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.

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Date April 29, 1977

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

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## 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.

TABLE 4. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF DIETS, TRIAL Ib (BOZ-1)

| Diet[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 10.0 | 1.6 | 3.4 | 72.0 | 9.3 | 3.7 | .54 | .57 |
| HPN | 9.4 | 1.6 | 2.6 | 72.4 | 10.0 | 4.0 | .57 | .59 |
| Carlsberg II | 10.2 | 1.8 | 4.0 | 71.2 | 7.9 | 4.9 | .51 | .60 |
| C/R 29 | 10.2 | 1.8 | 4.8 | 69.9 | 7.9 | 5.4 | .62 | .64 |
| C/R 56 | 10.5 | 1.7 | 4.2 | 71.2 | 7.6 | 4.8 | .57 | .60 |

[a]HP=Hiproly, HPN=Hiproly Normal 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 5. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF GRAINS, TRIAL II (BOZ-2) AND A PORTION OF TRIAL IIIa (BOZ-2 AND HUNT)

| Grain[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 19.3 | 1.8 | 2.8 | 66.7 | 7.1 | 2.3 | -- | -- |
| HPN | 17.8 | 1.6 | 1.9 | 69.3 | 7.1 | 2.3 | -- | -- |
| Normal maize | 9.3 | 3.2 | 2.6 | 73.6 | 9.9 | 1.4 | .01 | .33 |
| HL maize | 9.6 | 4.2 | 2.1 | 73.2 | 9.5 | 1.4 | .01 | .34 |
| Bomi | 13.1 | 1.5 | 4.8 | 70.1 | 7.9 | 2.6 | -- | -- |
| B/R 1508 | 13.1 | 1.9 | 5.3 | 69.5 | 7.6 | 2.6 | -- | -- |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi 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 6. PERCENTAGE COMPOSITION OF GRAIN AND CASEIN DIETS, TRIAL II (BOZ-2) AND A PORTION OF TRIAL IIIa (BOZ-2 AND HUNT)

| Diet[a] | Grain/ casein[b] | Maize starch | Maize oil | Mineral mixture[c] | Vitamin mixture[c] | $CaCO_3$[d] | Alphacel[c] | Anti-biotic[e] |
|---|---|---|---|---|---|---|---|---|
| HP | 44.82 | 44.91 | 2.97 | 2.00 | 2.00 | 0.80 | 2.25 | 0.25 |
| HPN | 48.60 | 40.77 | 3.00 | 2.00 | 2.00 | 0.80 | 2.58 | 0.25 |
| Normal maize | 93.07 | -- | 0.80 | 2.00 | 2.00 | 0.80 | 1.08 | 0.25 |
| HL maize | 90.10 | 3.24 | -- | 2.00 | 2.00 | 0.80 | 1.61 | 0.25 |
| Bomi | 66.03 | 25.80 | 2.79 | 2.00 | 2.00 | 0.80 | 0.33 | 0.25 |
| B/R 1508 | 66.03 | 26.39 | 2.53 | 2.00 | 2.00 | 0.80 | -- | 0.25 |
| Casein | 9.72 | 77.95 | 3.78 | 2.00 | 2.00 | 0.80 | 3.50 | 0.25 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi 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 7. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF DIETS, TRIAL II (BOZ-2) AND A PORTION OF TRIAL IIIa (BOZ-2 AND HUNT)

| Diet[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 8.7 | 3.7 | 1.9 | 76.0 | 6.7 | 3.0 | .51 | .53 |
| HPN | 9.0 | 3.7 | 1.7 | 76.2 | 6.5 | 2.9 | .48 | .51 |
| Normal maize | 9.0 | 3.4 | 2.4 | 72.1 | 9.3 | 3.8 | .60 | .71 |
| HL maize | 8.9 | 3.4 | 2.6 | 72.5 | 8.7 | 3.9 | .57 | .74 |
| Bomi | 9.2 | 3.6 | 2.8 | 72.7 | 7.1 | 4.6 | .59 | .65 |
| B/R 1508 | 9.1 | 3.9 | 3.5 | 71.8 | 6.9 | 4.8 | .57 | .66 |
| Casein | 9.1 | 3.7 | 1.1 | 76.8 | 6.8 | 2.5 | .58 | .50 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi 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 8. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF GRAINS, TRIAL III AND IIIa (HUNT)

| Grain[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 19.5 | 1.7 | 1.9 | 66.6 | 7.4 | 2.9 | .03 | .56 |
| HPN | 17.5 | 1.9 | 1.5 | 70.0 | 6.7 | 2.4 | .03 | .50 |
| Normal maize | 9.3 | 3.2 | 2.6 | 73.6 | 9.9 | 1.4 | .01 | .33 |
| HL maize | 9.6 | 4.2 | 2.1 | 73.2 | 9.5 | 1.4 | .01 | .34 |
| Bomi | 12.8 | 2.3 | 3.6 | 73.1 | 5.4 | 2.8 | -- | .47 |
| B/R 1508 | 11.6 | 2.7 | 3.3 | 74.3 | 5.4 | 2.7 | .02 | .44 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi 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 9. PERCENTAGE COMPOSITION OF GRAIN AND CASEIN DIETS, TRIAL III AND IIIa (HUNT)

| Diet[a] | Grain/ casein[b] | Maize starch | Maize oil | Mineral mixture[c] | Vitamin mixture[c] | $CaCO_3$[d] | Alphacel[c] | Anti-biotic[e] |
|---|---|---|---|---|---|---|---|---|
| HP | 41.64 | 48.81 | 2.84 | 2.00 | 2.00 | 0.80 | 1.66 | 0.25 |
| HPN | 46.40 | 44.13 | 2.67 | 2.00 | 2.00 | 0.80 | 1.75 | 0.25 |
| Normal maize | 94.42 | -- | 0.53 | 2.00 | 2.00 | 0.80 | -- | 0.25 |
| HL maize | 84.58 | 9.70 | -- | 2.00 | 2.00 | 0.80 | 0.67 | 0.25 |
| Bomi | 63.44 | 29.25 | 2.09 | 2.00 | 2.00 | 0.80 | 0.17 | 0.25 |
| B/R 1508 | 70.00 | 23.15 | 1.66 | 2.00 | 2.00 | 0.80 | 0.14 | 0.25 |
| Casein | 9.12 | 79.83 | 3.55 | 2.00 | 2.00 | 0.80 | 2.45 | 0.25 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi 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 10. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF DIETS, TRIAL III AND IIIa (HUNT)

| Diet[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 8.4 | 4.1 | 1.3 | 76.5 | 6.3 | 3.4 | .59 | .66 |
| HPN | 8.4 | 4.0 | 1.4 | 76.4 | 6.3 | 3.5 | .61 | .65 |
| Normal maize | 9.0 | 4.0 | 1.6 | 73.1 | 8.5 | 3.8 | .62 | .72 |
| HL maize | 8.1 | 3.3 | 2.0 | 75.6 | 7.2 | 3.8 | .57 | .74 |
| Bomi | 8.5 | 3.2 | 2.9 | 74.3 | 6.9 | 4.2 | .57 | .70 |
| B/R 1508 | 8.4 | 3.1 | 3.0 | 74.2 | 7.1 | 4.2 | .62 | .71 |
| Casein | 8.1 | 3.5 | 0.6 | 79.6 | 5.7 | 2.5 | .57 | .48 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi 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 11. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF GRAINS, TRIAL IV AND IVa (BOZ-2)

| Grain[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 20.6 | 2.1 | 2.1 | 66.3 | 5.8 | 3.1 | .02 | .58 |
| HPN | 19.0 | 1.8 | 1.7 | 69.3 | 5.7 | 2.5 | .01 | .53 |
| Normal maize | 8.6 | 4.1 | 2.0 | 76.3 | 7.6 | 1.4 | -- | .29 |
| HL maize | 9.6 | 4.4 | 2.0 | 76.4 | 5.8 | 1.8 | .01 | .33 |
| Carlsberg II | 13.3 | 2.0 | 3.3 | 72.8 | 6.0 | 2.6 | .04 | .38 |
| C/R 29 | 13.6 | 3.7 | 3.9 | 71.1 | 4.9 | 2.8 | .03 | .38 |
| C/R 86 | 14.0 | 3.7 | 3.6 | 72.2 | 3.7 | 2.8 | -- | .40 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, 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 12. PERCENTAGE COMPOSITION OF GRAIN AND CASEIN DIETS, TRIAL IV AND IVa (BOZ-2)

| Diet[a] | Grain/ casein[b] | Maize starch | Maize oil | Mineral mixture[c] | Vitamin mixture[c] | $CaCO_3$[d] | Alphacel[c] | Anti-biotic[e] |
|---|---|---|---|---|---|---|---|---|
| HP | 39.42 | 51.19 | 2.72 | 2.00 | 2.00 | 0.80 | 1.62 | 0.25 |
| HPN | 42.74 | 47.71 | 2.78 | 2.00 | 2.00 | 0.80 | 1.72 | 0.25 |
| Normal maize | 94.42 | -- | 0.53 | 2.00 | 2.00 | 0.80 | -- | 0.25 |
| HL maize | 84.58 | 9.70 | -- | 2.00 | 2.00 | 0.80 | 0.67 | 0.25 |
| Carlsberg II | 61.05 | 31.13 | 2.33 | 2.00 | 2.00 | 0.80 | 0.44 | 0.25 |
| C/R 29 | 59.71 | 33.78 | 1.34 | 2.00 | 2.00 | 0.80 | 0.12 | 0.25 |
| C/R 86 | 58.00 | 35.19 | 1.40 | 2.00 | 2.00 | 0.80 | 0.36 | 0.25 |
| Casein | 9.12 | 79.83 | 3.55 | 2.00 | 2.00 | 0.80 | 2.45 | 0.25 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, 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 13. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF DIETS, TRIAL IV AND IVa (BOZ-2)

| Diet[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 8.4 | 3.7 | 1.5 | 76.8 | 6.0 | 3.6 | .56 | .62 |
| HPN | 8.3 | 3.6 | 0.9 | 76.8 | 6.5 | 3.9 | .60 | .67 |
| Normal maize | 8.2 | 4.0 | 1.6 | 73.1 | 8.5 | 3.8 | .62 | .72 |
| HL maize | 8.1 | 3.3 | 2.0 | 75.6 | 7.2 | 3.8 | .55 | .70 |
| Carlsberg II | 8.6 | 3.0 | 2.6 | 74.7 | 6.2 | 4.9 | .61 | .62 |
| C/R 29 | 8.6 | 2.8 | 3.1 | 73.7 | 6.0 | 5.8 | .58 | .63 |
| C/R 86 | 8.3 | 2.6 | 3.2 | 73.3 | 6.5 | 6.1 | .58 | .62 |
| Casein | 8.1 | 3.5 | 0.6 | 79.6 | 5.7 | 2.5 | .57 | .48 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, 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 14. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF GRAINS, TRIAL V AND Va (HUNT)

