Effect of nitrogen fertilizer on selected barley cultivars : nutritional quality of protein and physical characteristics of the grain

by Gerald Joseph Killen

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
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Abstract:
Hiproly (HP), Hiproly Normal (HPN), Compana (C) and Unitan (U) barleys were fertilized with 0, 112 or 224 kg N/ha. Compana and U produced the greatest yields at all levels of fertilizer. Hiproly Normal yielded about 75% and HP about 33% as much as C and U. Hiproly had the greatest percentage protein followed by HPN, C and U in that order. Hiproly had the lightest kernels and least plump kernels. As nitrogen fertilizer increased yield and protein content of the barley increased while kernel weight, test weight and percentage plump kernels decreased. Hiproly remained nearly constant in protein at all fertilizer levels. Osborne fractionations of the barleys showed HP to have less hordeins and more glutelins, albumins and globulins than the other barleys. The protein composition of HP and HPN changed very little as fertilizer levels increased. In C and U, an increase in hordein with a decrease in salt-soluble proteins was observed between 0 and 112 kg/ha N fertilizer. Protein composition in these two barleys remained nearly constant at the two higher levels of fertilization. Hiproly contained more lysine (g/16g N), methionine and tryptophan and less glutamic acid and proline than the other barleys. Hiproly Normal had the highest amounts of glutamic acid and proline and the lowest lysine content of the four culti-vars. Nitrogen fertilization tended to decrease lysine, methionine and valine contents of the protein in C and U while glutamic acid and protein contents increased. Neither HP nor HPN showed consistent changes in amino acid composition with increased N fertilizer. Unitan barley produced greater gains and PER in rats than those produced by C and HPN. Gains and PER's of rats fed HP were intermediate between those fed U and C. HPN produced the lowest gains and PER's and poorest feed/gain ratios of all four cultivars. Averaged across all cultivars, rats performed poorer as N fertilization of the barley increased. Rat metabolism trials showed that BV of barley protein decreased with increased N fertilization. Unitan had the lowest TPD and NPU, C the highest. Hiproly had a higher NPU and lower TPD than HPN. Phenylalanine was positively correlated and cystine, serine, isoleucine and threonine were negatively correlated to protein percentage in all four cultivars. When HP is taken out of the correlation, a strong negative correlation is seen between lysine, methionine and arginine and protein percentage of the grains. Postitive correlations without HP were found for glutamic acid, phenylalanine and proline. When biological measurements from rat trials were correlated to amino acid (g/16g N) and total protein content, positive correlations between lysine, methionine, valine and threonine and gain, PER, BV and NPU were detected. These biological measurements were negatively related to glutamic acid, proline, phenylalanine and total protein. Amino acids affected TPD nearly opposite from the other measurements. Removing HP data from the correlation increased the magnitude of the correlations.

