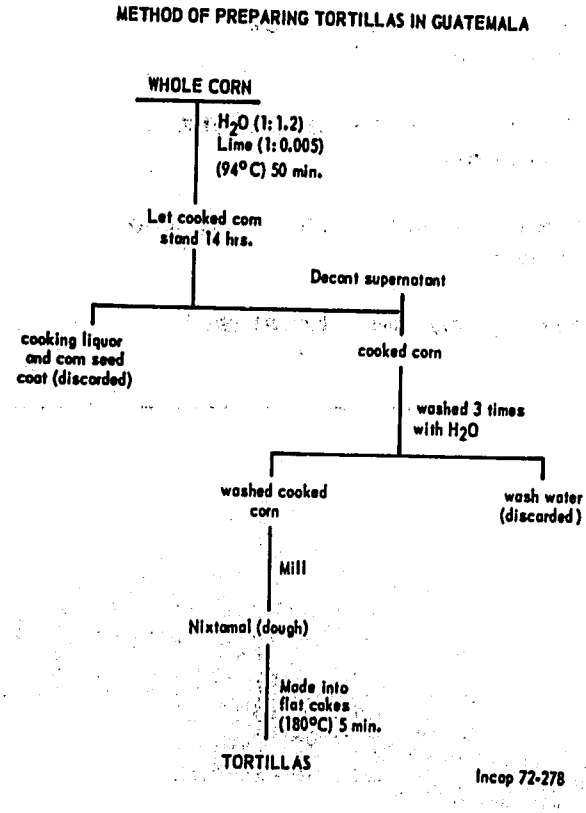


FIGURE 5

It consists in cooking the corn for one hour, using a calcium hydroxide solution. After cooking and cooling, the corn is washed with water, an operation which removes the seed coat and eliminates excess calcium hydroxide. The cooked maize is called nixtamal. It is then ground, an operation which was done by hand with a grinding stone. However, the grinding stone is no longer used; it has been replaced by a corn mill shown in Figure 6 which is distributed in many places, both in urban and rural areas.

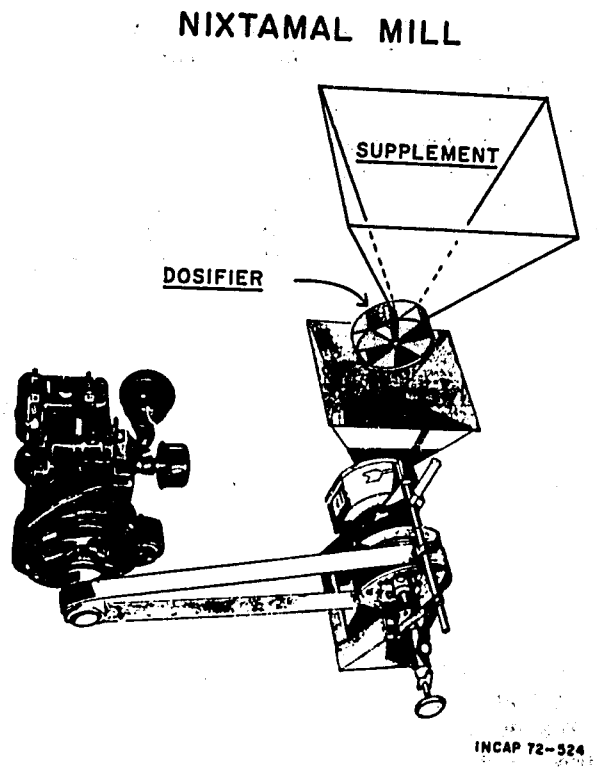


FIGURE 6

a. Industrial Level

The sequence of steps for the industrial preparation of precooked corn flours is basically the same as the one used at the home level when the alkaline treatment is included in the process. The main differences consist in the cooking time, which is around 1.5 hours, and the omission of the soaking step after cooking. After grinding, the nixtamal (dough) is dehydrated to give a precooked flour as a final product.