| Grain[b] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 19.3 | 1.9 | 1.7 | 67.8 | 6.6 | 2.7 | .02 | .57 |
| HPN | 17.8 | 1.9 | 1.4 | 70.1 | 6.4 | 2.4 | .02 | .52 |
| Normal maize | 8.6 | 4.1 | 2.0 | 76.3 | 7.6 | 1.4 | -- | .29 |
| HL maize | 9.6 | 4.4 | 2.0 | 76.4 | 5.8 | 1.8 | .01 | .33 |
| Carlsberg II | 11.0 | 2.0 | 3.3 | 75.6 | 5.4 | 2.7 | .03 | .43 |
| C/R 29 | 12.1 | 4.0 | 3.9 | 73.5 | 3.5 | 3.0 | .04 | .47 |
| C/R 86 | 12.4 | 3.8 | 3.1 | 74.4 | 3.3 | 3.0 | .04 | .47 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, 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 15. PERCENTAGE COMPOSITION OF GRAIN AND CASEIN DIETS, TRIAL V AND Va (HUNT)

| Diet[a] | Grain/ casein[b] | Maize starch | Maize oil | Mineral mixture[c] | Vitamin mixture[c] | $CaCO_3$[d] | Alphacel[c] | Anti-biotic[e] |
|---|---|---|---|---|---|---|---|---|
| HP | 41.97 | 48.04 | 3.05 | 2.00 | 2.00 | 0.80 | 1.89 | 0.25 |
| HPN | 45.51 | 44.48 | 2.99 | 2.00 | 2.00 | 0.80 | 1.97 | 0.25 |
| Normal maize | 94.22 | -- | -- | 2.00 | 2.00 | 0.80 | 0.73 | 0.25 |
| HL maize | 84.34 | 9.54 | 0.15 | 2.00 | 2.00 | 0.80 | 0.92 | 0.25 |
| Carlsberg II | 73.64 | 18.74 | 2.39 | 2.00 | 2.00 | 0.80 | 0.18 | 0.25 |
| C/R 29 | 66.94 | 26.83 | 1.18 | 2.00 | 2.00 | 0.80 | -- | 0.25 |
| C/R 86 | 65.32 | 27.66 | 1.38 | 2.00 | 2.00 | 0.80 | 0.59 | 0.25 |
| Casein | 9.10 | 79.38 | 3.86 | 2.00 | 2.00 | 0.80 | 2.61 | 0.25 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, 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 16. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHOROUS COMPOSITION OF DIETS, TRIAL V AND Va (HUNT)

| Diet[a] | Percentage[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C.P. | E.E. | C.F. | N.F.E. | $H_2O$ | Ash | Ca | P |
| HP | 8.6 | 4.2 | 1.6 | 76.6 | 5.6 | 3.4 | .56 | .60 |
| HPN | 8.2 | 4.0 | 1.0 | 77.8 | 5.5 | 3.5 | .57 | .62 |
| Normal maize | 8.5 | 3.5 | 1.9 | 73.4 | 8.9 | 3.8 | .58 | .62 |
| HL maize | 8.4 | 3.4 | 2.4 | 73.9 | 8.0 | 3.9 | .60 | .65 |
| Carlsberg II | 8.4 | 3.5 | 3.5 | 74.1 | 6.1 | 4.4 | .60 | .67 |
| C/R 29 | 8.7 | 2.8 | 3.6 | 74.0 | 6.5 | 4.4 | .58 | .66 |
| C/R 86 | 8.4 | 2.8 | 3.8 | 74.5 | 6.2 | 4.3 | .58 | .65 |
| Casein | 8.5 | 3.7 | 0.3 | 78.7 | 6.2 | 2.6 | .60 | .46 |

[a]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, 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 17. PERCENTAGE COMPOSITION OF THE NITROGEN-FREE DIET, TRIAL VI

| Ingredients | Percentage |
|---|---|
| Maize starch | 82.48 |
| Maize oil | 4.85 |
| Alphacel[a] | 4.85 |
| Vitamin mixture[a] | 1.94 |
| Mineral mixture[a] | 1.94 |
| Calcium carbonate[b] | 0.78 |
| Antibiotic[c] | 0.24 |
| Water | 2.92 |

[a]ICN Nutritional Biochemicals non-nutritive cellulose; vitamin fortification mixture; Bernhart-Tomarelli salt mixture, modified 1135-B (see appendix tables 49 and 50).

[b]Reagent grade.

[c]Chlortetracycline, penicillin and sulfamethazine, 220.5, 100.2 and 220.5 grams per kilogram of antibiotic mixture, respectively.

TABLE 18. AMINO ACID ANALYSIS OF BOMI AND BOMI DERIVED BARLEYS EXPRESSED AS A PERCENTAGE OF THE GRAIN

| | Bomi | | | B/R 7[a] | B/R 8[a] | B/R 9[a] | B/R 13[a] | B/R 1508[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | Boz-1 | Boz-2 | Hunt | Boz-1 | Boz-1 | Boz-1 | Boz-1 | Boz-1 | Boz-2 | Hunt |
| Amino acid | | | | | | | | | | |
| Alanine | .41 | .42 | .41 | .44 | .48 | .48 | .49 | .55 | .45 | .42 |
| Arginine | .52 | .55 | .55 | .56 | .67 | .55 | .61 | .71 | .62 | .59 |
| Aspartic acid | .64 | .66 | .63 | .71 | .84 | .71 | .85 | 1.02 | .78 | .71 |
| Cystine | .21 | .21 | .21 | .21 | .21 | .24 | .22 | .21 | .21 | .21 |
| Glutamic acid | 2.71 | 2.80 | 2.70 | 2.45 | 2.21 | 2.76 | 2.38 | 1.87 | 2.18 | 2.14 |
| Glycine | .35 | .37 | .37 | .42 | .41 | .45 | .41 | .50 | .41 | .39 |
| Histidine | .23 | .23 | .23 | .25 | .25 | .26 | .25 | .28 | .24 | .23 |
| Isoleucine | .39 | .40 | .39 | .36 | .38 | .41 | .39 | .38 | .36 | .34 |
| Leucine | .76 | .78 | .76 | .74 | .73 | .82 | .75 | .73 | .71 | .68 |
| Lysine | .38 | .38 | .38 | .42 | .49 | .43 | .50 | .58 | .44 | .42 |
| Methionine | .18 | .19 | .18 | .19 | .19 | .19 | .19 | .19 | .19 | .18 |
| Phenylalanine | .60 | .58 | .57 | .54 | .51 | .62 | .56 | .45 | .47 | .46 |
| Proline | 1.26 | 1.32 | 1.26 | 1.10 | 1.01 | 1.29 | 1.05 | .79 | .99 | .97 |
| Serine | .50 | .51 | .51 | .52 | .49 | .61 | .51 | .55 | .49 | .47 |
| Threonine | .38 | .38 | .38 | .42 | .40 | .43 | .42 | .46 | .40 | .38 |
| Tryptophan | .18 | .18 | .18 | .15 | .22 | .21 | .23 | .21 | .21 | .21 |
| Tyrosine | .33 | .30 | .29 | .34 | .28 | .34 | .34 | .32 | .26 | .26 |
| Valine | .51 | .50 | .49 | .51 | .53 | .56 | .53 | .55 | .48 | .46 |

[a]B/R=Bomi Riso mutant.

TABLE 19. AMINO ACID ANALYSIS OF BOMI AND BOMI DERIVED BARLEYS EXPRESSED AS A PERCENTAGE OF THE TOTAL PROTEIN RECOVERED

| | Bomi | | | B/R 7[a] | B/R 8[a] | B/R 9[a] | B/R 13[a] | B/R 1508[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | Boz-1 | Boz-2 | Hunt | Boz-1 | Boz-1 | Boz-1 | Boz-1 | Boz-1 | Boz-2 | Hunt |
| Amino acid | | | | | | | | | | |
| Alanine | 3.89 | 3.90 | 3.91 | 4.26 | 4.66 | 4.23 | 4.59 | 5.31 | 4.55 | 4.41 |
| Arginine | 4.93 | 5.11 | 5.24 | 5.42 | 6.50 | 4.84 | 5.71 | 6.86 | 6.27 | 6.20 |
| Aspartic acid | 6.07 | 6.13 | 6.01 | 6.87 | 8.16 | 6.25 | 7.96 | 9.86 | 7.89 | 7.46 |
| Cystine | 1.99 | 1.95 | 2.00 | 2.03 | 2.04 | 2.11 | 2.06 | 2.03 | 2.12 | 2.21 |
| Glutamic acid | 25.72 | 26.02 | 25.75 | 23.72 | 21.44 | 24.28 | 22.30 | 18.08 | 22.05 | 22.47 |
| Glycine | 3.32 | 3.44 | 3.53 | 4.07 | 3.98 | 3.96 | 3.84 | 4.83 | 4.15 | 4.10 |
| Histidine | 2.18 | 2.14 | 2.19 | 2.42 | 2.43 | 2.29 | 2.34 | 2.71 | 2.43 | 2.42 |
| Isoleucine | 3.70 | 3.72 | 3.72 | 3.48 | 3.69 | 3.61 | 3.65 | 3.67 | 3.64 | 3.57 |
| Leucine | 7.21 | 7.25 | 7.24 | 7.16 | 7.09 | 7.22 | 7.02 | 7.05 | 7.18 | 7.14 |
| Lysine | 3.61 | 3.53 | 3.62 | 4.07 | 4.76 | 3.79 | 4.68 | 5.60 | 4.45 | 4.41 |
| Methionine | 1.71 | 1.77 | 1.72 | 1.84 | 1.84 | 1.67 | 1.78 | 1.84 | 1.92 | 1.89 |
| Phenylalanine | 5.69 | 5.39 | 5.43 | 5.23 | 4.95 | 5.46 | 5.24 | 4.35 | 4.75 | 4.83 |
| Proline | 11.95 | 12.27 | 12.01 | 10.65 | 9.81 | 11.36 | 9.83 | 7.63 | 10.01 | 10.19 |
| Serine | 4.74 | 4.74 | 4.86 | 5.03 | 4.76 | 5.37 | 4.78 | 5.31 | 4.95 | 4.94 |
| Threonine | 3.61 | 3.53 | 3.62 | 4.07 | 3.88 | 3.79 | 3.93 | 4.44 | 4.04 | 3.99 |
| Tryptophan | 1.71 | 1.67 | 1.72 | 1.45 | 2.14 | 1.85 | 2.15 | 2.03 | 2.12 | 2.21 |
| Tyrosine | 3.13 | 2.79 | 2.76 | 3.29 | 2.72 | 2.99 | 3.18 | 3.09 | 2.63 | 2.73 |
| Valine | 4.84 | 4.65 | 4.67 | 4.94 | 5.15 | 4.93 | 4.96 | 5.31 | 4.85 | 4.83 |

[a]B/R=Bomi Riso mutant.