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by

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

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in

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ABSTRACT

Hiproly (HP), Hiproly Normal (HPN), Compana (C) and Unitan (U) barleys were fertilized with 0, 112 or 224 kg N/ha. Compana and U produced the greatest yields at all levels of fertilizer. Hiproly Normal yielded about 75% and HP about 33% as much as C and U. Hiproly had the greatest percentage protein followed by HPN, C and U in that order. Hiproly had the lightest kernels and least plump kernels. As nitrogen fertilizer increased yield and protein content of the barley increased while kernel weight, test weight and percentage plump kernels decreased. Hiproly remained nearly constant in protein at all fertilizer levels. Osborne fractionations of the barleys showed HP to have less hordeins and more glutelins, albumins and globulins than the other barleys. The protein composition of HP and HPN changed very little as fertilizer levels increased. In C and U, an increase in hordein with a decrease in salt-soluble proteins was observed between 0 and 112 kg/ha N fertilizer. Protein composition in these two barleys remained nearly constant at the two higher levels of fertilization. Hiproly contained more lysine (g/16g N), methionine and tryptophan and less glutamic acid and proline than the other barleys. Hiproly Normal had the highest amounts of glutamic acid and proline and the lowest lysine content of the four cultivars. Nitrogen fertilization tended to decrease lysine, methionine and valine contents of the protein in C and U while glutamic acid and protein contents increased. Neither HP nor HPN showed consistent changes in amino acid composition with increased N fertilizer. Unitan barley produced greater gains and PER in rats than those produced by C and HPN. Gains and PER's of rats fed HP were intermediate between those fed U and C. HPN produced the lowest gains and PER's and poorest feed/gain ratios of all four cultivars. Averaged across all cultivars, rats performed poorer as N fertilization of the barley increased. Rat metabolism trials showed that BV of barley protein decreased with increased N fertilization. Unitan had the lowest TPD and NPU, C the highest. Hiproly had a higher NPU and lower TPD than HPN. Phenylalanine was positively correlated and cystine, serine, isoleucine and threonine were negatively correlated to protein percentage in all four cultivars. When HP is taken out of the correlation, a strong negative correlation is seen between lysine, methionine and arginine and protein percentage of the grains. Postitive correlations without HP were found for glutamic acid, phenylalanine and proline. When biological measurements from rat trials were correlated to amino acid (g/16g N) and total protein content, positive correlations between lysine, methionine, valine and threonine and gain, PER, BV and NPU were detected. These biological measurements were negatively related to glutamic acid, proline, phenylalanine and total protein. Amino acids affected TPD nearly opposite from the other measurements. Removing HP data from the correlation increased the magnitude of the correlations.

INTRODUCTION

Cereal grains provide about 80 million tons of protein per year. This is about one-half of the annual human requirement. Because of this large amount of protein produced, a small increase in total protein of a cereal grain or improvement of the biological value of the protein could contribute significantly to solving the worldwide problem of protein malnutrition.

Barley ranks fourth in worldwide production and can be grown in a larger variety of climates and elevations than most other cereal grains. Barley is the major feed grain in Montana. Since wheat and rice are primarily used for human consumption and human consumption of maize is increasing, barley is becoming a prominent animal feed grain.

Many factors both environmental and genetic affect the protein quantity and quality of barley. Among the environmental factors are nitrogen and water availability, day length and temperature.

This thesis project was to investigate the effect of nitrogen fertilization on the protein of barley cultivars know to differ in protein. The yield and physical characteristics of the grain were also studied.

LITERATURE REVIEW

General Aspects

In 1816, Magendie, a French physiologist showed that nitrogen in the body had its origin in nitrogenous compounds of food. He established that proteins were the compounds concerned and that proteins are not of equal value (Maynard and Loosli, 1969).

It was later found that the difference between proteins was due to the balance of the 20 or more amino acids which make up protein. Some of these can be synthesized in the animal body and are termed non-essential or dispensable. The ones that cannot be synthesized in the body are called essential or indispensible and must be provided in an animal's diet. Rose et al. (1948) found 10 amino acids to be essential for the immature rat. They were valine, leucine, isoleucine, methionine, threonine, phenylalanine, tryptophan, lysine, histidine and arginine.

The synthesis of body protein is dependent on the concentrations of the most limiting amino acid in the diet. Howe et al. (1965) stated that the first limiting amino acid in barley proteins is lysine followed by threonine, methionine and tryptophan.

The availability or digestibility of the amino acids may also affect protein quality. Eggum (1973b) said that in barley protein lysine has a digestibility of 76% while glutamic acid is digested at 91.4%. The reason for this is that lysine is highest in the protein of the aleuron layer of the kernel which is less digestible than the endosperm proteins which are low in lysine and high in glutamic acid (Munck, 1964a).

Osborne (1895) divided cereal protein into four fractions depending on their solubility. They were albumins, globulins, prolamines and glutelins which are soluble in water, weak salt, alcohol and alkali solutions, respectively. Hordein is the name given the prolamine fraction in barley. Folkes and Yemm (1956) showed that the protein fractions of barley did not contain equal amounts of lysine expressed as a percent of the protein: Albumins have 7.9% lysine, glubulins 6.3%, glutelin 4.8% and prolamine .80%.