In this method of processing, the best stage to add the supplement is after grinding, which would include a mixing operation before packaging. Addition of the supplement before this stage would result in significant losses of nutrients because of the washing procedure used after cooking. Experimental data have shown that, for an efficient, homogenous distribution, addition of the supplement as a powder is better than in any other form.

b. Village Level

There are two possibilities of adding the supplement to corn in the rural areas - at home and at the mill used to grind the cooked corn (9, 11, 12). It is clear that the first procedure is very difficult since this mechanism will create numerous problems from the practical point of view. The second alternative is more feasible when carried out under controlled conditions, but still very difficult to be applied as a general procedure, due to the low educational level of the rural population. At the present time, the addition of 15% whole soybeans is being recommended.

c. Advantages and Disadvantages

The addition of the supplement either at the industrial or village level presents advantages and drawbacks. From the industrial point of view, the main advantage is the uniformity of the product and its constant availability in different convenient forms. However, the main problem consists in the fact that in developing countries food industries are generally located in the urban areas, the supplemented food will not reach the rural areas where undernutrition is more prevalent; of course, those problems can be solved either by locating the food industries in the rural area or by recommending adequate methods of transportation. However, the last alternative implies economic factors which are also important from the consumer's point of view.

V. Evaluation of the Supplemented Corn and of Opaque-2 Corn in Diets of Cereal Grain and Legume Foods

Results demonstrating the improvement of protein quality of lime-treated corn have already been shown in the preceding sections of this document. In this section, selected results will be shown to

indicate that the superior quality of supplemented corn and of Opaque-2 corn in comparison with common corn is also evident in mixed diets (8). Some biological results obtained are shown in Table 18.

TABLE 18

IMPROVEMENT OF THE PROTEIN QUALITY OF A CORN-BEAN DIET THROUGH AMINO ACID OR PROTEIN SUPPLEMENTATION

Supplement	Average wt. gain, g	PER	Utilizable protein, %
None	52 ± 4.4	1.75 ± 0.12	4.4
Corn + Lys + Tryp	80 ± 3.6	2.48 ± 0.06	6.3
Corn + 8% soy flour + 0.15% Lys	120 ± 5.4	2.50 ± 0.06	8.2
Corn + 8% skim milk + 0.10% Lys	111 ± 6.1	2.63 ± 0.07	7.7
Corn + 3% Torula + 0.10% Lys	85 ± 5.2	2.24 ± 0.08	6.2

In: Nutritional Improvement of Maize, 1972.

The data clearly indicate that the protein quality of the diet, in terms of protein efficiency and utilizable protein, is increased by various supplements added including that of the soybean flour. In all cases, small amounts of lysine were included with the protein supplement added because it was not able to meet all of the lysine needed in corn as shown by previous results (6). A second example is shown in Table 19.

Table 19

COMPARATIVE PROTEIN QUALITY OF CORN WITH AND WITHOUT SUPPLEMENTS AND OF OPAQUE-2 CORN

Protein	Protein %	PER	Relative Nutritive Value*, %	Utilizable Protein ** %
Corn***	7.9	1.26	33.7	2.66
Corn*** + 0.3% Lys + 0.1% Tryp	8.0	2.78	74.5	5.96
Corn*** + Soybean flour + 0.1% Lys	9.7	2.43	65.1	6.31
Opaque-2 corn***	10.1	2.66	71.2	7.19
Casein	9.8	2.80	75.0	7.35

* Relative nutritive value to casein
** Protein content x Relative Protein Quality
*** Corn masa.

In this case, the evaluation was done by nitrogen balance, which shows that the replacement of common corn by Opaque-2 corn induces a significant increase in nitrogen retention. This information demonstrates, therefore, that either Opaque-2 corn or protein supplemented corn improves the quality of simple diets based on cereal and beans. It is believed the same sort of improvement is expected in human populations, and reports to this effect will be presented elsewhere in this document.

Prospects

The evidence presented shows that a nutritional intervention such as the one described is of great value in improving the nutritional status of people consuming poor protein quality diets of low protein content. The problem, however, is how to achieve permanent acceptance for a

measure such as the one described, since it has economic implications which might not be possible for these low-income people and because they produce the corn they consume. If the lime-treated corn were industrially produced and consumed by all, not only by urban populations, the problem would have a simple solution. But, as it was indicated, this is not the case. There are no easy solutions to the above problem. However, one which we are beginning to develop consists in the use of whole soybeans together with corn. In this scheme, 85 parts of corn and 15 parts of whole soybeans are processed together as indicated (5). Material balance of this process averaged 85%. The 15% loss is accounted for by the losses of seed coats and poor quality kernels. Since soybeans are not normally grown in some of these countries, the success of this possibility would involve agricultural extension programs designed to teach soybean cultivation, as well as home economics type of education to show how to use this material together with corn. A small industry could be developed following a flow diagram as shown in Figure 7 which will permit not only the production of soybean fortified tortillas, but other food products of higher protein and energy content with good protein quality.

