Nutritional evaluation of selected high-lysine grains
by Jane Ellen Trotter Stobart

A thesis submitted in partial fulfillment of the requirement for the degree of MASTER OF SCIENCE in Animal Science
Montana State University

Abstract:
Female weanling rats were used in five growth trials to compare the nutritive value of Bomi and Carlsberg II barleys and their Riso mutants 7, 8, 9, 13, 1508, 29, 56 and 86. Hiproly and Hiproly Normal barleys were used for comparison in all trials. Normal maize and high-lysine maize were also used for comparison in four of the trials. A casein control diet was included in each of the five trials to permit calculation of adjusted protein efficiency ratios (PER). Each barley and maize cultivar was also tested in one of five nitrogen balance trials for determination of biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU). All diets were isonitrogenous and isocaloric with equalized fiber contents within each trial. Bomi Riso mutant 1508 (Boz-1) produced the best feed efficiency ratio and highest PER and BV of all barleys tested. It also showed an 80% reduction in the lysine poor hordeins and a 49% increase in the lysine rich albumins + globulins resulting in a lysine content (g/16 g N) of 5.60%. This mutant produced a yield that was only about 75% of that of the parent barley, Bomi. Bomi Riso mutant 7 also showed a high PER value while being the best producing Bomi Riso mutant. Bomi Riso mutant 8 resulted in a BV that was not different (P>'.05) from that of Bomi Riso mutant 1508. The Boz-2 and Hunt Bomi Riso mutant 1508 barleys were believed to be contaminated with an unknown barley. As a result of this, the feed efficiency and PER values of the mutants were higher (P<.05) than those of the Bomi barleys but not with the same magnitude as shown between the Boz-1 Bomi and Bomi Riso mutant 1508 barleys. The Carlsberg Riso mutants showed some improvements in the amino acid composition and Osborne protein fractions in regard to reduced hordeins and in the amino acid lysine (g/16 g N) over that of Carlsberg II. This small change was reflected by a slight improvement in the biological measurements of the mutants. Carlsberg Riso mutant 29 was shown to be the best yielding of the Carlsberg II derived mutant barleys followed by Carlsberg Riso mutant 56 with Carlsberg Riso mutant 86 producing the lowest yields.

The eighteen amino acids included in the multiple regression analysis accounted for over 92% of the variance of each of the biological measurements. In all of the measurements except feed consumption and NPU, the majority of that variance was accounted for by the first five amino acids. Lysine was the primary amino acid responsible for the variance in gain, PER and BV. It was also an important amino acid in the values of feed efficiency and NPU. Lysine was highly correlated (P .01) with gain, feed efficiency, PER, BV and TPD. In all trials where high-lysine maize was included, it produced the best PER and feed efficiency ratio (P<.05) of all of the grain diets, but lower (P<.05) biological values than any of the Riso mutants.

STATEMENT OF PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication in this thesis for financial gain shall not be allowed without my written permission.

Signature Jane E. Trotter Stobart

Date April 29, 1977

NUTRITIONAL EVALUATION OF SELECTED HIGH-LYSINE GRAINS

by

JANE ELLEN TROTTER STOBART

A thesis submitted in partial fulfillment
of the requirement for the degree

of

MASTER OF SCIENCE

in

Animal Science

Approved:

C W Newman
Chairperson, Graduate Committee

J. L. Van Horn
Head, Major Department

Henry L Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

April, 1977

ACKNOWLEDGEMENTS

I would like to express my appreciation to my graduate professor, Dr. C. W. Newman, for his assistance and patience throughout my graduate program and the writing of this thesis.

I would also like to thank Professor R. F. Eslick and staff of the Montana State University Plant and Soil Science Department and Mr. D. E. Baldridge and staff of the Southern Agricultural Research Center, Huntley, Montana, for the production of the barley and maize varieties used in my research. Credit also goes to Dr. C. McGuire and his technicians for the physical characterization of the grains.

Mrs. Shirley Gerhardt, Dr. Nancy Roth and Mrs. Dana Leininger deserve a very special thank you for their assistance in chemical analysis, care of the rats and their moral support.

My appreciation and thanks are also extended to Dr. E. Smith and his associates for the time spent with the statistical analysis of the thesis data.

My gratitude also is extended to Dr. S. Chapman, Dr. L. L. Jackson, Dr. B. R. Moss and Dr. O. O. Thomas for their participation in the graduate committee and suggestions in the writing of this thesis.

I am indebted to Dr. L. J. Nygaard of Fort Collins, Colorado for his enthusiasm and encouragement which stimulated my interest in the field of animal nutrition.

I am dedicating this thesis to my parents and to my husband, Bob,

for without them and their support, this thesis would never have been realized.