TABLE 20. AMINO ACID ANALYSIS OF CARLSBERG II AND CARLSBERG II DERIVED BARLEYS EXPRESSED AS A PERCENTAGE OF THE GRAIN

| | Carlsberg II | | | C/R 29[a] | | | C/R 56[a] | C/R 86[a] | |
|---|---|---|---|---|---|---|---|---|---|
| Location | Boz-1 | Boz-2 | Hunt | Boz-1 | Boz-2 | Hunt | Boz-1 | Boz-2 | Hunt |
| Amino acid | | | | | | | | | |
| Alanine | .36 | .42 | .38 | .34 | .49 | .46 | .58 | .50 | .46 |
| Arginine | .42 | .53 | .48 | .50 | .63 | .60 | .62 | .63 | .60 |
| Aspartic acid | .58 | .68 | .60 | .68 | .84 | .73 | .80 | .85 | .74 |
| Cystine | .21 | .21 | .21 | .16 | .16 | .16 | .22 | .21 | .21 |
| Glutamic acid | 2.09 | 2.78 | 2.23 | 1.94 | 2.56 | 2.26 | 2.40 | 2.67 | 2.22 |
| Glycine | .30 | .37 | .34 | .30 | .44 | .40 | .44 | .44 | .41 |
| Histidine | .19 | .23 | .20 | .21 | .25 | .23 | .26 | .26 | .24 |
| Isoleucine | .31 | .40 | .35 | .34 | .41 | .38 | .37 | .42 | .38 |
| Leucine | .61 | .76 | .66 | .64 | .79 | .73 | .74 | .81 | .73 |
| Lysine | .33 | .35 | .35 | .41 | .48 | .44 | .51 | .49 | .46 |
| Methionine | .16 | .20 | .18 | .17 | .21 | .19 | .21 | .22 | .20 |
| Phenylalanine | .46 | .58 | .47 | .45 | .57 | .50 | .66 | .59 | .50 |
| Proline | .92 | 1.30 | 1.04 | .86 | 1.19 | 1.05 | 1.15 | 1.22 | .98 |
| Serine | .40 | .51 | .45 | .45 | .56 | .50 | .54 | .58 | .51 |
| Threonine | .32 | .40 | .35 | .36 | .44 | .40 | .45 | .45 | .40 |
| Tryptophan | .12 | .12 | .12 | .17 | .17 | .17 | .20 | .22 | .22 |
| Tyrosine | .28 | .30 | .26 | .28 | .31 | .30 | .36 | .31 | .29 |
| Valine | .41 | .49 | .43 | .29 | .53 | .49 | .51 | .53 | .49 |

[a]C/R=Carlsberg Riso mutant.

TABLE 21. AMINO ACID ANALYSIS OF CARLSBERG II AND CARLSBERG II DERIVED BARLEYS EXPRESSED AS A PERCENTAGE OF THE TOTAL PROTEIN RECOVERED

| | Carlsberg II | | | C/R 29[a] | | | C/R 56[a] | C/R 86[a] | |
|---|---|---|---|---|---|---|---|---|---|
| Location | Boz-1 | Boz-2 | Hunt | Boz-1 | Boz-2 | Hunt | Boz-1 | Boz-2 | Hunt |
| Amino acid | | | | | | | | | |
| Alanine | 4.25 | 3.95 | 4.18 | 3.98 | 4.44 | 4.60 | 5.26 | 4.39 | 4.58 |
| Arginine | 4.96 | 4.99 | 5.27 | 5.85 | 5.71 | 6.01 | 5.63 | 5.53 | 5.98 |
| Aspartic acid | 6.85 | 6.40 | 6.59 | 7.95 | 7.62 | 7.31 | 7.26 | 7.46 | 7.37 |
| Cystine | 2.48 | 1.98 | 2.31 | 1.87 | 1.45 | 1.60 | 2.00 | 1.84 | 2.09 |
| Glutamic acid | 24.67 | 26.15 | 24.48 | 22.68 | 23.20 | 22.64 | 21.76 | 23.40 | 22.13 |
| Glycine | 3.54 | 3.48 | 3.74 | 3.51 | 3.99 | 4.00 | 3.99 | 3.86 | 4.08 |
| Histidine | 2.24 | 2.16 | 2.20 | 2.46 | 2.27 | 2.30 | 2.36 | 2.28 | 2.39 |
| Isoleucine | 3.66 | 3.76 | 3.85 | 3.98 | 3.72 | 3.80 | 3.36 | 3.68 | 3.78 |
| Leucine | 7.20 | 7.15 | 7.25 | 7.49 | 7.16 | 7.31 | 6.72 | 7.11 | 7.27 |
| Lysine | 3.90 | 3.29 | 3.85 | 4.80 | 4.35 | 4.40 | 4.63 | 4.30 | 4.58 |
| Methionine | 1.89 | 1.88 | 1.98 | 1.99 | 1.90 | 1.90 | 1.91 | 1.93 | 1.99 |
| Phenylalanine | 5.43 | 5.46 | 5.16 | 5.26 | 5.17 | 5.01 | 5.99 | 5.18 | 4.98 |
| Proline | 10.86 | 12.23 | 11.43 | 10.06 | 10.79 | 10.51 | 10.44 | 10.70 | 9.76 |
| Serine | 4.72 | 4.80 | 4.95 | 5.26 | 5.08 | 5.01 | 4.90 | 5.09 | 5.08 |
| Threonine | 3.78 | 3.76 | 3.85 | 4.21 | 3.99 | 4.00 | 4.08 | 3.95 | 3.98 |
| Tryptophan | 1.42 | 1.13 | 1.32 | 1.99 | 1.54 | 1.70 | 1.81 | 1.93 | 2.19 |
| Tyrosine | 3.31 | 2.82 | 2.86 | 3.27 | 2.81 | 3.00 | 3.27 | 2.72 | 2.89 |
| Valine | 4.84 | 4.61 | 4.73 | 3.39 | 4.81 | 4.90 | 4.63 | 4.65 | 4.88 |

[a]C/R=Carlsberg Riso mutant.

TABLE 22. AMINO ACID ANALYSIS OF GRAINS EXPRESSED AS A PERCENTAGE OF THE GRAIN

| Amino acid | Normal maize[a] | HL maize[a] | Hiproly[b] | HPN[b] |
|---|---|---|---|---|
| Alanine | .42 | .49 | .67 | .48 |
| Arginine | .27 | .52 | .81 | .65 |
| Aspartic acid | .47 | .89 | 1.06 | .78 |
| Cystine | .11 | .19 | .25 | .28 |
| Glutamic acid | 1.03 | 1.37 | 3.99 | 4.14 |
| Glycine | .20 | .34 | .53 | .44 |
| Histidine | .18 | .31 | .33 | .29 |
| Isoleucine | .21 | .29 | .57 | .49 |
| Leucine | .76 | .73 | 1.09 | .95 |
| Lysine | .18 | .38 | .67 | .45 |
| Methionine | .12 | .14 | .30 | .22 |
| Phenylalanine | .30 | .41 | .98 | .95 |
| Proline | .47 | .68 | 1.82 | 1.96 |
| Serine | .30 | .39 | .72 | .64 |
| Threonine | .22 | .33 | .56 | .48 |
| Tryptophan | .06 | .09 | .28 | .26 |
| Tyrosine | .21 | .23 | .54 | .54 |
| Valine | .27 | .42 | .75 | .59 |

[a]HL=high-lysine; represents Boz-2 and Hunt trials.
[b]HPN=Hiproly Normal; represents Boz-1 trials.

TABLE 23.. AMINO ACID ANALYSIS OF GRAINS EXPRESSED AS A PERCENTAGE OF THE TOTAL PROTEIN RECOVERED

| Amino acid | Normal maize[a] | HL maize[a] | Hiproly[b] | HPN[b] |
|---|---|---|---|---|
| Alanine | 7.27 | 5.98 | 4.21 | 3.29 |
| Arginine | 4.67 | 6.34 | 5.09 | 4.46 |
| Aspartic acid | 8.13 | 10.85 | 6.66 | 5.35 |
| Cystine | 1.90 | 2.32 | 1.57 | 1.92 |
| Glutamic acid | 17.83 | 16.71 | 25.06 | 28.37 |
| Glycine | 3.46 | 4.15 | 3.33 | 3.02 |
| Histidine | 3.11 | 3.78 | 2.07 | 1.99 |
| Isoleucine | 3.63 | 3.54 | 3.58 | 3.36 |
| Leucine | 13.15 | 8.90 | 6.85 | 6.51 |
| Lysine | 3.11 | 4.63 | 4.21 | 3.08 |
| Methionine | 2.08 | 1.71 | 1.88 | 1.51 |
| Phenyalanine | 5.19 | 5.00 | 6.16 | 6.51 |
| Proline | 8.13 | 8.29 | 11.43 | 13.43 |
| Serine | 5.19 | 4.76 | 4.52 | 4.39 |
| Threonine | 3.81 | 4.02 | 3.52 | 3.29 |
| Tryptophan | 1.04 | 1.10 | 1.76 | 1.78 |
| Tyrosine | 3.63 | 2.80 | 3.39 | 3.70 |
| Valine | 4.67 | 5.12 | 4.71 | 4.04 |

[a]HL=high-lysine; represents Boz-2 and Hunt trials.
[b]HPN=Hiproly Normal; represents Boz-1 trials.

## RESULTS OF BIOLOGICAL RAT TRIALS

Trial I, Ia, Ib. The rat growth data of trial I are shown in table 24. Bomi Riso mutant 1508 produced a higher ($P<.05$) PER than any other Bomi derived barley cultivar with the exception of Bomi Riso mutant 7 to which it was approximately equal. The feed efficiency ratio of Bomi Riso mutant 1508 was better ($P<.05$) than that of Bomi Riso mutants 9 and 13 and the parent line, Bomi, as well as Hiproly and Hiproly Normal. Of the Carlsberg lines, Carlsberg Riso mutant 56 had a superior ($P<.05$) feed efficiency ratio than the parent line but similar to Carlsberg Riso mutant 29. The same relationship held true for PER values. Carlsberg Riso mutants 29 and 56 and Bomi Riso mutants 7 and 1508 PERs and feed efficiencies were not different from casein as was the case for the feed efficiency of Bomi Riso mutant 8 ($P>.05$). Bomi Riso mutant 9 showed the poorest ($P<.05$) feed efficiency value of all the barleys except Bomi Riso mutant 13 and Hiproly Normal which were similar.

The biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU) values of trial Ia and Ib are shown in tables 25 and 26, respectively. In trials Ia and Ib there were no differences ($P>.05$) in NPU of the individual barleys. There were no differences ($P>.05$) in the biological values in trial Ib. Bomi Riso mutants 1508 and 8 produced similar biological values but were not different ($P>.05$) from the other mutants. However, Bomi Riso mutants 1508 and 8 were higher ($P<.05$) in regard to biological value when compared to that of

Bomi, Hiproly and Hiproly Normal. Hiproly Normal showed the highest ($P<.05$) true protein digestibility in trial Ia. In trial Ib, Carlsberg Riso mutant 29 showed a lower ($P<.05$) TPD than that of all other barleys except Carlsberg II (table 26). Hiproly, Hiproly Normal and Carlsberg Riso mutant 56 (table 26) were higher in TPD ($P<.05$) than the other barleys, but not different between themselves, or, with the exception of Hiproly Normal, from the parent line with respect to true protein digestibility. Bomi Riso mutant 13 had a higher ($P<.05$) BV than Hiproly Normal but both barleys were similar to Bomi Riso mutants 7 and 9, Bomi and Hiproly (table 25). Bomi Riso mutant 13 had a lower ($P<.05$) TPD than Bomi, Hiproly, Hiproly Normal and Bomi Riso mutant 9, but was similar to Bomi Riso mutants 7, 8 and 1508. Bomi Riso mutant 7 and 8 were also similar to Hiproly, Bomi, Bomi Riso mutants 9 and 1508.