PROPOSED VILLAGE CORN-SOYBEAN PRODUCTS INDUSTRY

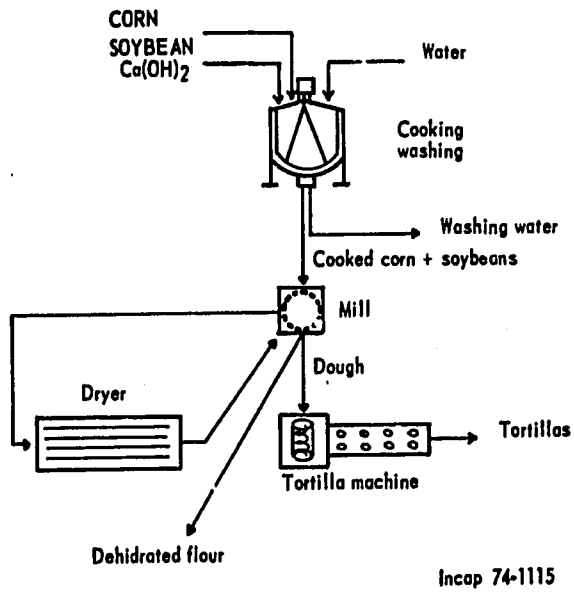


FIGURE 7

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THE RELATIVE IMPORTANCE OF PROTEIN AND ENERGY IN THE DIET OF INFANTS AND CHILDREN

George G. Graham, M.D.

Individuals responsible for the formulation of nutrition policy in the less developed countries have recently been placed in an extremely difficult position. They are being asked to abandon all concern for the quantity and quality of protein in the diet of their people and to concentrate on simply increasing the availability of the staple foods. The assumption is made that the needy, including infants and children, can and will increase their intakes of these foods, thus correcting the gross deficiencies of energy in their diets and automatically satisfying their previously "overestimated" protein requirements. It has even been suggested that the addition of sugar to these poor diets will increase the utilization of present protein intakes sufficiently to assure satisfactory growth and freedom from protein deficiency.

For some time there has been a legitimate concern over an exaggerated emphasis on protein as the major, if not only, deficient element in poor diets, neglecting the adverse effect on protein utilization of unmet energy needs. This concern was unfortunately translated into the frequently repeated statement that if energy needs were not fully satisfied, dietary protein would be used preferentially as a source of energy, a gross exaggeration of the facts. More recently the same concern has degenerated into strident denunciations of the "protein fiasco" which implies that there never was a protein problem and that all efforts directed at improving the protein content and quality of poor diets by breeding, fortification, or by complementary mixing of vegetable proteins, have been a waste of time, effort and resources. Plant breeders are now being told that all efforts must be directed at raising energy yields, disregarding protein content and quality. Occasionally the admission is made that diets based nearly exclusively on cassava and the sweet potato might just barely miss being adequate in protein if consumed in amounts sufficient to satisfy energy requirements.

The publication in 1973 (4) of "Recommended Energy and Protein Intakes" by an ad hoc Expert Committee of the FAO/WHO agencies has unfortunately seemded to legitimize these statements. This is a scholarly document of great value but seems to have been written more for the sophisticated scientist who is in a position to disagree with its assumptions than for the public health functionary who is already using its tables as if they had been handed down to Moses on Mt. Zion, and who has not bothered to read the text with its careful reasoning and qualifications.