TABLE OF CONTENTS

INDEX TO TABLES

TABLE

INDEX TO APPENDIX

ABSTRACT

Female weanling rats were used in five growth trials to compare the nutritive value of Bomi and Carlsberg II barleys and their Riso mutants 7, 8, 9, 13, 1508, 29, 56 and 86. Hiproly and Hiproly Normal barleys were used for comparison in all trials. Normal maize and high-lysine maize were also used for comparison in four of the trials. A casein control diet was included in each of the five trials to permit calculation of adjusted protein efficiency ratios (PER). Each barley and maize cultivar was also tested in one of five nitrogen balance trials for determination of biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU). All diets were isonitrogenous and isocaloric with equalized fiber contents within each trial. Bomi Riso mutant 1508 (Boz-1) produced the best feed efficiency ratio and highest PER and BV of all barleys tested. It also showed an 80% reduction in the lysine poor hordeins and a 49% increase in the lysine rich albumins + globulins resulting in a lysine content (g/16 g N) of 5.60%. This mutant produced a yield that was only about 75% of that of the parent barley, Bomi. Bomi Riso mutant 7 also showed a high PER value while being the best producing Bomi Riso mutant. Bomi Riso mutant 8 resulted in a BV that was not different ($P>.05$) from that of Bomi Riso mutant 1508. The Boz-2 and Hunt Bomi Riso mutant 1508 barleys were believed to be contaminated with an unknown barley. As a result of this, the feed efficiency and PER values of the mutants were higher ($P<.05$) than those of the Bomi barleys but not with the same magnitude as shown between the Boz-1 Bomi and Bomi Riso mutant 1508 barleys. The Carlsberg Riso mutants showed some improvements in the amino acid composition and Osborne protein fractions in regard to reduced hordeins and in the amino acid lysine (g/16 g N) over that of Carlsberg II. This small change was reflected by a slight improvement in the biological measurements of the mutants. Carlsberg Riso mutant 29 was shown to be the best yielding of the Carlsberg II derived mutant barleys followed by Carlsberg Riso mutant 56 with Carlsberg Riso mutant 86 producing the lowest yields. The eighteen amino acids included in the multiple regression analysis accounted for over 92% of the variance of each of the biological measurements. In all of the measurements except feed consumption and NPU, the majority of that variance was accounted for by the first five amino acids. Lysine was the primary amino acid responsible for the variance in gain, PER and BV. It was also an important amino acid in the values of feed efficiency and NPU. Lysine was highly correlated ($P .01$) with gain, feed efficiency, PER, BV and TPD. In all trials where high-lysine maize was included, it produced the best PER and feed efficiency ratio ($P<.05$) of all of the grain diets, but lower ($P<.05$) biological values than any of the Riso mutants.

INTRODUCTION

Agricultural production of cereal grains for human and livestock consumption is an international economic factor. Each year the number of agricultural producers and the area of land being farmed decreases while the world population steadily increases. Hence, extreme pressures are exerted on the producers to not only be more efficient in their yields but also to increase the quality of their yields.

Barley ranks fourth in world production of cereal grains preceeded by maize, rice and wheat. It is the major feed grain grown in Montana. Wheat and rice are currently utilized primarily in human diets, and with the trend in human population growth, a sizable portion of the world's maize production, previously fed to domestic farm animals, may be channeled more directly into the human food chain. This increases the need to produce other feed grains of high nutritional value. Barley appears to be the major candidate to fulfill this need, particularly in countries with semi-arid conditions, high altitudes or cool environments.

The environments in which barley can be grown are quite varied. It has been grown successfully in a wide range of climatic conditions and soil types and at more northernly latitudes and higher elevations than most other cereal grains.

The nutritional value of barley cultivars currently in commercial production varies with each individual in respect to protein quantity and quality. Lysine is the first limiting amino acid in barley, hence,

with an increase in the lysine content, an increased nutritional value of the grain is expected. Higher lysine content of the barley proteins may result from the presence of proteins that are absent in normal barley lines, or from a depression or increase in the content of a certain protein or group of proteins. In the latter case, some lysine rich proteins may be increased at the expense of some lysine poor proteins. These changes may be accomplished through the action of superior genes or mutations which alter the quantitative relationship among the reserve proteins.

The development of a strain or strains of barley which contains high quality protein along with desirable agronomic characteristics would be a tremendous boost to livestock industries that utilize barley and perhaps also have a positive influence on the world's food supply for a hungry population.

This thesis project was initiated to evaluate the nutritional value of high-lysine barley cultivars and their parent lines and the accepted superior lines of Hiproly and high-lysine hybrid maize.

LITERATURE REVIEW

Amino Acids and Proteins

Berzelius suggested the name "protein" from the Greek word proteios, meaning first or of primary importance (Scott et al. 1969). This name seems very appropriate when considering that proteins function in the body as enzymes, hormones and structural elements as well as having storage, transport, contractile and protective functions.

Proteins consist of a polymer of building-block molecules, known as amino acids, linked together by peptide bonds. There are twenty different amino acids commonly found in proteins, all of which contain at least one carboxyl group and one α-amino group. Proteins may vary in their chemical and physical properties due to the type, number and sequence of the amino acids of which they are composed. Proteins, upon hydrolysis, may yield only amino acids or they may yield amino acids and other organic or inorganic compounds. The former type are known as simple proteins while the latter are conjugated proteins (Lehninger, 1975). A portion of barley protein is glycoprotein in nature (Waldschmidt and Leitz, 1961) meaning it is a conjugated protein with a carbohydrate group as its prosthetic group (Harper, 1973).

Some amino acids can not be manufactured in adequate amounts by the body to fulfill its needs. These amino acids are designated the essential amino acids and must be supplied through the diet. The essential amino acids for the young rat (Rose et al. 1948) and most

other animals include lysine, methionine, phenylalanine, threonine, leucine, isoleucine, tryptophan, valine, arginine and histidine. If these amino acids are not found in adequate amounts in the diet, the maximum growth potential of the animal will not be achieved (Howe et al. 1965).