Ingversen and Koie (1973a) reported that the salt-soluble proteins (albumins and globulins) in an average barley contain 44% of the total lysine and compose only 24% of the total protein. The glutelins make up 33% of the barley proteins and contain 38% of the lysine in the barley. Hordein accounts for 9% of the lysine and 35% of the total protein.

Munck (1964a) found the outer aleuron layer is rich in salt-soluble proteins. The embryo of the kernel is rich in albumins and globulins (Pomeranz, 1975).

Methods of Protein Evaluation

Several methods of evaluating protein from its amino acid composition have been devised. Mitchell and Block (1946) developed the chemical score method of analysis. This method is based on the percent deficit of the limiting amino acid. The method does not take into account the availability of amino acids or the possibility of imbalance or antagonism between amino acids.

The Essential Amino Acid Index (Oser 1951) takes into account all essential amino acids. This may be an improvement over the chemical score method but its accuracy is still questionable for the same reasons as chemical score.

Biological value (BV) is the percent of nitrogen which is absorbed into the body that is retained (Mitchell 1924). There is a problem with determining the metabolic fecal nitrogen and the endogenous urinary nitrogen when using this method. Using rats fed a nitrogen-free diet Mitchell (1924) determined the metabolic fecal nitrogen in terms of nitrogen per gram food consumed and applied this to the calculation of BV. Endogenous urinary nitrogen was also determined by feeding a nitrogen-free diet and was expressed in grams per day. Danish workers used a 4% freeze dried egg protein diet to determine the metabolic fecal and endogenous urinary nitrogen (Eggum 1969).

Using a multiple regression analysis, Hansen and Eggum (1973) developed an equation that would provide the closest agreement between measured and calculated biological value. This was designated the Total Amino Acid Value (TAAV). When feedstuffs were categorized they found a correlation between TAAV and measured BV (r=.85). They also devised an equation for the calculations of BV using only the essential amino acids and termed this value Essential Amino Acid Value (EAAV) a correlation of .82 was found between EAAV and BV. This means that the nonessential amino acids had some effect on BV, although it was small. Using the same data, the correlation coefficient between BV and chemical score was .65 and BV with EAAV was .71. The total amino acid value provided the best agreement between calculated value and measured BV.

Chapman et al. (1959) described the Protein Efficiency Ratio as the weight gain of experimental animals divided by the weight of protein consumed. Variation in PER is seen between different levels of protein in the diet, age and sex of test animals and length of test period. Morrison and Campbell (1959) pointed out that maximal PER's were obtained with female rats fed low protein diets. Because of this, it has been highly criticized. It is also based on the assumption that body weight increase is an index of protein synthesis. The validity of this assumption has been challenged (Eggum 1969).

Bender and Doll (1957) used a comparison between rats fed a non-protein diet and rats fed the test protein diet to determine the Net Protein Ratio (NPR). NPR is described as (gain in weight of test group + loss of weight of nonprotein rats) ÷ protein intake. These researchers further stated that this method may be more accurate than PER and correlated better with NPU.

High Lysine Barley

Munck et al. (1969) described Hiproly barley as a naked two-rowed barley with short straw. They reported Hiproly barley to contain up to 50% more crude protein and 30% more lysine (g/16g N) than normal barleys. The lysine in Hiproly ranged from 4.2 to 4.4% of the protein compared to 3.7% for normal barley (Eggum 1969). Pomeranz et al. (1972) reported 4.3% lysine and 2.9% methionine + cysteine in Hiproly barley. The increased lysine content was found to be partially due to decreased hordein and increased albumins in the endosperm (Munck 1972). Ingverson and Koie (1971) reported that there were five new proteins in addition to the seven normal proteins found in the albumins and globulin fractions of Hiproly. Another reason for the increased lysine in Hiproly is the change in synthesis of b-proteins of the salt-soluble fraction (Ingversen and Koie 1973b).