Trial II, III, IIIa. Performance of rats in growth trials II and III are shown in table 27. High-lysine maize showed the highest PER and best feed efficiency ratio ($P<.05$) compared to all other cereal diets, however, both measurements were not different ($P>.05$) from those of the casein diet. Bomi Riso mutant 1508 showed a better ($P<.05$) feed efficiency ratio than Bomi but neither were different from Hiproly ($P>.05$). The same relationship was exibited for high PER values with only the Bomi Riso mutant 1508 being similar to Hiproly. Normal maize exibited the poorest ($P<.05$) feed efficiency ratio and the lowest ($P<.05$) PER of all the diets compared.

The measurements from nitrogen balance trial IIIa are shown in table 28. No differences were shown in the NPU of the individual grains. Hiproly and high-lysine maize were not different ($P > .05$) from Hiproly Normal and normal maize in TPD and were higher ($P < .05$) in this respect than the other barleys. The TPD of both Bomi barleys and both Bomi Riso mutant 1508 barleys was not different from each other or from Hiproly Normal and normal maize. The BV of Bomi Riso mutant 1508 (Boz-2) was numerically higher than all other diets. It was however, not different ($P > .05$) from Bomi (Hunt) or Bomi Riso mutant 1508 (Hunt), but higher ($P < .05$) in BV than the two maize diets, Hiproly, Hiproly Normal and Bomi (Boz-2).

Trial IV, IVa, V, Va. The results of trials IV and V are shown in table 29. In trials IV and V, Carlsberg Riso mutant 86 appeared to have a higher PER than Carlsberg Riso mutant 29, but statistically there was no difference ($P > .05$). The PER of Carlsberg Riso mutant 86 was higher ($P < .05$) than the parent line, Carlsberg II, Hiproly, Hiproly Normal and normal maize but lower ($P < .05$) than high-lysine maize and casein. The best feed efficiency ratio belonged to casein but was not significantly different from high-lysine maize ($P > .05$). The feed efficiency ratio of high-lysine maize appeared to be superior to Carlsberg Riso mutant 29, but the difference was not significant ($P > .05$). Carlsberg Riso mutants 29 and 86 showed similar ($P > .05$) feed efficiency ratios and Carlsberg Riso mutant 86 was also similar to Carlsberg II ($P > .05$) in regard to

feed efficiency ratio. Hiproly, Hiproly Normal and normal maize showed poorer ($P<.05$) feed efficiency ratios than any other diet but they were not different from each other.

Biological values, true protein digestibilities and net protein utilization values of trials IVa and Va are shown in tables 30 and 31, respectively. The BV of both Boz-2 and Hunt Carlsberg Riso mutant 86 barleys were numerically highest but not different ($P>.05$) from either of the Carlsberg Riso 29 mutants. It was however, higher ($P<.05$) in BV than Carlsberg II and all other barley and maize cultivars. Hiproly, Hiproly Normal, normal maize and high-lysine maize showed the lowest ($P<.05$) biological values of trial IVa with all four showing a similarity ($P>.05$) to Carlsberg II as did Carlsberg Riso mutant 29. In both trials no differences ($P>.05$) were shown between NPU values. Trial IVa showed no differences ($P>.05$) among the TPD values for the various diets. The TPD values of Carlsberg Riso mutant 29 (Hunt) and Carlsberg Riso mutant 86 (Hunt) were lower ($P<.05$) than any other diet in trial Va except for the similarity between Carlsberg II and Carlsberg Riso mutant 86. Normal maize showed the highest TPD although it was the same as that of Hiproly, Hiproly Normal and high-lysine maize ($P>.05$), the latter of which was also similar to Carlsberg II ($P>.05$). Carlsberg Riso mutant 29 had a similar BV to Carlsberg II with only Carlsberg II being the same as high-lysine maize and Hiproly ($P>.05$). In turn, Hiproly was the same ($P>.05$) as normal maize and high-lysine maize while Hiproly Normal, nor-

mal maize and high-lysine maize showed similarities (P>.05).

Trial VI. The results of the determinations for metabolic fecal nitrogen (MN) and endogenous urinary nitrogen (EN) are shown in table 32. The average MN and EN were .014 g/g dry matter consumed and .065 g N/rat/4 days, respectively. All rats lost weight while being fed the nitrogen-free diet (table 17).

TABLE 24. LEAST SQUARES MEANS OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS GRAIN AND CASEIN DIETS FOR 28 DAYS, TRIAL I (BOZ-1)

| Diet[g] | No. rats | Gain, g | Feed consumed, g | Feed/gain ratio | Adjusted PER |
|---|---|---|---|---|---|
| HP | 10 | $71.8^{bc}$ | $366.1^{ab}$ | $5.13^{bcd}$ | $2.05^{de}$ |
| HPN | 10 | $65.2^{cd}$ | $360.5^{abc}$ | $5.60^{ab}$ | $1.79^{f}$ |
| Bomi | 10 | $68.5^{bcd}$ | $338.3^{bc}$ | $5.08^{cd}$ | $2.13^{de}$ |
| B/R 7 | 10 | $74.2^{abc}$ | $323.8^{bc}$ | $4.42^{ef}$ | $2.39^{abc}$ |
| B/R 8 | 10 | $76.9^{abc}$ | $360.7^{abc}$ | $4.71^{def}$ | $2.25^{bcd}$ |
| B/R 9 | 10 | $59.5^{d}$ | $336.9^{bc}$ | $5.74^{a}$ | $1.83^{f}$ |
| B/R 13 | 10 | $74.2^{abc}$ | $393.3^{a}$ | $5.36^{abc}$ | $1.99^{ef}$ |
| B/R 1508 | 10 | $84.4^{a}$ | $358.4^{abc}$ | $4.32^{f}$ | $2.48^{a}$ |
| Carlsberg II | 10 | $66.5^{cd}$ | $320.8^{c}$ | $4.86^{cde}$ | $2.20^{cde}$ |
| C/R 29 | 10 | $80.1^{ab}$ | $352.4^{abc}$ | $4.39^{ef}$ | $2.37^{abc}$ |
| C/R 56 | 10 | $84.8^{a}$ | $359.9^{abc}$ | $4.29^{f}$ | $2.44^{a}$ |
| Casein | 10 | $79.9^{ab}$ | $338.7^{bc}$ | $4.31^{f}$ | $2.50^{a}$ |

[abcdef] Means with different superscript letters in the same column are significantly different (P<.05).

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

TABLE 25. LEAST SQUARES MEANS OF BIOLOGICAL VALUE (BV), TRUE PROTEIN DIGESTIBILITY (TPD) AND NET PROTEIN UTILIZATION (NPU) OF RATS, TRIAL Ia (BOZ-1)

| Diet[d] | No. rats | BV | TPD | NPU |
|---|---|---|---|---|
| HP | 4 | $75.8^{bc}$ | $76.0^{b}$ | $57.8^{a}$ |
| HPN | 4 | $73.5^{c}$ | $86.8^{a}$ | $64.0^{a}$ |
| Bomi | 4 | $75.1^{bc}$ | $75.4^{b}$ | $56.6^{a}$ |
| B/R 7 | 4 | $84.4^{abc}$ | $74.0^{bc}$ | $62.3^{a}$ |
| B/R 8 | 4 | $91.7^{a}$ | $69.3^{bc}$ | $63.3^{a}$ |
| B/R 9 | 4 | $82.4^{abc}$ | $77.7^{b}$ | $64.0^{a}$ |
| B/R 13 | 4 | $87.1^{ab}$ | $65.9^{c}$ | $57.2^{a}$ |
| B/R 1508 | 4 | $91.6^{a}$ | $69.2^{bc}$ | $63.3^{a}$ |

[abc]Means with different superscript letters in the same column are significantly different (P<.05).

[d]HP=Hiproly, HPN=Hiproly Normal, B/R=Bomi Riso mutant.

TABLE 26. LEAST SQUARES MEANS OF BIOLOGICAL VALUE (BV), TRUE PROTEIN DIGESTIBILITY (TPD) AND NET PROTEIN UTILIZATION (NPU) OF RATS, TRIAL Ib (BOZ-1)

| Diet[d] | No. rats | BV | TPD | NPU |
|---|---|---|---|---|
| HP | 4 | 71.0[a] | 85.3[ab] | 60.6[a] |
| HPN | 4 | 66.4[a] | 86.8[a] | 57.5[a] |
| Carlsberg II | 4 | 75.7[a] | 79.0[bc] | 59.7[a] |
| C/R 29 | 4 | 75.7[a] | 76.3[c] | 58.0[a] |
| C/R 56 | 4 | 76.6[a] | 85.8[ab] | 65.8[a] |

[abc]Means with different superscript letters in the same column are significantly different (P<.05).
[d]HP=Hiproly, HPN=Hiproly Normal, C/R=Carlsberg Riso mutant.

TABLE 27. LEAST SQUARES MEANS OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS GRAIN AND CASEIN DIETS FOR 28 DAYS, TRIAL II (BOZ-2) AND III (HUNT)

| Diet[f] | No. rats | Gain, g | Feed consumed, g | Feed/gain ratio | Adjusted PER |
|---|---|---|---|---|---|
| HP | 20 | 67.2[cd] | 394.1[ab] | 6.00[cd] | 2.06[b] |
| HPN | 20 | 56.4[e] | 395.3[ab] | 7.12[b] | 1.74[c] |
| Normal maize | 20 | 43.6[f] | 338.8[c] | 7.93[a] | 1.56[d] |
| HL maize | 20 | 84.0[a] | 427.3[a] | 5.15[e] | 2.39[a] |
| Bomi | 20 | 60.3[de] | 372.4[bc] | 6.25[c] | 1.85[c] |
| B/R 1508 | 20 | 75.1[bc] | 408.4[ab] | 5.66[d] | 2.16[b] |
| Casein | 20 | 78.3[ab] | 378.9[b] | 4.86[e] | 2.50[a] |
| Trial II | 70 | 65.7[a] | 392.5[a] | 6.30[a] | 2.00[b] |
| Trial III | 70 | 67.1[a] | 383.3[a] | 5.98[b] | 2.08[a] |

[abcde]Means in the same column with different superscript letters are significantly different (P<.05).
[f]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi Riso mutant

TABLE 28. LEAST SQUARES MEANS OF BIOLOGICAL VALUE (BV), TRUE PROTEIN DIGESTIBILITY (TPD) AND NET PROTEIN UTILIZATION (NPU) OF RATS, TRIAL IIIa (BOZ-2 AND HUNT)

| Diet[d] | No. rats | BV | TPD | NPU |
|---|---|---|---|---|
| HP | 4 | $69.3^{bc}$ | $91.0^{a}$ | $61.5^{a}$ |
| HPN | 4 | $64.7^{c}$ | $87.0^{ab}$ | $56.7^{a}$ |
| Normal maize | 4 | $63.6^{c}$ | $86.6^{ab}$ | $55.4^{a}$ |
| HL maize | 4 | $66.2^{bc}$ | $91.5^{a}$ | $60.8^{a}$ |
| Bomi (Boz-2) | 4 | $68.2^{bc}$ | $82.6^{b}$ | $56.4^{a}$ |
| B/R 1508 (Boz-2) | 4 | $80.2^{a}$ | $82.0^{b}$ | $65.9^{a}$ |
| Bomi (Hunt) | 4 | $73.8^{abc}$ | $80.9^{b}$ | $59.7^{a}$ |
| B/R 1508 (Hunt) | 4 | $75.7^{abc}$ | $82.5^{b}$ | $62.5^{a}$ |

[abc]Means with different superscript letters in the same column are significantly different ($P<.05$).
[d]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, B/R=Bomi Riso mutant.