Amino acid availability to the animal is a consideration when evaluating a cereal grain. Amino acids are found in a complex form known as peptide chains rather than as free amino acids which are readily available to the animal. Hydrolysis of the peptide bonds must be accomplished by the digestive processes before any amino acids can be used in the body's metabolic processes. A certain proportion of the dietary proteins is not hydrolyzed to free amino acids for use by the body. This may be due to incomplete digestion, changes in the chemical composition due to processing of the feedstuff, amino acid antagonisms, amino acid imbalances, amino acid toxicities or inhibitors of digestive enzymes (Scott et al. 1969).

Nitrogen utilization is dependent upon the amino acid composition of the proteins, or, in other words, the low concentration of the first limiting amino acids. The limiting essential amino acids, or those found in deficient amounts in barley are lysine, methionine, threonine and tryptophan, in that order (Howe et al. 1965). Since lysine is the first limiting essential amino acid of barley, it is essentially the cause of any nitrogen wastage by the animal's body.

The National Academy of Science (1971) reports barley to contain 13.0% protein and .52% lysine on a dry matter basis. These figures refer to the average commercial barley variety currently in production.

Osborne (1895) described proteins as having four fractions based on solubility. They are the water soluble albumins, the globulins which are soluble in weak salt solution, the alcohol soluble prolamines (known as hordeins in barley) and the alkali soluble glutelins. The globulins of barley have been further classified into three fractions by ultracentrifugation (Danielson, 1949). They are the heavy gamma-globulins (MW 270,000), the intermediate beta-globulins (MW 113,000) and the lighter alpha-globulins (MW 24,000). Ingverson (1973b) suggests the existance of three high-lysine protein groups in the globulins of barley.

Expressed as nitrogen as a percent of protein, the albumins and globulins have higher lysine contents than the prolamines while the glutelins have intermediate lysine contents. The prolamines generally have high contents of proline and amide groups, hence the name, as well as glutamic acid, but low contents of lysine, methionine, threonine, histidine, valine and arginine.

Munck (1964a) divided the barley kernel into two segments, the outer aleuron and the inner endosperm. He found barley's multicellular aleuron to be nutritionally superior to the endosperm since the former contained higher levels of lysine, arginine, threonine and histidine as well as the important enzymes associated to the salt soluble globulins.

The endosperm was rich in the lysine poor hordeins and contained a stronger concentration of phenylalanine than the aleuron.

Protein quality, defined as the essential amino acid composition of the protein, is a critical parameter in the nutritional evaluation of cereal grains. Commercial barley varieties are primarily deficient in lysine and methionine and secondarily in threonine and tryptophan.

Generally speaking, as the percent of total protein in a cereal grain increases, the lysine content (g/16 g N) decreases (Eggum, 1973a). This trend may be explained by a decrease in the globulins and an increase in the lysine poor prolamines with the glutelins remaining constant at various protein levels (Munck, 1964b).

Methods of Protein Evaluation

Various methods of evaluating the nutritional value of a feedstuff from a protein standpoint have evolved over time. The methods may be chemical or biological. Most chemical methods are relatively easy to obtain rapidly since they are based on an amino acid analysis. Biological methods are more accurate, however they take longer to obtain.

Mitchell and Block (1946) developed a method known as chemical score for estimating protein utilization. This method is based on the principle that the utilization of the protein is solely dependent upon the limiting essential amino acids. It does not account for availability of the amino acids in relation to the animal's requirements.

The Essential Amino Acid Index (Oser, 1951) incorporates all of the

essential amino acids into the calculation. This method is an improvement over chemical score, however, it still exibits the same restrictions and problems as seen with chemical score.

Total Amino Acid Value (TAAV) was developed by Hansen and Eggum (1973) for estimation of protein from the amino acid composition. This method reduces the effects of imbalance, antagonism and availability of amino acids to the organism. TAAV is an equation which provides the closest agreement (highest correlation coefficient and lowest deviation) between the measured and calculated biological value. The multiple correlation coefficient thus provides an expression of the degree of agreement, which can be obtained between the measured and calculated biological value on the basis of the amino acid composition. Hansen and Eggum (1973) also performed a regression analysis without the non-essential amino acids and termed the value the Essential Amino Acid Value (EAAV). They found the non-essential amino acids to exert a small influence on the biological value of proteins. These researchers found a TAAV for barley of 71.95% and an EAAV of 72.24% as compared to a value of 73.50% obtained in biological experiments with rats.

Chapman et al. (1959) evaluated proteins of food with a value known as protein efficiency ratio (PER). This method also has its drawbacks since results vary with the level of protein in the diet and the food intake, and the assumption that weight increase is an index of protein synthesis is not necessarily valid (Eggum, 1969). Also, it has been

discovered that younger rats show higher PER values than older rats (Chapman et al. 1959) and female rats gave maximal PER values at lower protein levels as compared to males (Morrison, 1960).

Biological value (BV) is the percentage of digested and absorbed nitrogen which is retained (Mitchell, 1924). Mitchell and Bert (1954) found that for the growing albino rat, a linear relationship was expected between the ratio of fecal nitrogen to dry matter consumed and the level of dietary protein within a range of 0 to 20%. Biological value is regarded as being directly dependent upon amino acid balance. This assumes that all protein is completely hydrolyzed to free amino acids in the digestive tract and that these acids are absorbed by the organism (Hansen and Eggum, 1973). Biological value measurements are useful in giving relative values to an individual protein inasmuch as they give a percentage of the actually digested nitrogen that is utilized for both growth and maintenance.