Tallberg (1973) reported no change in the physical makeup of the endosperm in Hiproly and concluded that the increase in lysine was due primarily to the increase in water soluble proteins. Munck et al. (1970) found that Hiproly barley gave significantly greater PER's for mice and BV and NPU for rats. They also reported an increased true digestibility for protein and lysine. Newman et al. (1974) also reported increased gain, feed efficiency and PER for rats fed Hiproly barley. However, Newman et al. (1974) reported that the protein of Hiproly was not as digestible as that of normal barley. The growth performance of rats fed Hiproly was judged to be similar to rats fed normal barley supplemented with lysine or lysine, methionine and threonine according to these authors.

Newman et al. (1977) showed that a Hiproly barley could provide a major portion of the total protein and amino acid requirements of growing-finishing pigs. Thomke and Widstromer (1975) found that Hiproly barley and some of its crosses had higher N digestibility, BV, NPU and utilizable nitrogen than reference barley fed to piglets and growing-finishing pigs. Hiproly barley has a shrunken kernel and very poor yield. The poor yield is due in part to a low number of kernels per spike and sterility (Munck et al., 1970). It does, however, provide genetic potential for use in plant breeding programs.

Nitrogen Fertilizer: Effect on Yield and Percentage Protein in Barley

In general, there is a negative relationship between grain yield and protein content of cereal grain. This is because conditions that cause increased grain yield increase the amounts of carbohydrates more than proteins (Pomeranz 1975).

The effect of nitrogen fertilizer on yield and protein content of the grain has been subject to many studies. Foote and Batchelder (1953) stated that nitrogen application at seeding time had more effect on yield than later application. McBeath and Toogood (1960) reported that application of nitrogen fertilizer raised both yield and protein content regardless of when the fertilizer was applied. However, when a large response in yield was obtained, protein content was only slightly increased and when the yield increase was small, protein content was higher. Applications of fertilizer closer to heading tended to raise protein content more than earlier applications. Both of the above mentioned researchers pointed out the importance of the relationship between time of application of fertilizer and precipitation or irrigation. Gately (1968 and Woodham et al. (1972) reported an increase in both yield and percent nitrogen in grain by increasing nitrogen fertilization.

Kirby (1968) reported no response in yield to added nitrogen fertilizer but it did increase the number of spikes by 16% and the nitrogen content of the grain.

Bishop and MacEarchen (1971) found that increasing nitrogen fertilizer on barley increased yield in only one of the three years. Each year they found a linear increase in percentage nitrogen in the grain in response to increased nitrogen fertilizer. A linear relationship between percentage nitrogen in the grains and total available nitrogen was also found by Walker (1975). He also discovered that grain yield and total nitrogen produced increased quadratically with increased available soil nitrogen. Macleod and Suzuki (1972) reported that percentage protein nitrogen and nonprotein nitrogen increased in grain and vegetative tissue with successive rates of nitrogen fertilizer whereas they decreased as phosphorus and potassium fertilizer was increased. The application of large amounts of nitrogen fertilizer resulted in inefficient use of the nitrogen unless sufficient phosphorus and potassium were provided.

It is generally thought that nitrogen fertilization decreases the kernel weight. Anderson and Koie (1975) found this to be true for Bomi and its mutant 1508. Gately (1958) however said that nitrogen fertilization increased kernel weight unless lodging occurred.

Nitrogen Fertilizer: Effect on Protein Quality

Protein quality decreases in the normal barley as percentage protein in the grain increases due to nitrogen fertilization or other environmental factors. This is due to the decrease in lysine (g/16g N) as total protein increases. Pomeranz (1975) showed a curvilinear relationship between percent protein and lysine (g/16g N). The decrease in lysine may be explained by the simultaneous decrease in lysine rich salt-soluble proteins and increase in lysine poor prolamines. The glutelins which are intermediate in lysine content, remain fairly constant. Nitrogen fertilization causes nitrogen to be passed more to the endosperm portion rather than the aleuron part of the seed. Since the endosperm is rich in prolamine this explains the shift in protein fractions (Munck 1964b). Prolamine is considered to be the storage protein of barley. Munck et al. (1969) reported a correlation of -.89 between crude protein and g lysine/100 g protein for commercial barley cultivar grown at two locations. In a fertilizer experiment, the correlation was -.43 when nitrogen gertilizer was varied and phosphorus and potassium were held constant. Other amino acids that showed negative relationships between crude protein and amino acid content of the protein were histidine, cysteine, threonine, valine and alanine. Viuf (1969) with 650 barley cultivars showed low protein barleys (9.5 to 11% CP) to have 4.2% lysine while high protein barleys 17.8 to 18.1% CP) had 2.7 to 3.1% lysine in the protein.