Tables 7 and 25 of the above publication are more frequently quoted and are being used as absolute standards by which to judge the adequacy of local diets. The first is a listing of recommended <u>mean</u> energy intakes for males and females of all ages. It is derived from measured intakes of boys and girls in developed societies whose size corresponds to the 50th percentile of a well-known U.S. standard, all of which is made clear in the text. This also recognizes that the much small body size of children and adults in the developing world might call for a scaling down of the recommendations, which indeed is done for children over the age of 13 and for adults. For those 13 and under it insists that age and not size be used as the determinant of the appropriate requirement, on the very

questionable assumption that this will allow for "catch-up" growth. Even if we assume that it is possible for children to satisfy these very high energy "requirements" from their present dietary sources, there is much evidence to suggest that they would merely become obese. Most important is the dangerous assumption that there is something seriously wrong with being small and that it is desirable for all populations to achieve a body size comparable to ours. Dr. Gershoff has just shown us a remarkably healthy and happy population of children from Thailand who have nutritionally determined short stature and who could not be made to consume any more of their predominantly rice diet. When their energy intakes are stacked up against the recommendations in Table 7 of the FAO/WHO publication, they seem grossly inadequate, at the near-starvation level, and this is what is happening all over the world.

Shortly after the publication of the FAO/WHO recommendations, Durnin and associates (1) reported that adolescent boys and girls in Great Britain who on the average were taller and heavier than the 50th percentile of the same U.S. standard, were consuming 88 and 80%, respectively, of the energy recommended by that publication. Undoubtedly distressed by the implications and the use being given to this publication, WHO in 1975 convened another expert committee (5) to "clarify" its meaning. At one point this new committee recommends a correction for "height age," at another it recommends that true age be used until 19 years (again to allow for "catch-up"), and still further it urges that the 1973 recommendations be multiplied by 0.8, in deference to Durnin's very precise data. At this point our public health functionary must really be ready to throw up his arms in disgust and total confusion, that is, if he has seen this new report.

The "safe levels" of protein intake recommended in Table 25 of the 1973 publication are not means, but those estimated to meet the needs of 97.5% of the population. They were derived from estimated obligatory nitrogen losses and an allowance for growth in children, with 30% added for the "inefficiency" of utilization of whole egg protein by young adults found in the Berkeley and other studies. A further 30% was added for individual variation in requirements, thus presumably bringing the recommendations to a safe level. The reader has be be distrubed by the fact that the 30% "inefficiency" was an average figure, determined in one age group and with a single source of high quality protein. The extrapolation to growing children and to all proteins requires a great deal of faith. More recently the M.I.T. group has reported that for young adults to be "in balance" with the 0.57 g egg protein/kg/day originally derived from balance data and used in the FAO/WHO publication, their energy intakes had to be increased well above their previously determined needs, casting serious doubts on the adequacy of the protein intakes (3). Furthermore, they found that if such intakes were prolonged, there was a deterioration of body composition (7).

This same Table 25 (4) contains three columns of recommended "safe" protein intakes from diets whose protein quality is inferior to that of the rferenced proteins, egg, or milk. For diets with mixed animal and vegetable proteins they recommend the arbitrary use of a "score" of 80, for predominantly cereal diets a score of 70, and for predominantly root protein diets, one of 60. The text indicates that if the amino acid composition of the diet is known, this should be used for making the correction, ignoring the problem of digestibility, a matter of considerable concern to previous FAO/WHO committees. The text does state that if the relative net protein utilization of the diet has been determined in humans

of appropriate age groups, that this should be used. This would be ideal, as it takes into account both amino acid composition and digestibility, but such data are practically non-existent. As reliable data on amino acid composition of diets are also not readily available, those involved in the assessment of local or national diets are using the arbitrary corrections, most commonly that of 70. If the requirement of high quality protein has been under-estimated, as there is much reason to believe, and if the correction of 70 fails to take digestibility into account, which is the case, it is not at all surprising that false conclusions about the adequacy of protein in many diets are being reached. Another factor not considered in these recommendations is the probable metabolic penalty imposed by the consumption of marked excesses of other amino acids when the needs for each are met by consuming proteins limiting in one essential amino acid (9).

Because of these concerns, we have re-examined data obtained in the process of evaluating the quality of various dietary proteins, in the hope that we might throw some light on the question of dietary protein and energy needs.

Approximately 15 years ago, in convalescent malnourished infants, we compared at isoenergetic and isocaloric levels, the protein in a well-known modified cow milk formula with a vegetable protein mixture (cottonseed flour, quinoa and lima beans; protein score approximately added fat ans sugar, 85) and noodles made with 90% semolina and 10% fish protein concentration (score approximately 80), all providing 2.0 gm. protein and 150 kl/kg body weight/ day. Table 1 lists the number of studies with each, their duration, and the percentage of that time during which metabolic collections were made. Table 2 lists the rates of weight gain, the estimated percentages of nitrogen in the weight gains, and the changes in serum albumin, a sensitive indicator of body composition (8).