High-Lysine Barley Cultivars

Hiproly, a high protein, high lysine cultivar, was found to be an exception to the generality of decreasing lysine accompanying an increase in protein. It was selected from the World Barley Collection by the dye-binding-capacity (DBC) technique (Hagberg and Karlson, 1969). Dye-binding-capacity indicates a high basic amino acid content. The acid dye, Acilane Orange G, combines at pH 2.6 with basic groups of amino acids from the cereal proteins in suspension. This method has

been recommended for use as a mass screening technique to recognize barley varieties with a high lysine content (Mossberg, 1969). Because this technique is not completely selective for basic amino acids, which include lysine, confirmative analysis is necessary. Chromatographic amino acid analysis and a Kjeldahl analysis will establish if the basic amino acid content is due to a change in the amino acid pattern or to an increase in total protein.

Hiproly (CI 3947) is an Ethiopian barley. Munck et al. (1970) describes it as being of an erectoid type with naked, slightly shriveled seeds and requiring a long photoperiod. A morphologically similar line of barley was discovered with Hiproly and has been designated Hiproly Normal (CI 4362). It is described as having similar habit to Hiproly but with longer, smoother seeds and an inferior nutritional value.

Hiproly contains up to 50% more protein and 30% more lysine, as a percent of total protein, than commercial varieties (Munck et al. 1969). The researchers postulated the increase in lysine content of Hiproly is due to either a single recessive gene or a complex of genetic factors while Ingverson et al. (1973c) describes one reason for the increase as a change in the control mechanism regulating the synthesis of b-proteins which are rich in the basic amino acids. Munck (1972), using a scanning electron microscope, found no apparent change in the morphology of protein bodies in Hiproly due to the high lysine gene when compared to

Hiproly Normal. He found the higher lysine content in the endosperm to depend on an almost doubled amount of water soluble proteins (albumins) and to be only slightly affected by a small reduction of the ethanol soluble proteins (hordeins).

Newman et al. (1974) found the nutritional value of Hiproly fed to rats to be similar to that of normal commercial varieties of barley supplemented with free amino acids. Munck et al. (1970) also found the nutritional value of Hiproly to be superior to that of a commercial barley when fed to rats and mice.

The major setback of Hiproly is that the high lysine character may be linked to a detrimental character, namely low yield (Ingverson et al., 1973a). However, the high lysine trait is still of value as it can be selectively bred into other cultivars (Munck et al., 1970).

Since the commercial production of a high protein, high lysine barley cultivar is the ultimate goal, the search for barley varieties with the nutritional qualities of Hiproly and the desirable agronomic qualities of commercial varieties began.

Bomi Riso mutant 1508 is a barley line which appears to meet the qualifications. The mutant shows only a 10% reduction in grain yield (Ingverson et al., 1973d) and an 18% reduction in kernel yield per acre (Doll et al., 1973) as compared to the parent variety and an absolute lysine yield of 30% above that of the parent.

Bomi Riso mutant 1508 is an induced mutant of the 2-rowed Swedish

barley, Bomi. Treatment of the parent was with ethyleneimine and discovery of the mutant was by DBC in 1970 (Ingverson et al., 1973d). Oram et al. (1975) reports that grain plumpness, hordein pattern and lysine:ammonia ratio of the endosperm are pleiotropic effects of the same recessive mutant allele. Bomi Riso mutant 1508 has correspondingly a shrunken endosperm, a 20% reduction in hordeins and a 44% increase in lysine (g/16 g N) over the parent variety (Ingverson et al., 1973d). Doll (1973) reports the high lysine character of Bomi Riso mutant 1508 is due to a single recessive gene since the ratio of high and low lysine:ammonia F_2 generation of seeds approximates that of the expected 1:3 ratio.

The principle protein stores of cereal endosperm are the protein bodies. Barley endosperm consists of a granular component in which are embedded homogenously structured spheres which correspond to maize protein bodies. Ingverson (1975) found the protein bodies from 13 day old Bomi endosperms and Bomi Riso mutant 1508 endosperms were similar. However, at 28 days those of the mutant consisted of mainly a granular component with a few embedded spheres. When fractionated according to solubility, Bomi contained a large amount of prolamines and some glutelins while the mutant had glutelins as the major component and little prolamines. Tallberg (1973) found no reduction in size or number of protein bodies in Hiproly when compared to Hiproly Normal. However, when comparing Bomi and Bomi Riso mutant 1508, the number and size of

protein bodies in the latter were greatly reduced. Small protein bodies observed in Bomi Riso mutant 1508 are similar to those of Opaque-2 maize, suggesting that Bomi Riso mutant 1508 is analogous to Opaque-2 maize in which the reduced prolamine content corresponds to a reduced size of protein bodies.

The 44% increase in lysine in Bomi Riso mutant 1508 over Bomi (Ingverson et al., 1973d) is explained as being the result of a simultaneous increase in the soluble proteins and reduction in the prolamines. The albumins and globulins increased from 27% in Bomi to 46% in Bomi Riso mutant 1508 while hordeins in the mutant were 20% less than those of the parent. Bomi Riso mutant 1508 also showed a 36% increase in threonine, one of barley's limiting essential amino acids, over Bomi (Ingverson et al., 1973d).

Doll et al. (1974) reported a 14% increase in biological value for rats fed Bomi Riso mutant 1508 along with a 7% improvement in net protein utilization as compared to Bomi. Only a slight improvement was seen in the net protein utilization because of a reduced true digestibility of the mutant.

Another barley cultivar resulting in high lysine mutants when treated mutagenically is Carlsberg II. When treated with ethyl methanesulphonate, Carlsberg Riso mutants 29 and 86 resulted. Treatment with gamma radiation resulted in Carlsberg Riso mutant 56. All mutants show increased lysine contents over that of the parent line, however,

Carlsberg Riso mutant 56 also showed a 23% decrease in yield (Doll, 1972).