TABLE 29. LEAST SQUARES MEANS OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS GRAIN AND CASEIN DIETS FOR 28 DAYS, TRIAL IV (BOZ-2) AND V (HUNT)

| Diet[f] | No. rats | Gain, g | Feed consumed, g | Feed/gain ratio | Adjusted PER |
|---|---|---|---|---|---|
| HP | 16 | $48.4^{b}$ | $346.6^{c}$ | $7.27^{a}$ | $1.72^{e}$ |
| HPN | 17 | $53.4^{b}$ | $392.0^{b}$ | $7.50^{a}$ | $1.68^{e}$ |
| Normal maize | 18 | $48.3^{b}$ | $342.6^{c}$ | $7.28^{a}$ | $1.74^{e}$ |
| HL maize | 20 | $79.5^{a}$ | $426.3^{ab}$ | $5.41^{de}$ | $2.36^{b}$ |
| Carlsberg II | 19 | $66.9^{b}$ | $411.5^{ab}$ | $6.23^{b}$ | $2.00^{d}$ |
| C/R 29 | 19 | $75.0^{a}$ | $433.5^{ab}$ | $5.80^{cd}$ | $2.08^{cd}$ |
| C/R 86 | 20 | $74.3^{a}$ | $439.9^{ab}$ | $5.93^{bc}$ | $2.13^{c}$ |
| Casein | 18 | $79.8^{a}$ | $401.4^{ab}$ | $5.07^{e}$ | $2.50^{a}$ |
| Trial IV | 71 | $64.9^{a}$ | $391.9^{a}$ | $6.20^{b}$ | $2.03^{a}$ |
| Trial V | 76 | $66.5^{a}$ | $405.8^{a}$ | $6.42^{a}$ | $2.02^{a}$ |

[abcde]Means with different superscript letters in the same column are significantly different ($P<.05$).
[f]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, C/R=Carlsberg Riso mutant.

TABLE 30. LEAST SQUARES MEANS OF BIOLOGICAL VALUE (BV), TRUE PROTEIN DIGESTIBILITY (TPD) AND NET PROTEIN UTILIZATION (NPU) OF RATS, TRIAL IVa (BOZ-2)

| Diet[d] | No. rats | BV | TPD | NPU |
|---|---|---|---|---|
| HP | 4 | $66.9^{c}$ | $83.4^{a}$ | $55.7^{a}$ |
| HPN | 4 | $69.5^{c}$ | $82.8^{a}$ | $58.3^{a}$ |
| Normal maize | 4 | $64.8^{c}$ | $77.8^{a}$ | $50.9^{a}$ |
| HL maize | 4 | $69.0^{c}$ | $85.3^{a}$ | $58.6^{a}$ |
| Carlsberg II | 4 | $73.9^{bc}$ | $85.6^{a}$ | $63.3^{a}$ |
| C/R 29 | 4 | $82.4^{ab}$ | $72.2^{a}$ | $59.5^{a}$ |
| C/R 86 | 4 | $86.7^{a}$ | $77.9^{a}$ | $67.7^{a}$ |

[abc]Means with different superscript letters in the same column are significantly different ($P<.05$).

[d]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, C/R=Carlsberg Riso mutant.

TABLE 31. LEAST SQUARES MEANS OF BIOLOGICAL VALUE (BV), TRUE PROTEIN DIGESTIBILITY (TPD) AND NET PROTEIN UTILIZATION (NPU) OF RATS, TRIAL Va (HUNT)

| Diet[f] | No. rats | BV | TPD | NPU |
|---|---|---|---|---|
| HP | 4 | $77.0^{cd}$ | $83.0^{ab}$ | $63.8^{a}$ |
| HPN | 4 | $67.6^{e}$ | $84.5^{ab}$ | $57.0^{a}$ |
| Normal maize | 4 | $69.6^{de}$ | $90.7^{a}$ | $63.3^{a}$ |
| HL maize | 4 | $74.7^{cde}$ | $86.5^{ab}$ | $64.6^{a}$ |
| Carlsberg II | 4 | $79.9^{bc}$ | $79.5^{bc}$ | $63.4^{a}$ |
| C/R 29 | 4 | $86.4^{ab}$ | $67.3^{d}$ | $58.2^{a}$ |
| C/R 86 | 4 | $89.8^{a}$ | $69.9^{cd}$ | $62.7^{a}$ |

[abcde]Means with different superscript letters in the same column are significantly different ($P<.05$).

[f]HP=Hiproly, HPN=Hiproly Normal, HL=high-lysine, C/R=Carlsberg Riso mutant.

TABLE 32. METABOLIC FECAL NITROGEN (MN) AND ENDOGENOUS URINARY NITROGEN (EN) EXCRETION BY RATS FED A NITROGEN-FREE DIET, TRIAL VIa

| Rat no. | MN (g/g dm consumed) | EN (g/rat/day) |
|---|---|---|
| 1 | .0012 | .120 |
| 2 | .0024 | .130 |
| 3 | .0015 | .170 |
| 4 | .0007 | .230 |
| 5 | .0005 | .107 |
| 6 | .0024 | .143 |
| 7 | .0008 | .127 |
| 8 | .0027 | .170 |
| 9 | .0008 | .167 |
| 10 | .0010 | .250 |
| Average | .0140 | 1.614 |

## AMINO ACID COMPOSITION AND PROTEIN FRACTIONS IN RELATION TO BIOLOGICAL MEASUREMENTS

Trial I, Ia, Ib. Bomi Riso mutant 8 showed a 10% reduction in hordeins and an 11% increase in the water and salt soluble fractions over its parent, Bomi (Boz-1) (table 33). The lysine content (g/16 g N) of Bomi Riso mutant 8 was 4.76%, compared to 3.61% for Boz-1 Bomi. Bomi Riso mutant 1508 showed the highest lysine content (g/16 g N) of all the barleys with a value of 5.60%. This mutant also showed an 80% reduction in the lysine poor hordeins and a 49% increase in the lysine rich albumin + globulin fraction over the parent. Bomi Riso mutants 7, 9 and 13 showed only slight changes in their Osborne fractions compared to Bomi. The lysine content (g/16 g N) of these three mutants ranged from increases of 5% (Bomi Riso mutant 9) to 30% (Bomi Riso mutant 13) over Bomi. Carlsberg Riso mutants 29 and 56 showed lysine contents (g/16 g N) of 4.80% and 4.63%, respectively, compared to 3.90% for Carlsberg II (table 34). Both mutants exhibited an 18% increase in the albumin + globulin fraction with a 39% reduction in the hordeins in Carlsberg Riso mutant 29 and a 21% reduction in Carlsberg Riso mutant 56. Carlsberg Riso mutant 29 also showed an increase of 37% of glutelins over that of Carlsberg II.

The reduction in hordeins and increase in albumins + globulins and the extremely high lysine content (g/16 g N) of Bomi Riso mutant 1508 (Boz-1) are reflected in the biological measurements of feed efficiency, PER and BV. Bomi Riso mutant 8 showed a fairly high lysine content

(g/16 g N) as compared to Bomi and the changes in the Osborne fractions of the mutant should have resulted in above average biological measurements. The BV of Bomi Riso mutant 8 was very high, however, the PER value was not what was expected on the basis of its chemical composition. Since a duplicate of Bomi Riso mutant 8 was not run in a future trial, it is not possible to say at this time if the low PER value reflects the barley's true value or if something was amiss elsewhere. Bomi Riso mutant 7 did not show large differences in its chemical composition compared to Bomi. It did however have a feed efficiency ratio and PER which were not different ($P>.05$) from those of casein and a BV which was lower but not different ($P>.05$) from that of Bomi Riso mutant 1508. The Carlsberg Riso mutants did not result in exceptional biological measurements. There was some improvement in the amino acid compsition and Osborne fractions in regard to reduced hordeins and in the amino acid lysine (g/16 g N) over that of Carlsberg II and this small change was reflected by a slight improvement in the biological measurements of the mutants. Of the two Carlsberg Riso mutants, Carlsberg Riso mutant 56 appeared to have a slight advantage from a nutritional viewpoint.

Trial II, III, IIIa. The two Bomi Riso mutant 1508 barleys (Boz-2 and Hunt) were respectively 32% and 22% higher in albumin + globulins and approximately 27% lower in hordeins than the two parent Bomi barleys. The amino acid composition of the mutants reflected higher levels of

alanine, aspartic acid, glycine, histidine, methionine, threonine and tryptophan and lower levels of glutamic acid, phenylalanine and proline. Lysine (g/16 g N) was also increased in the mutants by 26% for Bomi Riso mutant 1508 (Boz-2) and 22% for Bomi Riso mutant 1508 (Hunt) over that of their respective parents. Although the changes in lysine content (g/16 g N) of the mutants were not very large, the PER values of the parents and mutants were different ($P<.05$); however, the PERs of the mutants were also lower ($P<.05$) than casein and high-lysine maize. The BV of Bomi Riso mutant 1508 (Boz-2) reflected its greatest lysine content compared to Bomi Riso mutant 1508 (Hunt) and both Bomi barleys used in trial IIIa. Both mutants and one parent (Hunt) showed higher ($P<.05$) BV values than high-lysine maize also which was not expected since high-lysine maize contained more lysine (g/16 g N) (table 23) than any of those barleys.

Trial IV, IVa, V, Va. Both Boz-2 and Hunt Carlsberg Riso mutant 86 barleys and the Boz-2 Carlsberg Riso mutant 29 showed a 17% increase in the albumin + globulin fractions over the Carlsberg II lines. Carlsberg Riso mutant 29 (Hunt) showed a 19% increase in that fraction. The Boz-2 and Hunt Carlsberg Riso mutant 29 barleys showed a greater decrease in the lysine poor hordeins (22%) than that of Carlsberg Riso mutant 86 (Boz-2) (9%) and Carlsberg Riso mutant 86 (Hunt) (14%). The Boz-2 mutants also showed a 14% increase in their glutelin content compared to Carlsberg II (table 34). The amino acid compositions of the Carlsberg II and

Carlsberg II derived mutants are shown in tables 20 and 21. The Boz-2 Carlsberg Riso mutants were lower in lysine content (g/16 g N) than the Hunt Carlsberg Riso mutants. The increase in lysine content (g/16 g N) of the Boz-2 Carlsberg Riso mutants 29 and 86 over their parent line was, however, more than the increase of the Hunt Carlsberg Riso mutants over their parent line.