Woodham et al. (1972) looked at the effect of nitrogen fertilizer on 14 cultivars of barley grown at different locations throughout the United Kingdo . They also showed that lysine content was inversely proportioned to crude protein percentage, especially for barleys between 8 and 11% crude protein. It is interesting to note that the locality in which the barley was grown had more effect on total protein than did nitrogen fertilizer. An effect due to locality was also seen for lysine content.

Munck (1964a) used five barley cultivars and four levels of nitrogen fertilizer to study the effect of added nitrogen on chemical composition and nutritive value for rats and mice. There was an increase in total protein (N x 6.25) with increased nitrogen fertilizer. Calculated as percentage of total protein the salt-soluble protein decreased and amide nitrogen (an estimation of hordein) increased. Calculated as percentage of dry matter both fractions increased but amide nitrogen increased faster. There was a weak tendency for lysine to decrease as a percentage of the protein but it increased as a percentage of dry matter. Protein efficiency ratio values for mice varied significantly with barley cultivar but were not affected by nitrogen fertilizer.

This lack of effect on PER by fertilization is contradictory to the effect of nitrogen fertilizer on chemical composition. This reflects a difference in availability of amino acids. In vitro digestibility with pepsin (24h) showed that the protein from low protein barley was less digestible than that from high protein samples. This was explained by the difference in digestibility of the fractions discussed earlier. Eggum (1973a) points out that BV decreased at a slower rate in relation to protein content than the rate of which true digestibility increased. This caused a positive correlation between protein content and NPN (r=.50).

Buchmann (1977) in an experiment in which barley was grown in pots with different levels of nitrogen, showed a positive correlation (r=.73) between in vitro digestibility of protein and percentage protein in the grain. A correlation (r=.83) was found between hordein content of protein and protein digestibility. The increase in hordein in the protein paralleled the increase of total protein with increased fertilization.

Hiproly barley does not show this general relationship between protein content and lysine content. As Hiproly barley varies in protein content, its amino acid content remains rather constant (Rhodes et al., 1974). There is not a large decrease in digestilibity of Hiproly due to the change in protein fractions. This allows lysine to be fairly available and BV values to be high, Eggum (1973b). When Hiproly was included in the correlation between amino acid content and protein content, a reduction in the magnitude of the correlation was seem for threonine, valine, and alanine. Only cysteine increased in magnitude, changing from -.59 to -.70. Lysine changed from a -.37 to .11 when Hiproly was included in a correlation between protein, (Munck et al., 1969).

Many factors other than nitrogen fertilizer affect the protein and lysine content of barley. As mentioned earlier, Woodham et al. (1972) saw differences in protein and lysine content of barley due to the location on which it was grown. Munck et al. (1969) reported a correlation of only -.37 between crude protein and lysine content for barleys grown worldwide. This is considerably less than the -.89 correlation for barleys grown only in Sweden. The differences between locations may be due to day length and temperature. Munck et al. (1969) reported a study in which lysine as a percentage of protein content and quality of barley, much more research and study on these factors should be done.

MATERIALS AND METHODS

Barleys

Four different barley cultivars, Hiproly (CI 3947), Hiproly Normal (CI 4362), Compana, (CI 5438), and Unitan (CI 10421) were produced on the same location at the Montana Agricultural Experiment Station farm west of Bozeman, Montana in the summer of 1976. Barleys were seeded on May 19 in plots 6.1 m x 9.15 m. Nitrogen fertilizer (NH_3NO_3) was added 30 days after planting at the rates of 0, 112 and 224 kg/ha. Four replications were planted for each barley and fertilizer level. Unitan is a six-rowed barley and the other three are two-rowed cultivars.