Ingverson et al. (1971) found no differences in the albumin + globulin pattern or in the hordein composition between the Carlsberg II mother line and the Carlsberg Riso mutants 29 and 86. However, of seven proteins, the Carlsberg II lines did not have five proteins which were found in Hiproly. A minor change in the control mechanism regulating b-protein synthesis may have occurred in these mutants (Ingverson and Koie, 1973c).

Notch-1 and Notch-2 mutants were identified at the Indian Agricultural Research Institute by the DBC method from ethyl methanesulphonate treated populations of the variety NP-113 (Bansal, 1970). These mutants derive their name from the dorsal depression found on the kernels. Bansal (1970 and 1972) reported increased protein and lysine contents of the mutants of about 40% and 20% respectively over the parent. Notch mutants show a 30% reduction in yield compared to NP-113, however, it is still a better yielding variety than Hiproly.

Balaravi et al. (1976) reported on the chemical characterization, protein quality and biological value of the Notch mutants and NP-113. Notch mutants contained nearly 18% more lysine (g/16 g N) than the parent. Notch-1 had 34% more and Notch-2 25% more seed protein than NP-113. Aside from the increased lysine, the mutants also showed increased isoleucine, tyrosine, valine, aspartic acid, glycine and ala-

nine. Only Notch-1 showed increased leucine and phenylalanine. The mutants also showed a 10 to 16% reduction in proline content, a fact of importance due to a corresponding reduction in the prolamine fraction. The albumin + globulin fraction of Notch-1 and Notch-2 increased 9% and 8% respectively over the parent. Very small differences existed between the mutants and parent in the glutelin contents. Notch-2 shows a biological value 11.8% higher than that of NP-113. Notch-1 has a low true protein digestibility which leads to a lower net protein utilization value compared to Notch-2.

A mutant of the six-rowed variety Glacier (CI 9676), designated Glacier Ac 38, was described by Merritt (1967). The interest in this mutant was concerned primarily with its high amylose content. In 1972, Pomeranz, Eslick and Robbins found the mutant to contain a greater percentage of lysine (g/16 g N) than normal Glacier. Calvert (1975) found more lysine, arginine, cystine/2, threonine and tryptophan and less proline and glutamic acid in the mutant. The Osborne fractions of the mutant showed a small increase in the albumin + globulin fraction and a reduction in the hordeins. Studies by Calvert (1975) with weanling rats and growing swine showed Glacier Ac 38 to produce better feed efficiency and PER values for rats and slightly better feed efficiency values for swine when compared to normal Glacier.

Although Glacier Ac 38 appears superior to Glacier from a nitrogen evaluation, it does not appear to be a barley cultivar which will merit

much attention since it has been determined that the starch structure of Glacier Ac 38 is the least desirable of the three types of starch structures which are presently known (Calvert, 1975).

High-Lysine Maize Cultivars

The terms floury-2 maize or Opaque-2 maize refer to maize seeds homozygous for the floury-2 or the Opaque-2 mutation, respectively (Nelson and Mertz, 1972). These mutants are characterized by a dull, opaque appearing kernel with a larger embryo than that of normal maize.

The amino acid composition as well as the Osborne fractions of Opaque-2 maize differ from those of normal maize. According to Mertz et al. (1964), Opaque-2 maize contained more than twice the lysine content of normal maize in the endosperm. When they compared Opaque-2 and normal maize with identical protein contents on a moisture and fat free basis, the Opaque-2 mutant contained 69% more lysine (g/100 g protein), more histidine, arginine, aspartic acid, glycine and cystine and less glutamic acid, alanine, methionine, leucine and tyrosine than the normal maize, They also reported a reduction in the amount of zein (prolamine) synthesis in Opaque-2 with a relative increase of the albumins, globulins and glutelins. The glutelin fraction of the mutant contained a higher proportion of such amino acids as lysine than normal maize. Mertz et al. (1964) found Opaque-2 maize to have a reduced zein:glutelin fraction and a reduction of the ratio of zein to glutelin. Nelson (1969) concluded that mutations which reduce the concentration of synthesized

zein proteins, as in the case of Opaque-2 maize, and that allow increased synthesis of other protein fractions are the most effective way of altering amino acid composition of seeds of cereal grains with a high concentration of alcohol-soluble proteins.

In the mature endosperm of normal maize, the largest protein granules are found in the cells just under the aleuron with the size and number decreasing in successive cells going toward the center of the endosperm (Duvick, 1955). Duvick (1961) offers proof that protein granules may be composed largely, or only, of the complex of proteins presently designated as zein. Wolf et al. (1967) found the subcellular protein granules of high-lysine maize to be smaller in size when compared to normal maize. They state that a change in size of the protein granule represents a corresponding change in zein content, and the protein granules are largely the site of zein storage in the maize endosperm. Their work suggests that, while the mechanism for deposition of subcellular bodies in which zein is stored is greatly impaired in high-lysine mutants, deposition of non-granular storage protein high in lysine content is simultaneously increased.