Carlsberg Riso mutant 86 (Boz-2 and Hunt) averaged slightly more lysine (g/16 g N) than the two Carlsberg Riso mutant 29 and the two Carlsberg II barleys. This resulted in a higher PER value for Carlsberg Riso mutant 86 than for Carlsberg Riso mutant 29 ($P<.05$) and Carlsberg II ($P<.05$). The high lysine content (g/16 g N) of high-lysine maize (table 23) was reflected in the PER value of that diet which was the highest ($P<.05$) of the grain diets in trials IV and V. The BV of Carlsberg Riso mutant 86 (Boz-2 and Hunt) was higher than the Carlsberg II and high-lysine maize ($P<.05$) and Carlsberg Riso mutant 29 ($P<.05$) barleys which was not an indication of the relationship of the amino acid composition (g/16 g N) of the grains.

TABLE 33. PERCENTAGE OF PROTEIN, LYSINE AND PROTEIN FRACTIONS OF BOMI AND BOMI DERIVED CULTIVARS

| Barley[a] | % Protein[b] | % Lysine[c] | % Al/Glo[d] | % Hord[d] | % Glu[d] | % Resi[d] |
|---|---|---|---|---|---|---|
| Bomi (Boz-1) | 12.43 | 3.61 | 37 | 25 | 24 | 13 |
| Bomi (Boz-2) | 12.21 | 3.53 | 39 | 21 | 28 | 13 |
| Bomi (Hunt) | 12.16 | 3.62 | 36 | 20 | 32 | 11 |
| B/R 7 (Boz-1) | 12.12 | 4.07 | 41 | 19 | 24 | 15 |
| B/R 8 (Boz-1) | 13.00 | 4.76 | 48 | 15 | 25 | 13 |
| B/R 9 (Boz-1) | 13.17 | 3.79 | 37 | 21 | 31 | 10 |
| B/R 13 (Boz-1) | 13.09 | 4.68 | 45 | 18 | 24 | 13 |
| B/R 1508 (Boz-1) | 13.13 | 5.60 | 55 | 5 | 24 | 15 |
| B/R 1508 (Boz-2) | 12.60 | 4.45 | 49 | 15 | 19 | 18 |
| B/R 1508 (Hunt) | 11.29 | 4.41 | 45 | 15 | 25 | 16 |

[a]B/R=Bomi Riso mutant.
[b]Protein=N x 6.25.
[c]Lysine=g/16 g N.
[d]Al/Glo=albumins + globulins, Hord=hordeins, Glu=glutelins, Resi=residue.

TABLE 34. PERCENTAGE OF PROTEIN, LYSINE AND PROTEIN FRACTIONS OF CARLSBERG II AND CARLSBERG II DERIVED CULTIVARS

| Barley[a] | % Protein[b] | % Lysine[c] | % Al/Glo[d] | % Hord[d] | % Glu[d] | % Resi[d] |
|---|---|---|---|---|---|---|
| C II (Boz-1) | 10.72 | 3.90 | 38 | 28 | 19 | 15 |
| C II (Boz-2) | 12.65 | 3.29 | 35 | 27 | 21 | 16 |
| C II (Hunt) | 10.02 | 3.85 | 36 | 22 | 26 | 16 |
| C/R 29 (Boz-1) | 11.11 | 4.80 | 45 | 17 | 26 | 12 |
| C/R 29 (Boz-2) | 12.95 | 4.35 | 41 | 21 | 24 | 14 |
| C/R 29 (Hunt) | 11.55 | 4.40 | 43 | 20 | 25 | 13 |
| C/R 56 (Boz-1) | 13.13 | 4.63 | 45 | 22 | 20 | 13 |
| C/R 86 (Boz-2) | 13.39 | 4.30 | 41 | 21 | 24 | 13 |
| C/R 86 (Hunt) | 11.42 | 4.58 | 42 | 19 | 26 | 13 |

[a]C=Carlsberg, C/R=Carlsberg Riso mutant.
[b]Protein=N x 6.25.
[c]Lysine=g/16 g N.
[d]Al/Glo=albumins + globulins, Hord=hordeins, Glu=glutelins, Resi=residue.

## RESULTS OF PHYSICAL CHARACTERIZATION

Of the Bomi derived mutants, Bomi Riso mutant 7 was the best yielding variety as compared to the parent variety (table 35). It was slightly lower in test weight and the ratio of plump to thin kernels was decreased from about 4:1 in Bomi to 2:1 in the mutant. Bomi Riso mutant 1508 showed almost a 25% decrease in yield compared to Bomi. The test weight and kernel weight of Bomi Riso mutant 1508 was also lower than that of the parent and it averaged almost equal percentages of plump and thin kernels in the nine samples grown. Bomi Riso mutant 9 showed the lowest average yield of all the Bomi Riso mutants while Bomi Riso mutant 8 and 13 barleys averaged about 31.5 q/ha. Bomi Riso mutant 8 averaged the lowest test weight and kernel weight of all the Bomi and Bomi derived mutant barleys.

Carlsberg Riso mutant 86 was the lowest yielding Carlsberg II derived barley followed by Carlsberg Riso mutants 56 and 29 in that order (table 36). Carlsberg Riso mutant 86 showed a reversal in the ratio of plump to thin kernels as compared to Carlsberg II as well as the lowest test and kernel weights of all the mutants. Carlsberg Riso mutants 29 and 56 showed decreases in kernel weight of approximately 2.4 mg and in test weight of 5.0 and 6.6 kg/hl, respectively.

TABLE 35. PHYSICAL CHARACTERIZATION OF BOMI AND BOMI DERIVED MUTANTS

| Barley[a] | No. obs. | % plump | % thin | Yield (q/ha)[b] | Test weight (kg/hl)[c] | Kernel weight (mg) |
|---|---|---|---|---|---|---|
| Bomi | 9 | 60.5 | 16.4 | 38.5 | 62.9 | 39.8 |
| B/R 7 | 8 | 52.0 | 22.5 | 38.4 | 52.3 | 38.7 |
| B/R 8 | 8 | 14.9 | 58.1 | 31.5 | 53.7 | 30.8 |
| B/R 9 | 8 | 30.0 | 33.8 | 26.4 | 56.4 | 33.7 |
| B/R 13 | 8 | 11.1 | 65.1 | 31.4 | 56.0 | 33.1 |
| B/R 1508 | 9 | 35.0 | 33.8 | 29.0 | 55.7 | 32.0 |

[a]B/R=Bomi Riso mutant.
[b]q/ha=quintals per hectare.
[c]kg/hl=kilograms per hectoliter.

TABLE 36. PHYSICAL CHARACTERIZATION OF CARLSBERG II AND CARLSBERG II DERIVED MUTANTS

| Barley[a] | No. obs. | % plump | % thin | Yield (q/ha)[b] | Test weight (kg/hl)[c] | Kernel weight (mg) |
|---|---|---|---|---|---|---|
| C II | 9 | 56.7 | 20.9 | 35.1 | 62.4 | 36.1 |
| C/R 29 | 9 | 32.6 | 40.7 | 32.4 | 57.4 | 33.8 |
| C/R 56 | 8 | 22.3 | 44.5 | 29.4 | 55.8 | 33.6 |
| C/R 86 | 9 | 20.0 | 53.3 | 25.3 | 51.7 | 29.0 |

[a]C=Carlsberg, C/R=Carlsberg Riso mutant.
[b]q/ha=quintals per hectare.
[c]kg/hl=kilograms per hectoliter.

## RESULTS OF LEAST SQUARES ANALYSIS

Least squares analysis for trials I through V are shown in appendix tables 37, 38 and 39. Data from trial I was analyzed separately, whereas data from trials II and III were combined as were data from trials IV and V. This was possible since the same barley and maize cultivars were fed in trials II and III and in trials IV and V, respectively. The cultivars in the combined trials differed only in the location where they were grown.

There was a significant ration x location interaction ($P<.05$) in both analyses for feed efficiency ratio and adjusted PER. In the data from trials II and III, there was a significant ($P<.01$) effect due to ration and location for these measurements. The feed efficiency ratio and PER favored trial III for Hiproly, Hiproly Normal, normal maize and high-lysine maize whereas in trial II these data were superior for Bomi, Bomi Riso mutant 1508 and the casein diets.

Ration but not location showed a significant ($P<.01$) effect in trials IV and V. However, there was a significant ($P<.05$) ration x location interaction for feed efficiency ratio and adjusted PER as in trials II and III. The feed efficiency ratio for Hiproly, Hiproly Normal, normal maize and Carlsberg Riso mutant 86 was best in trial IV whereas it was best in trial V for Carlsberg II and Carlsberg Riso mutant 29. Differences between trials for feed efficiency ratio for high-lysine maize and the casein diets were negligible. The effect on

PER was similar to the effect on feed efficiency ratio except there was no difference in PER for the Hiproly Normal diet in trials IV and V.

## STEPWISE MULTIPLE REGRESSION ANALYSIS OF AMINO ACIDS (G/16 G N) ON BIOLOGICAL MEASUREMENTS

A stepwise multiple regression analysis was computed using the amino acids (g/16 g N) as the independent variable and gain, feed consumption, feed efficiency, PER, BV, TPD and NPU as the dependent variables (tables 40 through 46, respectively).

Lysine was the primary amino acid which accounted for the variance in three measurements. They were gain (R=.7814), protein efficiency ratio (R=.7751) and biological value (R=.6199). Lysine also exerted a secondary effect on feed efficiency and was the third most important amino acid contributing to the net protein utilization values. The most influencial amino acids on feed consumption, feed efficiency, true protein digestibility and net protein utilization were tyrosine (R= .5043), leucine (R=.4254), tryptophan (R=.5226) and leucine (R=.4254), respectively.

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 parameters except feed consumption and net protein utilization, the majority of that variance was accounted for by the first five amino acids.

## RESULTS OF CORRELATIONS

Correlation coefficients of amino acids with the parameters from the growth and nitrogen balance trials are shown in tables 47 and 48. Tyrosine, with an r=-.50, was the only amino acid to be correlated ($P<.05$) with feed consumption. The differences between consumption of the various diets must therefore be attributed to other influencing factors such as palatability and fiber of barley origin. Seven amino acids were significantly correlated to gain. They were arginine (r=.72), glycine (r=.60), lysine (r=.78), threonine (r=.56) ($P<.01$), aspartic acid (r=.50), leucine (r=-.50) and tryptophan (r=.38) ($P<.05$). Arginine (r=-.44) ($P<.05$) and leucine (r=.68) ($P<.01$) were also correlated with feed efficiency ratios. Protein efficiency ratios were correlated with arginine (r=.67), glycine (r=.57), lysine (r=.78), threonine (r=.64) ($P<.01$) and aspartic acid (r=.42) ($P<.05$). Biological value was highly ($P<.01$) correlated with arginine (r=.53), glycine (r=.59), lysine (r=.62) and tryptophan (r=.62) and less correlated ($P<.05$) with leucine (r=-.50), phenylalanine (r=-.41) and threonine (r=.47). True protein digestibility was negatively correlated ($P<.01$) with lysine and tryptophan (r=-.52 for each). Glycine (r=.41) and leucine (r=-.43) were correlated ($P<.05$) with net protein utilization.