The plots were harvested on October 1 with a small combine that could be easily cleaned between plots. Barleys were air dried and weighed to obtain yield data. The grain was cleaned and samples were taken for Kjeldahl (A.O.A.C. 1970) and Udy (Mossberg 1969) protein analysis. Percentage plump and thin kernels, kernel weight, and test weight of the barley from each plot were also measured. Udy analysis, percentage plump and thin kernels, and kernel weight was accomplished by the Cereal Quality Laboratory at Montana State University.

Since Kjeldahl protein analysis of the barley from the four replications of the same cultivar with the same level of nitrogen fertilizer were fairly similar (table 1), replications were pooled. Samples of the composited barleys were taken for proximate analysis (modified A.O.A.C. 1970) calcium, phosphorus and amino acid determination. Calcium analysis was performed by the modified Kramer-Tisdall method of Clark and Collip (1925) and phosphorus by the technique of Fiske and Subbarow (1925). Amino acid analysis[1] was done on an acid hydrolysate of each pooled sample according to the method of Spackman et al. (1970). Separate analysis of tryptophan (Hulgi and Moore 1972) and cystine/2 (cystine + 2 x cysteine) (Hirs, 1967) were performed. Proximate analysis, calcium and phosphorus are given in table 2.

Osborne protein fractions were determined on a composite sample of the variety from each nitrogen fertilizer treatment using a modification of the Osborne technique (Ingverson and Koie, 1973c).

Diets

Isonitrogenous, 8.1% protein, were formulated for all trials. Corn starch was used at the expense of barley to balance diets for nitrogen. The level of dietary protein was dictated by the barley having the lowest percentage of protein. Purified cellulose was used to equalize fiber contents of the diets. An antibiotic mixture, vitamins, minerals and reagent grade calcium carbonate was added in equal amounts of each diet. The antibiotic was a commercial mixture of aureomycin, penicillin and sulfamethazine. Vitamins were provided by ICN Nutritional Biochemicals[2]

[1]Amino acid analyses were performed by AAA Laboratories, 6206 89th Avenue Southeast, Mercer Island, Washington 98040.

[2]ICN Nutritional Biochemicals, Pharmaceuticals Inc., Life Sciences Group 26201 Miles Road, Cleveland, Ohio 44128.

vitamin fortification mixture for rats. Minerals were provided by the Bernhardt-Tomarelli salt mixture prepared by ICN Nutritional Biochemicals. A casein control diet was mixed using the afore mentioned ingredients. Equal amounts of each diet were mixed before each of two growth trials and were also used for metabolism trials. The composition of each diet is given in table 3. Complete chemical analysis as previously explained was done only on the first diet mix. Only protein (N x 6.25) and moisture were done on the second mix (table 4).

Growth Trials

In each of two growth trials, five female weanling (21 to 24 days of age) Sprague-Dawley rats of the Holtzman strain were assigned according to initial weight to each diet. Rats were individually caged in three racks and assigned to different levels on the cage rack as equally as possible. Each rack had five levels and five cages per level. The cages measured 20 x 24 x 18 cm and were constructed of woven wire. Feed and water were given *ad libitum*. Rats were kept in an environmentally controlled room that provided 12 hr of light and 12 hr of darkness per day. Rats were weighed weekly and the growth trial was terminated after 28 days. Total gain and total feed consumption were determined. Feed efficiency (units of feed consumed per unit of gain), protein efficiency ratio (PER), and adjusted PER (PER x 2.50 ÷ casein PER) (Chapman, *et al*. 1959) were calculated. Protein efficiency ratio is defined as the units of gain per unit of protein consumed over a specified period of time. This measurement is most valid when determined with young growing rats fed an 8 to 10% protein diet for a period of 28 days (Chapman, et al. 1959).

Nitrogen Balance Trials

Each diet was fed to eight female weanling (21 to 24 days of age) Sprague-Dawley rats of the Holtzman strain. Rats were individually caged and fed their respective diets for an adjustment period of four days. Rats were fed 11.5 g feed per day to provide 150 mg nitrogen per day. After the adjustment period, urine and feces were collected for four days. Water was available at all times.