Opaque-2 maize has been shown to be nutritionally superior to normal maize. Mertz et al. (1965) found the protein digestibilities of the two maize cultivars to be very similar; however, the PER values of rats fed the mutant was 2.8 compared to 1.6 for the normal maize. Gipp et al. (1968) showed superior feed efficiency ratios of rats and swine fed the

Opaque-2 mutants. Bressani et al. (1969) showed a PER value of Opaque-2 fed to rats to be 2.79 compared to 2.88 for casein. Mertz et al. (1965) and Pickett (1966) showed weanling rats and pigs grew approximately 3.5 times faster when fed Opaque-2 maize as when fed normal maize. Veron (1976) found Opaque-2 maize to be nutritionally superior for monogastric animals than normal maize or another type of high-lysine maize. The concensus of these researchers was that increased lysine contents of the mutant maize resulted in the superior feeding values. Bressani et al. (1969) also found the niacin content of raw Opaque-2 maize to be greater and more available than that of normal maize. Niacin is synthesized from tryptophan, the second limiting essential amino acid of maize, hence, increased niacin availability may spare the tryptophan requirement. Since lysine and tryptophan are the first two limiting essential amino acids of maize for monogastrics, the Opaque-2 mutation appears to meet the qualifications of a superior cereal grain from the standpoint of amino acid balance.

Floury-2 maize shows the same changes in the Osborne fractions and amino acid composition as seen in Opaque-2 with the addition of an increased methionine content. The floury-2 mutation also acts to increase protein quantity in maize (Nelson, 1969).

The nutritional value of floury-2 maize to monogastrics has been shown to be superior to that of normal maize but inferior to that of Opaque-2 maize (Nelson and Mertz, 1972). However, the floury-2 mutant

is superior for growing chicks due to the increased methionine content (Nelson, 1969).

EXPERIMENTAL PROCEEDURE

Trial Identification

Trials designated with a Roman numeral were 28-day growth trials, those with a Roman numeral followed with "a" or "b" were nitrogen balance trials.

Trial I included Bomi (MT 486124) and its Riso mutants 7, 8, 9, 13 and 1508; Carlsberg II (CI 10114) and its Riso mutants 29 and 56; Hiproly (CI 3947) and its sisterline, CI 4362, referred to as Hiproly Normal and a maize starch-casein control diet. Trial Ia included Bomi and its five Riso mutants fed in trial I along with Hiproly and Hiproly Normal. In trial Ib, Carlsberg II and its two Riso mutants fed in trial I were compared along with Hiproly and Hiproly Normal. All barleys were grown in 1975 at the Montana State University Agronomy Farm, west of Bozeman, Montana (designated Boz-1). Proximate analysis and calcium and phosphorous composition of the barleys, percentage composition of the diets and proximate analysis and calcium and phosphorous composition of the diets fed in trials I, Ia and Ib are shown in tables 1, 2, 3 and 4, respectively.

Trial II compared Bomi and its Riso mutant 1508, Hiproly, Hiproly Normal, Trojan hybrid maize (TX-90) (hereafter referred to as normal maize), its isogene, a high-lysine Trojan maize (LTX-90) with the Opaque-2 gene (hereafter referred to as high-lysine maize) and a maize starch-casein control. These barleys were also grown in 1975 on the

Montana State Agronomy Farm near Bozeman, Montana, but in a slightly different location from those in trials I, Ia and Ib (designated Boz-2). Proximate analysis and calcium and phosphorous composition of the grains, percentage composition of the diets and proximate analysis and calcium and phosphorous composition of the diets fed in trial II and in a portion of trial IIIa are shown in tables 5, 6 and 7, respectively.

Trial III consisted of the same barleys, maize cultivars and control used in trial II except they were grown in 1975 at the Southern Agricultural Research Center, Huntley, Montana (designated Hunt). Trial IIIa included the Bomi and Riso 1508 barleys used in trial II and all of the grain diets fed in trial III. Proximate analysis and calcium and phosphorous composition of the grains, percentage composition of the diets and proximate analysis and calcium and phosphorous composition of the diets used in trial III and in a portion of trial IIIa are shown in tables 8, 9 and 10, respectively.

Trial IV consisted of a growth trial utilizing Carlsberg II, Carlsberg Riso mutants 29 and 86, Hiproly, Hiproly Normal, normal maize, high-lysine maize and a maize starch-casein control. These barleys were grown in 1975 on the Montana State University Agronomy Farm west of Bozeman, Montana (Boz-2). Trial IVa included the same diets as used in trial IV with the exclusion of the control diet. Proximate analysis and calcium and phosphorous composition of the grains, percentage composition of the diets and proximate analysis and calcium and phosphorous

composition of the diets used in trial IV and IVa are shown in tables 11, 12 and 13, respectively.

Trial V included the same barleys, maize cultivars and control used in trial IV except they were grown in 1975 at the Southern Agricultural Research Center at Huntley, Montana (Hunt). Trial Va consisted of the same diets used in trial V with the exclusion of the casein control. Proximate analysis and calcium and phosphorous composition of the grains, percentage composition of the diets and proximate analysis and calcium and phosphorous composition of the diets used in trial V and Va are shown in tables 14, 15 and 16, respectively.

Trial VI consisted of a vitamin and mineral fortified maize starch nitrogen-free diet fed to determine metabolic fecal nitrogen and endogenous urinary nitrogen. Percentage composition of the nitrogen-free diet is shown in table 17.