Table 1

Heightage (± S.D.), duration of diet periods, in thirteen convalescent malnourished infants receiving a modified cow milk formula.

Protein Source	No. of Periods	Ht. Age Months	Study Days	Balances % of Days
Similac	13	8.8±12	32±12	50±18
Vegetable*	7	8.5±5.3	31±9	40±13
Wheat-FPC	9	7.6±3.6	30±10	39±13
V or WF	16	8.1±4.3	30±9	39±12

*Cottonseed flour, lima beans, quinoa.

Table 2

Gains in body weight, nitrogen content of the gain, and changes in serum albumin of the infants' on diets.

Protein Source	Wt. g/kg/day	% N in Wt. Gain	Serum Albumin - g/dl Initial		Final
Similac (S)	6.35 ±1.94	2.07 ±0.55	3.45 ±0.74	0.47 ±0.46	3.92 0.52
Veg. Mix (V)	5.97 ±1.30	1.72 ±0.32	3.10 ±0.52	0.06 ±0.33	3.04 ±0.31
Wheat-FPC (WF)	5.36 ±1.32	2.23 ±1.00	3.09 ±0.86	-0.23 ±0.39	2.86 ±0.63
V, WF	5.63 ±1.30	2.01 ±0.80	3.10 ±0.72	-0.16 ±0.37	2.94 ±0.57
S vs V, WF	N.S.	N.S.	N.S.	0.01	0.01

Although rates of weight gain were only moderately lower, the nitrogen content of the weight gains was very similar, being much like that of normal infants in the first four months of life, when only 12% of weight gained represents protein, and over 40% represents fat stored in adipose tissue (2). The striking difference in the serum albumin levels suggests that although all threee diets resulted in striking gains in weight, primarily fat, the two non-milk diets were associated with significant deterioration in lean body mass. This occurred despite the fact that both energy and protein intakes exceeded the FAO/WHO recommendations for proteins of this quality.

Table 3

Stool wet weights and apparent nitrogen absorptions and retentions in 7 children, all of whom received diets as outlined in Table 1.

Protein Source	No. of 3-Day Balances	Stool Wet Wt. g/day	Nitrogen Balance Absorption % Intake	Retention % Intake
Similac	33	69.9 ±42.9	82.0 ± 6 5	45.8 ±10.6
Wheat-FPC	33	48.1 ±32.7	83.3 ± 6.8	38.0 ±11.8
Veg. Mix	28	135.8 ±78.8	70.5 ± 7.3	29.6 ±10.6

Table 3 illustrates the difficulties encountered when digestibility of both the protein and carbohydrate components are not taken into account. From the same studies as above, it shows a significantly greater stool weight and inferior nitrogen absorption from the vegetable mixture, indicative of inferior digestibility of both carbohydrate and protein. This is reflected in a much lower nitrogen retention, despite the superior amino acid score, when comparing with the wheat-FPC mixture.

Some years ago we carried out studies of the effect of different levels of lysine supplementation on the weight gain and apparent nitrogen retention of infants consuming wheat as the only source of protein. We used a high protein flour made by air classification of ordinary white flour. Stool weights were not different from those of the wheat-FPC studies, suggesting that the starch was equally well digested. Apparent absorption of nitrogen was moderately higher than from wheat-PFC. The different levels of lysine did not affect stool weight or nitrogen absorption but they had a progressively favorable effect on weight gain and apparent nitrogen retention. The retention from the highest level of supplementation did not reach the levels seen with the wheat-FPC, which had a significantly better amino acid score despite its slightly inferior digestibility. When a vegetable protein has a digestibility similar to that of milk or egg proteins, as in this case, then relative protein value can be predicted from amino acid score.

More recently we studied the nutritive value of three wheat varieties, both as whole wheat flour and as white flour of 70% extraction. Because the results with the three varieties did not differ among themselves, we have in Table 4 combined the results for the whole wheat flours in one line and for the white flours in the other.