Urine was automatically collected through a glass wool filter into a flask containing 25 ml of 5% sulfuric acid. Urine was then diluted to 200 ml and a 15 ml sample was taken for Kjeldahl analysis to determine urinary nitrogen (UN). Feces was collected in a beaker containing 50 ml of 5% sulfuric acid. To form a homogenous solution, 100 ml of concentrated sulfuric acid was added at the end of the collection period. The solution was diluted to 500 ml with tap water, cooled and 100 ml aliquote was taken for Kjeldahl analysis. Both urinary and fecal nitrogen were expressed as grams nitrogen per rat per collection period. Biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU) were claculated for each rat and averaged for each diet (Mitchell, 1924 and Eggum 1973a). Values for endogenous urinary nitrogen and metabolic fecal nitrogen used were those found by Stobart (1977) using a nitrogen-free diet.

Statistical Analysis

Barley yield and physical data, BV, TPD and NPU were analyzed according to the least squares method of Harvey (1960). Yields from only the first three replications were used in the analysis due to a change in soil condition and an abundance of weeds in the fourth replication. Growth trial data were analyzed by analysis of variance (Steel and Torrie, 1960). Newman-Keuls multiple range test was used to separate the means if there was a signficant difference (Snedecor and Cochran, 1967).

TABLE 1. EFFECT OF CULTIVAR AND NITROGEN FERTILIZER ON KJELDAHL PROTEIN (N x 6.25) OF BARLEYS

Barley cultivar	Nitrogen Fert. level (kg/ha)	Replication				
		1	2	3	4	Avg.
Hiproly	0	16.3	16.7	16.9	17.2	16.8
	112	15.9	16.3	16.1	16.2	16.1
	224	17.2	16.8	16.8	17.1	17.0
Hiproly Normal	0	14.5	13.5	14.3	14.5	14.2
	112	14.7	14.7	15.0	14.8	14.8
	224	16.0	15.7	15.7	16.4	16.0
Compana	0	8.8	9.4	9.5	9.5	9.3
	112	12.0	12.7	12.3	12.5	12.4
	224	13.6	14.0	13.7	13.6	13.7
Unitan	0	9.1	8.8	9.5	9.1	9.1
	112	11.4	11.8	11.7	11.1	11.5
	224	12.6	12.8	12.2	12.3	12.5

TABLE 2. EFFECT OF CULTIVAR AND NITROGEN FERTILIZER ON THE PROXIMATE ANALYSIS[a] AND CALCIUM AND PHOSPHORUS OF BARLEYS

Barley cultivar	Nitrogen Fert. level (kg/ha)	Percentage[b]						
		C.P.	E.E.	C.F.	N.F.E.	Ash	Ca	P
Hiproly	0	18.1	1.6	2.5	75.3	2.5	.02	.49
	112	17.3	1.5	2.7	76.0	2.5	.05	.50
	224	17.8	1.5	2.9	75.3	2.5	.05	.49
Hiproly Normal	0	15.2	1.4	2.1	79.0	2.3	.02	.48
	112	15.7	1.4	2.5	78.1	2.3	.03	.47
	224	17.0	1.3	2.4	77.0	2.3	.04	.45
Compana	0	9.8	1.0	4.6	81.9	2.7	.01	.38
	112	12.5	1.0	4.6	79.3	2.6	.02	.36
	224	14.5	1.0	5.0	76.8	2.7	.02	.36
Unitan	0	9.6	1.1	5.6	81.1	2.6	.01	.37
	112	12.0	1.1	5.8	78.6	2.5	.03	.35
	224	13.3	1.1	6.1	76.9	2.6	.03	.33

[a] On a dry matter basis

[b] C.F.=crude protein, E.E.=ether extract, C.F.=crude fiber, N.F.E.=nitrogen free extract, Ca=calcium, P=phosphorus.