Diet Preparation

A proximate analysis of all barley and maize cultivars were obtained according to a modified proceedure of A.O.A.C. (1970). Protein was calculated from Kjeldahl nitrogen using the correction value of 6.25. Calcium percentages were determined by a modified Kramer and Tisdall proceedure of Clark and Collip (1925) and phosphorous by the method of Fiske and Subbarow (1925). Amino acid composition of acid hydrolysates of each grain was obtained by the method of Spackman et al. (1958). Separate analyses were necessary for cystine/2 (cystine + 2 x

cysteine) (Hirs, 1967) and tryptophan (Hulgi and Moore, 1972) (tables 18, 19, 20, 21, 22 and 23). All amino acid analyses were accomplished by AAA Laboratories.[1]

Isonitrogenous, isocaloric rations were formulated within each growth trial. Maize starch was added at the expense of barley to bring each diet to an isonitrogenous level and maize oil was added to equalize the caloric levels. Diets contained equal amounts of calcium carbonate, vitamins, mineral mixture and antibiotic. The calcium carbonate was added to provide for proper calcium:phosphorous ratios. The antibiotic consisted of a commercial mixture of chlortetracycline, penicillin and sulfamethazine added to rations to discourage respiratory infections. Purified wood cellulose was included in diets as required to equalize the amount of crude fiber in each diet within trials. A control diet using casein (89% protein) as the sole protein source and the aforementioned ingredients was also included for each growth trial. All diets were mechanically mixed to ensure uniform mixing of ingredients and stored in a refrigerated environment (-3^{o} C) to prevent rancidity. Chemical analysis as previously described was performed on each diet. Sufficient diet was mixed at the beginning to complete the growth trial and its corresponding nitrogen balance trial.

[1] AAA Laboratories, 6206 89th Avenue Southeast, Mercer Island, Washington 98040

Growth Trials

Ten female weanling Holtzman strain rats were assigned to each diet according to initial weight in each growth trial. The total rat weight per diet per trial was adjusted to vary no more than ±0.1 gram initially. Rats were individually caged and allotted to a position on the cage rack such that each diet was represented in each of the five horizontal cage levels and six vertical positions. Feed and water were offered *ad libitum*. All rats were maintained in an environmentally controlled room with automatic lighting which provided 12 hours of light and 12 hours of darkness. Weight gain and feed consumption were recorded weekly for each rat and summed at the end of the 28-day trial period to obtain total gain and total feed consumption for each individual. Feed efficiency ratios (feed consumed/gain) and protein efficiency ratios (PER) were then calculated. Adjusted PER's were then obtained by multiplying the PER of each observation by the factor obtained from dividing the average casein PER in each trial by the factor 2.50 (Chapman *et al.* 1959). All data were then averaged to obtain values for each diet.

Nitrogen Balance Trials

Four female weanling Holtzman strain rats were assigned to each diet according to initial weight in each nitrogen balance trial. The total rat weight of each diet per trial was such that they did not vary by ±1.0 gram. Rats were individually housed and fed their respective diets for a four day adjustment period. After adaptation, they were

weighed and allotted according to diet for a four day collection period. Each rat received ten grams of diet that provided 160 milligrams of nitrogen daily. Water was available at all times throughout the trial.

Proceedures for urine and fecal collection were patterned after those of Eggum (1973b); however, due to differences in cage construction, methods were slightly modified. Urine was automatically collected through a glass wool filter into a flask containing 25 ml of 5% sulfuric acid. The glass wool and cage bottoms were rinsed daily with distilled water to ensure that all nitrogen was collected. Feces either fell directly onto a screen beneath the cage where they were collected daily and placed into separate beakers containing 50 ml of 5% sulfuric acid or they fell directly into beakers containing the acid as they were voided from the rat.

At the conclusion of the collection period, Kjeldahl nitrogen was determined for each rat's urinary and fecal excretion. Duplicate analyses were conducted on each sample and an average nitrogen excretion was calculated. The proceedure for determining urinary nitrogen (UN) included dilution of each urine collection to 200 ml with tap water and sampling a 15 ml aliquot for macro-Kjeldahl. Fecal preparation for fecal nitrogen (FN) analysis included the addition of 100 ml of concentrated sulfuric acid to form a honogenous solution, dilution to 500 ml with tap water and taking a 100 ml aliquot for macro-Kjeldahl analysis. Urinary nitrogen and fecal nitrogen were calculated for each rat to express grams

of nitrogen excreted per rat per four days. Biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU) were then calculated for each rat and averaged for each diet (Mitchell, 1924 and Eggum, 1973b).

Nitrogen-Free Balance Trial

Ten female weanling Holtzman strain rats were utilized to determine metabolic nitrogen (MN) and endogenous nitrogen (EN). Nitrogen losses through the feces which originate from the body and digestive processes and not of food origin represent MN while EN measures nitrogen losses in the urine which are not of dietary origin. A nitrogen-free diet was formulated (table 17) and a Kjeldahl nitrogen analysis performed on the diet to ensure the nitrogen-free status. The experimental proceedure of this trial was identical to that of the previously described nitrogen balance trials with the exception of a three day collection period. Following Kjeldahl analysis of urine and feces collected for each rat, average values for EN and MN were determined from the total of ten rats. Endogenous nitrogen was expressed as grams of nitrogen per rat per day while MN was expressed as grams of nitrogen per gram of dry matter consumed. Trial VI was completed prior to the other nitrogen balance trials and the EN and MN figures obtained were then used to calculate biological values in trials Ia, Ib, IIIa, IVa and Va.

Data Analysis

Data were analyzed according to the least-squares method of Harvey (1960) and resulted in least squares means for gain, feed consumption, feed efficiency, PER, BV, TPD and NPU. Duncan's multiple range test (Snedecor and Cochran, 1967) was computed in the event of a significant difference shown by analysis of variance.