Table 4

Stool wet weights and apparent nitrogen absorptions and retentions in 4 children receiving whole wheat flours and 4 children receiving 70% extraction white flours from the same wheats.

Protein Source	No. of 3-Day Balances	Stool Wet Wt. g/day	Nitrogen Balance Absorption & Intake	Nitrogen Balance Retention & Intake
Whole wheat	24	211.8 ± 43.4	77.1 ± 2.7	26.8 ± 5.5
White Flour	22	157.7 ± 72.6	83.2 ± 3.5	27.5 ± 5.2

Stool wet weights during the white flour periods were significantly much lower than during the whole wheat flour periods but were twice as high as during the previously described wheat-FPC and wheat-lysine studies, indicative of an important effect of processing on the digestibility of the starch component. Apparent nitrogen absorption from the white flour in these studies was equal to that from the wheat-FPC but significantly better than from whole wheat flour. On the basis of relative digestibilities it would be logical to expect superior nitrogen retentions from the white flours. That this did not occur is undoubtedly due to the higher lysine content and consequent amino acid score of the whole wheat flours. In these studies

it is apparent that both digestibility and amino acid score played equally important roles in determining relative protein value, which could not have been predicted from either alone.

Some years ago we attempted to carry out studies with white rice (variety unknown) commercially available in Peru. Table 5 summarizes our experiences with two children.

Table 5

Studies in two convalescent infants receiving cooked white rice as the only source of protein in the diet.

Case #	Age Mo.	Prot. & Kcal	On Days	Stool g/day	Nitrogen Balance Absorption & Intake	Nitrogen Balance Retention & Intake
041	5	2.0/150	1-3	122	55	27
			4-6	178	42	18
			7	266	28	-3
			11-12	258	24	-2
			1-3	113	48	12
			4-6	132	36	20
			7-9	116	46	25
			10-12	131	31	3
			1-2	167	33	-4
			3-4	199	24	-6
			5-6	183	35	-7
			7-8	155	39	0

In order to provide 2.0 g rice protein/kg body weight/day to the first infant, we had to feed him approximately 30 g rice/kg/day. Because of its great bulkiness, feeding was a very slow process. We were immediately struck by the pregressive increase in stool wet weight, indicative of very incomplete starch digestion. As soon as we had the results of the balance studies, indicative of a very poor and pregressively decreasing nitrogen absorption and retention, the studies were discontinued. The second child was much older and we have him only 1.0 g rice protein/kg/day on two different occasions. In the first study stool weights were only slightly elevated and did not increase with time, suggesting satisfactory digestion of the smaller amount of starch by a more mature digestive system. Apparent nitrogen absorption, however, turned out to be very poor and was associated with variable but generally poor retentions. In the second study stool weights were significantly higher and apparent nitrogen absorption even lower than in the first study, resulting in negative nitrogen balance and necessitating discontinuation of the study. Subsequent experience suggests that cooking time employed for the rice was far too short but nevertheless these studies are indicative of difficulties that might be encountered in actual practice.

Table 6

Studies in two convalescent children receiving adequately cooked white rice (variety unknown) with added amylase, and with or without added lysine, as the only source of dietary protein.

Case #	Age Mo.	Prot. & Kcal	On Days	Stool g/day	Nitrogen Balance Absorption & Intake	Nitrogen Balance Retention & Intake
288	19	1.65/100*	7-9	339	49	9
			10	343	61	23
	21	1.65/100	4-6	122	70	34
			7-9	152	65	25
	22	1.65/100*	4-6	122	67	33
			7-9	165	70	34
296	27	1.65/100*	4-6	40	75	36
			7-9	74	74	30
		1.65/100	4-6	38	72	30
			7-9	42	72	35

*Lysine added

Table 6 summarizes studies carried out much later, in which the cooking time of the rice was much longer and an amylase (Miles HT 440) was added to assure complete starch digestion. In the first child the very high stool weights of the first period were probably due to an intercurrent diarrheal infection. Subsequently, stool weights were more reasonable and apparent nitrogen absorption, though quite low when compared to that from white flour, was in an acceptable range. Apparent nitrogen retentions were satisfactory but the fact that lysine produced no supplementary effect suggests that these were limited more by digestibility than by the amino acid composition. Although the stool weights of the second child were much lower and suggestive of complete starch digestion, nitrogen absorptions and retentions were very similar to those of the second child, with again no apparent effect of lysine.