Lysine was highly correlated ($P<.01$) with gain, feed efficiency and PER as well as with biological value and true protein digestibility. In each instance, the correlation coefficients of lysine with these

measurements were or approached the highest values, thus showing the importance of this amino acid for proper nutrition.

## DISCUSSION

None of the Hiproly barleys fed in the rat growth or metabolism trials performed as expected based on the findings of Munck et al. (1970) and Newman et al. (1974). The protein and lysine content (g/16 g N) (table 23) of Hiproly was higher (4.21%) than that of Hiproly Normal (3.08%) and in most cases, the feed efficiency, PER and BV of Hiproly were slightly higher than those of Hiproly Normal. There were no obvious reasons for the poor performance of the Hiproly barleys used for this thesis work.

It was believed following completion of the trials that the Boz-2 and Hunt Bomi Riso 1508 mutants were not pure samples. Screening showed the samples to contain two different sized kernels, one which contained a high lysine content (presumably the mutant) and one with a normal lysine content (possibly Bomi). Further evidence was established when it was seen that a pure sample of Bomi Riso mutant 1508 contained less than 1% plump kernels while the samples used in trials II, III and IIIa contained 25% plump kernels. The Osborne fractions of all three Bomi Riso mutant 1508 barleys, shown in table 33, indicated lower albumin + globulin fractions and higher hordeins for the Boz-2 and Hunt mutants than for the Boz-1 mutant which was a pure seed sample. The amino acid composition (g/16 g N) (table 19) of the mutants from Boz-2 and Hunt locations also showed increased glutamic acid, proline and tryptophan and decreased alanine, arginine, aspartic acid, glycine, lysine, serine,

threonine, tyrosine and valine as compared to the Boz-1 Bomi Riso mutant 1508. These facts may very well explain the differences in PER and BV obtained for Bomi Riso mutant 1508 used in trial I and Ia and the two Bomi Riso mutant 1508 barleys fed in trials II, III and IIIa.

Regardless of the possible dilution of the two Bomi Riso mutant 1508 samples, the barleys did give impressive biological measurements. The average yields were however not as good as those obtained by Ingverson et al. (1973d). These discrepancies may be due to differences in the growing conditions and management practices between the two countries. Ingverson et al. (1973d) made no mention of the number of locations from which he obtained his figures. When considering the values in table 35 were derived from only nine locations, it seems apparent that more samples need to be planted and grown before an accepted "average" can be placed on the growing capabilities of Bomi Riso mutant 1508. The BV of Bomi Riso mutant 1508 from trial I was 91.6% which is higher than the BV found by Doll et al. (1974). These researchers found a seed size of 41 mg, a lysine content of 5.30 g/16 g N and a TPD of 78% for Bomi Riso mutant 1508 which corresponds to the data found in this thesis work of 32.0 mg, 5.60 g/16 g N and 69.2%, respectively. Since the lysine content (g/16 g N) of Boz-1 Bomi Riso mutant 1508 was higher than that of the barley used by Doll and his coworkers (1974), it is logical that the BV of the Boz-1 Bomi Riso mutant was greater since lysine is highly correlated ($P < .01$) with BV

(r=.62).

Bomi Riso mutant 7 shows promise from the single set of data obtained from trial I. The agronomic characteristics shown in table 35 were very good compared to the parent line. This barley also produced a high PER value (table 24) and a BV that was not different (P>.05) from that of the other Bomi Riso mutant barleys fed in trial Ia. Doll et al. (1974) found Bomi Riso mutant 7 to contain 4.03% lysine (g/16 g N) which is comparable to the value of 4.07% found for Boz-1 Bomi Riso mutant 7.

The BV of Bomi Riso mutant 8 was 91.7% which was very high, however, the PER value obtained in trial I (table 24) as well as the test weight, kernel weight and grain yield (table 35) from eight locations were lower than those values for Bomi Riso mutant 7. From these data it would seem that the future of Bomi Riso mutant 8 is somewhat doubtful. Doll et al. (1974) found Bomi Riso mutant 8 to contain 4.55% lysine (g/16 g N) which was lower than the value of 4.76% found for Boz-1 Bomi Riso mutant 8.

The Osborne fractions of the Carlsberg II and Carlsberg II derived mutants (table 34) show the same pattern as those described by Ingverson et al. (1971). Doll (1972) described Carlsberg Riso mutant 56 to show the lowest yield of the three Carlsberg Riso mutants with a yield of 77% of that of Carlsberg II. The data obtained from eight locations in Montana (table 36) show, as a percent of the Carlsberg II yield, Carlsberg Riso mutant 86 to have produced the lowest yields (72.1%) with Carlsberg Riso mutant 29 producing the best yield of the three mutants

(92.3%) and Carlsberg Riso mutant 56 being intermediate (83.8%). The BV of all the Carlsberg II derived mutants in trial Ib were not different from that of the parent. In trials IVa and Va, Carlsberg Riso mutant 29 and Carlsberg II had similar ($P>.05$) BV values while Carlsberg Riso mutant 86 had a higher BV ($P<.05$) than Carlsberg II. Doll *et al.* (1974) found the BV of Carlsberg Riso mutants 29 and 56 to be 87% and 79%, respectively, which were higher than those found in trials Ib, IVa and Va. This caused some concern since the Montana barleys showed slightly higher lysine contents (g/16 g N) than those used by Doll *et al.* (1974). A pattern similar to that exibited by biological values was seen in the PER values of trial IV and V with Carlsberg Riso mutant 86 being different ($P<.05$) from that of Carlsberg II but similar ($P>.05$) to Carlsberg Riso mutant 29. From these data it appears that Carlsberg Riso mutant 86 is a better barley nutritionally than Carlsberg II, but whether or not it is better than Carlsberg Riso mutant 29 can not be determined from these data.

Numerous dilemmas were encountered during the course of the research work involved in this thesis. The major problem was the assumed contamination of the Bomi Riso mutant 1508 barleys used in trials II, III and IIIa which has been previously discussed. Another disturbance was when a number of rats in trials IV and V contracted a respiratory infection prior to completion of these trials. Due to a limited supply of barley, the trials could not be discarded and restarted with new,

healthy rats. Consequently, those rats which died or were very obviously inflicted with the ailment such that it effected their consumption and gains were removed from the trial. The question still remains however, how many rats which completed the trial contracted the disease and how did it effect the results of the trials? By comparing some PER values of trial I to those of trials IV and V, the values for the latter were lower numerically, but what part of that difference can be attributed to variations in the different barleys and what part to illness in rats can not be determined. The final problem was mentioned briefly, namely, a limited supply of certain barleys. It is impossible at this point in time to raise unlimited quantities of experimental barleys, but it would seem feasible to have on hand at least enough of each to complete duplicate growth and metabolism trials for each. The reference in this case is made to Bomi Riso mutants 7, 8, 9 and 13 and Carlsberg Riso mutant 56.

In spite of the problems which occurred, the data obtained from the growth and nitrogen balance trials did have value. They offered comparative data on Bomi and Carlsberg Riso mutants which have been previously limited.

The data offered in this thesis are far from being the final touches on answering all the questions relating to the nutritional value of these high-lysine barleys. They do however enhance the credibility of the data which have been offered by the Danish and Swedish research-

ers and will provide more groundwork for future research in this field.

SUMMARY

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.

A nitrogen-free balance trial was conducted with ten female weanling rats to determine metabolic fecal nitrogen and endogenous urinary

nitrogen. Average metabolic fecal nitrogen was .014 g N/g dry matter consumed and endogenous urinary nitrogen was .065 g N/rat/4 days.

# APPENDIX

APPENDIX TABLE 37. LEAST SQUARES ANALYSIS OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS GRAIN AND CASEIN DIETS FOR 28 DAYS, TRIAL I.

| Source of variation | d.f. | Mean Squares | | | |
|---|---|---|---|---|---|
| | | Gain | Feed consumed | Feed/gain ratio | Adjusted PER |
| Total | 120 | -- | -- | -- | -- |
| Total Reduction | 15 | 483.8 | 3730.4 | 2.174 | .451 |
| MU-Y | 1 | 324.0 | 3890.8 | .148 | .045 |
| Ration | 11 | 616.7[a] | 4256.8[a] | 2.737[a] | .590[a] |
| Cage Level | 3 | 42.1 | 1952.3 | .850[a] | .093 |
| Error | 105 | 133.5 | 1861.1 | .273 | .053 |

[a]P<.01

APPENDIX TABLE 38. LEAST SQUARES ANALYSIS OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS GRAIN AND CASEIN DIETS FOR 28 DAYS, TRIAL II AND III.

| Source of variation | d.f. | Mean Squares | | | |
|---|---|---|---|---|---|
| | | Gain | Feed consumed | Feed/gain ratio | Adjusted PER |
| Total | 140 | -- | -- | -- | -- |
| Total reduction | 22 | 1278.0 | 8228.3 | 7.745 | .739 |
| MU-Y | 1 | 3.8 | 21747.3[b] | 2.724[b] | .000 |
| Ration | 6 | 3913.8[a] | 15913.6[a] | 23.151[a] | 2.345[a] |
| Location | 1 | 65.6 | 2923.1 | 3.525[a] | .228[a] |
| L/L1[c] | 4 | 43.2 | 676.7[b] | .774 | .039 |
| L/L2[c] | 4 | 621.0[a] | 8203.4 | 2.172[a] | .194[a] |
| Ration x Location | 6 | 192.2 | 2837.3 | 1.806[a] | .118[b] |
| Error | 118 | 187.1 | 3001.9 | .483 | .051 |

[a]P<.01
[b]P<.05
[c]L/L=cage level within location.