Characterization of Grains

Bomi, Carlsberg II and their mutants were physically characterized as to percent plump and thin kernels, yield, test weight and kernel weight courtesy of Dr. C. McGuire and his technicians of the Montana State University Plant and Soil Science Department.

TABLE 1. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF BARLEYS, TRIAL I, Ia AND Ib (BOZ-1)

Barley[a]	Percentage[b]							
	C.P.	E.E.	C.F.	N.F.E.	H_2O	Ash	Ca	P
HP	18.9	2.3	1.6	66.5	8.2	2.5	.03	.54
HPN	16.5	2.1	1.5	69.6	7.9	2.4	.03	.52
Bomi	12.4	1.9	5.2	70.2	7.4	2.9	.02	.38
B/R 7	11.8	2.1	3.9	71.3	8.0	2.9	.01	.39
B/R 8	12.5	3.4	5.5	67.4	7.8	3.4	.03	.46
B/R 9	13.0	2.1	5.6	68.8	6.9	3.6	.02	.47
B/R 13	12.7	4.2	6.1	66.4	7.1	3.5	.02	.45
B/R 1508	12.7	3.1	6.5	66.8	7.3	3.6	.03	.43
Carlsberg II	10.5	2.0	4.6	71.7	8.1	3.1	--	.38
C/R 29	10.8	3.2	5.1	70.0	7.9	3.0	.02	.40
C/R 56	12.9	2.2	4.4	69.7	7.8	3.0	.02	.45

[a]HP=Hiproly, HPN=Hiproly Normal, B/R=Bomi Riso mutant and C/R=Carlsberg Riso mutant.

[b]C.P.=crude protein, E.E.=ether extract, C.F.=crude fiber, N.F.E.=nitrogen free extract, H_2O=moisture.

TABLE 2. PERCENTAGE COMPOSITION OF BARLEY AND CASEIN DIETS, TRIAL I, Ia AND Ib (BOZ-1)

Diet[a]	Barley/ casein[b]	Maize starch	Maize oil	Mineral mixture[c]	Vitamin mixture[c]	$CaCO_3$[d]	Alphacel[c]	Anti-biotic[e]
HP	51.59	37.17	2.03	2.00	2.00	0.80	4.16	0.25
HPN	59.10	29.77	1.98	2.00	2.00	0.80	4.10	0.25
Bomi	78.63	13.69	1.73	2.00	2.00	0.80	0.90	0.25
B/R 7	82.63	9.07	1.48	2.00	2.00	0.80	1.77	0.25
B/R 8	78.00	15.68	0.57	2.00	2.00	0.80	0.70	0.25
B/R 9	75.00	17.52	1.64	2.00	2.00	0.80	0.79	0.25
B/R 13	76.78	17.86	--	2.00	2.00	0.80	0.31	0.25
B/R 1508	76.78	17.33	0.84	2.00	2.00	0.80	--	0.25
Carlsberg II	92.85	--	1.36	2.00	2.00	0.80	0.74	0.25
C/R 29	90.28	3.95	0.33	2.00	2.00	0.80	0.39	0.25
C/R 56	75.59	16.14	1.56	2.00	2.00	0.80	1.66	0.25
Casein	10.96	75.78	3.22	2.00	2.00	0.80	4.99	0.25

[a]HP=Hiproly, HPN=Hiproly Normal, B/R=Bomi Riso mutant, C/R=Carlsberg Riso mutant.
[b]ICN Nutritional Biochemicals; casein, purified, high nitrogen; used only in casein diet.
[c]ICN Nutritional Biochemicals; Bernhart-Tomarelli salt mixture, modified 1135-B, vitamin diet fortification mixture (see appendix tables 49 and 50) and non-nutritive cellulose.
[d]Reagent grade calcium carbonate.
[e]Chlortetracycline, penicillin and sulfamethazine, 220.5, 110.2 and 220.5 grams per kilogram of antibiotic mixture, respectively.

TABLE 3. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF DIETS, TRIAL I AND Ia (BOZ-1)

Diet[a]	Percentage[b]							
	C.P.	E.E.	C.F.	N.F.E.	H_2O	Ash	Ca	P
HP	10.3	3.6	2.1	73.0	7.4	3.6	.53	.65
HPN	10.5	3.6	4.6	71.8	5.9	3.6	.55	.68
Bomi	10.2	3.4	3.7	72.3	6.0	4.4	.49	.68
B/R 7	10.2	3.3	3.7	71.3	7.1	4.4	.50	.64
B/R 8	10.2	3.4	4.8	70.1	6.4	5.1	.64	.75
B/R 9	10.2	3.2	4.4	71.5	5.6	5.1	.51	.76
B/R 13	10.2	3.2	2.3	74.4	5.4	4.5	.56	.81
B/R 1508	10.3	3.3	2.9	72.8	6.3	4.4	.53	.70
Carlsberg II	10.4	1.9	2.6	73.4	6.2	5.5	.64	.78
C/R 29	10.4	3.4	4.6	70.7	6.2	4.7	.50	.70
C/R 56	10.3	3.4	4.1	70.0	7.6	4.6	.51	.73
Casein	10.5	3.3	1.4	75.7	6.4	2.7	.56	.52

[a]HP=Hiproly, HPN=Hiproly Normal, B/R=Bomi Riso mutant and C/R=Carlsberg Riso mutant.

[b]C.P.=crude protein, E.E.=ether extract, C.F.=crude fiber, N.F.E.=nitrogen-free extract, H_2O=moisture.