Studies very recently completed in our unit have demonstrated an important effect of the intake of high quality protein (casein) on the absorption of fat (soy and cottonseed oils) by infants. Thus there is a direct effect of protein intake on the efficiency of energy utilization.

Public health functionaries of developing countries who are responsible for nutrition policy must have an extremely difficult time in reconciling the recommendations made by august committees and the child feeding practices of the developed countries whose scientists make most of the recommendations. I can imagine their confusion on seeing a recent article authored by a member of the Protein-Energy Advisory Group of the United Nations (6). In making recommendations for the daily menu of children, he suggests: "Three or four cups a day of milk or its equivalent in other forms - yogurt, ice cream, cheese ... Two or more servings of meat, poultry, fish, egg, dried beans, or peats, and nuts ... One potato a day ... Three or more servings of whole grains and cereals, and products from them or enriched flours." In Table 7 I have calculated the protein intake from such a menu. Over 75% of the protein is from animal sources. I suspect that our public health functionaries might think that someone is ripping them off.

Table 7

Estimated daily intake of protein from diet recommended by Mayer (ref. 9).

Protein Source	Portion Size-Grams	Portion g Protein	No. of Portions	Grams Protein
Milk	244 (cup)	8.54	3	25.62
Meat	86	18.92	2	37.84
Potato	100 (1)	2.40	1	2.40
Oatmeal	238 (cup)	5.47	1	5.47
Rice	191 (cup)	14.51	1/3	4.83
Whole Wheat	23 (slice)	2.13	4	8.55
Daily Total*				84.71 gm

*If 4 cups milk used, total = 93.75 grams

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Closing Remarks

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I would just like to say that even though I missed the first two days of this Conference, I have observed enough during the final two days that I am very much impressed with the results that you are coming up with. The quality of your reports and recommendations is a reflection of the high quality of the participants, who are from among the most outstanding people around the world who are concerned with the issues at hand. The reports will be of great value to us in the Office of Agriculture, A.I.D., as we plan for the future, and I am confident that they will be of much value also to the Office of Nutrition and the Office of Food for Peace.

Throughout the various discussions, I have heard comments to the effect that the results of this Conference were for the Agency for International Development. As I just indicated, it will be of considerable value to us, but I believe it would be a mistake to consider it solely, or even primarily, a product for A.I.D. I say that for two reasons. First, the reason I was not here for the first two days of this meeting is that I was participating in annual budget reviews, and I believe it is correct to say that we cannot expect a large flood of new money for research projects, in the conventional contract research mode. We are faced with a tight budget situation this year, and while we have flexibility to implement a few new contract research activities in selected priority areas, we will not be able to finance very many of even the best ideas in your report.

Secondly, it seems to me that what you have outlined is a series of research programs, as opposed to more narrowly defined research projects, and that these research programs that are described will be of interest and value to a number of agencies and institutions. It seems to me it could be especially useful to some of the universities, for instance, who are looking for structured ideas for proposals under the programmatic research grant element of Title XII of the Foreign Assistance Act. The report contains a framework for research that may usefully be carried out by any number of scientists or institutions, no matter what the source of financing. In that sense, it is your report, not A.I.D.'s.

In conclusion, let me emphasize once again how very much we appreciate the time and hard work you all have put into this Conference. It is truly an outstanding contribution.

CLOSING REMARKS

Irwin Hornstein

The Conference program has been summarized, so there is nothing substantive for me to add. I admire the capacity and tenacity of the group, you have been very patient; either the meeting was extremely interesting or you had no where else to go! In either event, we are certainly glad you stayed through the week.

I would like to thank the Task Group Chairmen and the Task Group participants and that means all of you, for the excellent work that you have done in making this workshop successful. We should give Hugh Roberts a hand for the fine job he did in pulling this workshop together and to Harold Wilcke for the excellent summary and finally for the record, we have had excellent support from the Conference facility here. We appreciate their cooperation.

I believe the results of this workshop will have an effect on ongoing breeding and fortification programs as well as on programs to be initiated during the next several years. A similar meeting 3-5 years from now will provide a forum to assess at that time the impact on our recommendations not only on R&D, but on worldwide efforts to overcome malnutrition.

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