APPENDIX TABLE 39. LEAST SQUARES ANALYSIS OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS GRAIN AND CASEIN DIETS FOR 28 DAYS, TRIAL IV AND V

| Source of variation | d.f. | Mean Squares | | | |
|---|---|---|---|---|---|
| | | Gain | Feed consumed | Feed/gain ratio | Adjusted PER |
| Total | 147 | -- | -- | -- | -- |
| Total Reduction | 24 | 1098.6 | 9354.8 | 5.404 | .558 |
| MU-Y | 1 | 40.5 | 362.6 | .123 | .013 |
| Ration | 7 | 3357.0[a] | 25119.9[a] | 15.026[a] | 1.603[a] |
| Location | 1 | 84.2 | 6703.3 | 1.770[b] | .005 |
| L/L1[c] | 4 | 35.2 | 2916.7 | .357 | .052 |
| L/L2[c] | 4 | 41.9 | 562.0 | .417 | .041 |
| Ration x Location | 7 | 270.7 | 4355.1 | 1.934[b] | .176[b] |
| Error | 123 | 138.2 | 2714.7 | .368 | .034 |

[a] $P < .01$

[b] $P < .05$

[c] L/L=cage level within location

APPENDIX TABLE 40. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON GAIN OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Lysine | .7814 | .6106 |
| Serine | .8460 | .7157 |
| Threonine | .8652 | .7486 |
| Leucine | .8924 | .7964 |
| Glutamic acid | .9094 | .8270 |
| Proline | .9187 | .8440 |
| Tyrosine | .9249 | .8554 |
| Methionine | .9344 | .8731 |
| Alanine | .9490 | .9006 |
| Phenylalanine | .9554 | .9128 |
| Cystine | .9579 | .9176 |
| Arginine | .9609 | .9233 |
| Tryptophan | .9767 | .9539 |
| Histidine | .9808 | .9620 |
| Glycine | .9843 | .9688 |
| Valine | .9933 | .9866 |
| Aspartic acid | .9964 | .9928 |
| Isoleucine | .9965 | .9930 |

APPENDIX TABLE 41. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON FEED CONSUMPTION OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Tyrosine | .5043 | .2543 |
| Cystine | .5573 | .3106 |
| Histidine | .6140 | .3770 |
| Serine | .6329 | .4006 |
| Methionine | .6696 | .4484 |
| Leucine | .6764 | .4575 |
| Threonine | .6961 | .4846 |
| Phenylalanine | .7077 | .5008 |
| Lysine | .7254 | .5262 |
| Glutamic acid | .7521 | .5657 |
| Valine | .7974 | .6358 |
| Arginine | .8143 | .6631 |
| Aspartic acid | .8779 | .7707 |
| Isoleucine | .8976 | .8057 |
| Tryptophan | .9232 | .8523 |
| Glycine | .9272 | .8597 |
| Alanine | .9272 | .8597 |
| Proline | .9273 | .8599 |

APPENDIX TABLE 42. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON FEED EFFICIENCY OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Leucine | .6780 | .4597 |
| Lysine | .8264 | .6829 |
| Tyrosine | .8913 | .7944 |
| Histidine | .9184 | .8435 |
| Tryptophan | .9261 | .8577 |
| Aspartic acid | .9312 | .8671 |
| Alanine | .9345 | .8733 |
| Isoleucine | .9434 | .8900 |
| Cystine | .9467 | .8962 |
| Proline | .9518 | .9059 |
| Glutamic acid | .9545 | .9111 |
| Glycine | .9652 | .9316 |
| Methionine | .9702 | .9413 |
| Serine | .9743 | .9493 |
| Phenyalanine | .9799 | .9602 |
| Threonine | .9847 | .9696 |
| Valine | .9853 | .9708 |
| Arginine | .9857 | .9716 |

APPENDIX TABLE 43. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON PROTEIN EFFICIENCY RATIO OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Lysine | .7751 | .6008 |
| Leucine | .8294 | .6879 |
| Tryptophan | .8727 | .7616 |
| Cystine | .8912 | .7942 |
| Aspartic acid | .9018 | .8132 |
| Histidine | .9157 | .8385 |
| Valine | .9223 | .8506 |
| Serine | .9273 | .8599 |
| Threonine | .9435 | .8902 |
| Alanine | .9453 | .8936 |
| Isoleucine | .9477 | .8981 |
| Proline | .9509 | .9042 |
| Methionine | .9522 | .9067 |
| Tyrosine | .9570 | .9158 |
| Arginine | .9608 | .9231 |
| Glutamic acid | .9713 | .9434 |
| Phenylalanine | .9842 | .9686 |
| Glycine | .9846 | .9694 |

APPENDIX TABLE 44. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON BIOLOGICAL VALUE OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Lysine | .6199 | .3843 |
| Histidine | .7799 | .6082 |
| Glycine | .8467 | .7169 |
| Valine | .8563 | .7333 |
| Serine | .8681 | .7536 |
| Tryptophan | .8713 | .7592 |
| Tyrosine | .8748 | .7652 |
| Isoleucine | .8853 | .7837 |
| Alanine | .8896 | .7914 |
| Glutamic acid | .8964 | .8036 |
| Phenylalanine | .9059 | .8207 |
| Proline | .9099 | .8279 |
| Cystine | .9142 | .8357 |
| Threonine | .9177 | .8422 |
| Arginine | .9182 | .8430 |
| Methionine | .9184 | .8435 |
| Leucine | .9193 | .8452 |
| Aspartic acid | .9225 | .8510 |

APPENDIX TABLE 45. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON TRUE PROTEIN DIGESTIBILITY OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Tryptophan | .5226 | .2731 |
| Isoleucine | .6453 | .4165 |
| Valine | .7447 | .5546 |
| Tyrosine | .8649 | .7480 |
| Cystine | .9065 | .8218 |
| Leucine | .9420 | .8873 |
| Threonine | .9481 | .8988 |
| Alanine | .9573 | .9164 |
| Glycine | .9609 | .9233 |
| Lysine | .9616 | .9246 |
| Proline | .9644 | .9301 |
| Aspartic acid | .9649 | .9310 |
| Serine | .9651 | .9314 |
| Arginine | .9659 | .9331 |
| Phenylalanine | .9669 | .9348 |
| Glutamic acid | .9671 | .9353 |
| Methionine | .9672 | .9354 |
| Histidine | .9690 | .9390 |

APPENDIX TABLE 46. MULTIPLE R AND $R^2$ OF BARLEY AMINO ACID EFFECT ON NET PROTEIN UTILIZATION OF RATS (RISO MUTANTS)

| Amino acid | R | $R^2$ |
|---|---|---|
| Leucine | .4254 | .1810 |
| Glycine | .5620 | .3159 |
| Lysine | .6447 | .4156 |
| Valine | .6950 | .4830 |
| Alanine | .7698 | .5925 |
| Cystine | .7897 | .6236 |
| Tryptophan | .8063 | .6501 |
| Aspartic acid | .8183 | .6696 |
| Methionine | .8518 | .7256 |
| Phenylalanine | .8804 | .7751 |
| Serine | .8883 | .7890 |
| Arginine | .8982 | .8067 |
| Histidine | .9243 | .8544 |
| Isoleucine | .9261 | .8577 |
| Threonine | .9310 | .8667 |
| Tyrosine | .9314 | .8675 |
| Glutamic acid | .9315 | .8676 |
| Proline | .9316 | .8679 |

APPENDIX TABLE 47. CORRELATION COEFFICIENTS OF AMINO ACIDS WITH GAIN, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY OF RATS FED 23 BARLEYS

| Amino acid | Gain | Feed consumption | Feed/gain ratio | Adjusted PER |
|---|---|---|---|---|
| | r | r | r | r |
| Alanine | -.02 | .11 | .33 | -.09 |
| Arginine | .72[a] | .37 | -.44[b] | .67[a] |
| Aspartic acid | .50[b] | .29 | -.20 | .42[b] |
| Cystine | .07 | -.17 | -.07 | .20 |
| Glutamic acid | -.32 | -.21 | .00 | .27 |
| Glycine | .60[a] | .26 | -.35 | .57[a] |
| Histidine | .17 | .20 | .11 | .12 |
| Isoleucine | -.12 | .28 | .15 | -.01 |
| Leucine | -.50[b] | -.02 | .68[a] | -.51 |
| Lysine | .78[a] | .21 | -.65[a] | .78[a] |
| Methionine | .04 | .24 | .20 | .12 |
| Phenylalanine | -.27 | -.33 | -.01 | -.31 |
| Proline | .32 | -.15 | .05 | -.31 |
| Serine | -.02 | .04 | .08 | .11 |
| Threonine | .56[a] | .18 | -.38 | .64[a] |
| Tryptophan | .38[b] | .02 | -.40 | .34 |
| Tyrosine | -.23 | -.50[b] | -.02 | -.20 |
| Valine | .25 | .15 | -.03 | .15 |

[a] $P < .01$
[b] $P < .05$

APPENDIX TABLE 48. CORRELATION COEFFICIENTS OF AMINO ACIDS WITH BIOLOGICAL VALUE (BV), TRUE PROTEIN DIGESTIBILITY (TPD) AND NET PROTEIN UTILIZATION OF RATS FED 23 BARLEYS

| Amino acid | BV | TPD | NPU |
|---|---|---|---|
| Alanine | -.19 | .18 | -.16 |
| Arginine | .53[a] | -.38 | .26 |
| Aspartic acid | .13 | -.06 | .02 |
| Cystine | -.12 | .31 | .26 |
| Glutamic acid | -.08 | .03 | .03 |
| Glycine | .59[a] | -.36 | .41[b] |
| Histidine | -.27 | .31 | -.11 |
| Isoleucine | .18 | -.40 | -.31 |
| Leucine | -.50[b] | .34 | -.43[b] |
| Lysine | .62[a] | -.52[a] | .22 |
| Methionine | .11 | -.16 | -.05 |
| Phenylalanine | -.41[b] | .35 | -.10 |
| Proline | .19 | .18 | .05 |
| Serine | .32 | -.27 | .13 |
| Threonine | .47[b] | -.33 | .25 |
| Tryptophan | .62[a] | -.52[a] | .28 |
| Tyrosine | -.27 | .09 | -.34 |
| Valine | .37 | -.28 | .13 |

[a] $P < .01$
[b] $P < .05$

APPENDIX TABLE 49. COMPOSITION OF ICN NUTRITIONAL BIOCHEMICALS TOTAL VITAMIN SUPPLEMENT[a]

| Ingredients | g/45.4 kg diet | mg/45.4 kg diet |
|---|---|---|
| Vitamin A acetate (200,000 IU/g) | 4.50 | -- |
| Vitamin D calciferol (400,000 IU/g) | 0.25 | -- |
| Alpha-tocopherol (100 IU/g) | 5.00 | -- |
| L-Ascorbic acid | 45.00 | -- |
| i-Inositol | 5.00 | -- |
| Choline Chloride | 75.00 | -- |
| Menadione | 2.25 | -- |
| p-Aminobenzoic acid | 5.00 | -- |
| Niacin | 4.50 | -- |
| Riboflavin | 1.00 | -- |
| Pyridoxine Hydrochloride | 1.00 | -- |
| Thiamin Hydrochloride | 1.00 | -- |
| D-Calcium Pantothenate | 3.00 | -- |
| Biotin | -- | 20.00 |
| Folic Acid | -- | 90.00 |
| Vitamin $B_{12}$ | -- | 1.35 |

[a]Vitamins titrated in dextrose to make 1 kilogram which makes 45.4 kilograms of diet.

APPENDIX TABLE 50. COMPOSITION OF ICN NUTRITIONAL BIOCHEMICALS BERN-HART-TOMARELLI SALT MIXTURE, MODIFIED 1135-B

| Ingredients | % |
|---|---|
| Calcium Carbonate | 2.100 |
| Calcium Phosphate | 73.500 |
| Citric acid | 0.227 |
| Cupric Citrate · $2\frac{1}{2}$ $H_2O$ | 0.046 |
| Ferric Citrate · 5 $H_2O$ | 0.558 |
| Magnesium Oxide | 2.500 |
| Manganese Citrate | 0.835 |
| Potassium Iodide | 0.001 |
| Potassium Phosphate dibasic | 8.100 |
| Potassium Sulfate | 6.800 |
| Soldium Chloride | 3.060 |
| Sodium Phosphate | 2.140 |
| Zinc Citrate · 2 $H_2O$ | 0.133 |

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