TABLE 3. PERCENTAGE COMPOSITION OF BARLEY AND CASEIN DIETS

Diet: Barley cultivar	Diet: Nitrogen Fert. level (kg/ha)	Barley/ Casein[a]	Corn starch	Corn oil	Mineral premix[b]	Vitamin premix[c]	$CaCO_3$[d]	Alphacel[e]	Antibiotic[f]
Hiproly	0	48.50	38.86	3.50	2.00	2.00	.80	4.09	.25
	112	50.95	36.44	3.50	2.00	2.00	.80	4.06	.25
	224	49.39	37.98	3.50	2.00	2.00	.80	4.08	.25
Hiproly Normal	0	57.86	29.60	3.50	2.00	2.00	.80	3.99	.25
	112	55.86	31.58	3.50	2.00	2.00	.80	4.01	.25
	224	51.59	35.81	3.50	2.00	2.00	.80	4.05	.25
Compana	0	90.00	0.93	3.50	2.00	2.00	.80	.52	.25
	112	69.83	20.19	3.50	2.00	2.00	.80	1.43	.25
	224	60.45	29.15	3.50	2.00	2.00	.80	1.85	.25
Unitan	0	91.45	0.00	3.50	2.00	2.00	.80	0.00	.25
	112	72.97	17.56	3.50	2.00	2.00	.80	.92	.25
	224	65.85	24.32	3.50	2.00	2.00	.80	1.28	.25
Casein		9.76	77.12	3.50	2.00	2.00	.80	4.57	.25

[a] ICN Nutritional Biochemicals; casein, purified, high nitrogen.

[b] Contributed mg/kg of diet; 420 calcium carbonate, 14,700 calcium phosphate, 45.4 citric acid, 9.2 cupric citrate·2½ H_2O, 111.6 ferric citrate·5 H_2O, 500 magnesium oxide, 167 manganese citrate, 0.2 potassium iodide, 1,620 potassium phosphate dibasic, 1,360 potassium sulfate, 612 sodium chloride, 428 sodium phosphate, 26.6 zinc citrate·2 H_2O.

[c] Contributed per kg of diet: 19,824 IU vitamin A acetate, 2,203 IU vitamin D calciferol, 11 IU alpha tocopherol, 991 mg L-ascorbic acid, 11 mg i-inositol, 1,652 mg choline chloride, 50 mg menadione, 110 mg p-aminobenzoic acid, 99.1 mg niacin, 22 mg riboflavin, 22 mg pyrodoxine hydrochloride, 22 mg thiamine hydrochloride, 66 mg D-calcium pantothenate, 0.44 mg biotin, 2.0 mg folic acid, 0.03 mg vitamin B_{12}.

[d] Reagent grade calcium carbonate.

[e] ICN Nutritional Biochemicals; non-nutritive cellulose.

[f] Contributed mg/kg of diet: 110.2 chlortetracycline, 110.2 penicillin and 55.1 sulfamethazine.

TABLE 4. PROXIMATE ANALYSIS AND CALCIUM AND PHOSPHORUS COMPOSITION OF DIETS (MIX 1 AND 2)

Diet		Percentage[a]									
Barley cultivar	Nitrogen Fert. level, (kg/ha)	C.P. (1)[b]	C.P. (2)[b]	E.E.	C.F.	N.F.E.	H_2O (1)[b]	H_2O (2)[b]	Ash	CA	P
Hiproly	0	8.7	8.6	3.0	2.6	74.6	7.3	6.6	3.8	.58	.65
	112	8.6	8.6	2.8	2.8	74.5	7.5	6.8	3.8	.58	.65
	224	8.5	8.6	2.8	2.9	74.4	7.5	6.7	3.9	.58	.65
Hiproly Normal	0	8.6	8.7	4.0	2.1	74.2	7.2	6.5	3.9	.59	.71
	112	8.6	8.7	4.0	2.3	74.0	7.3	6.5	3.8	.55	.68
	224	8.5	8.6	3.7	2.7	74.1	7.2	6.3	3.8	.59	.66
Compana	0	8.4	8.4	4.5	3.8	71.3	7.4	7.3	4.6	.55	.65
	112	8.7	8.7	3.0	3.7	73.6	6.8	6.9	4.2	.57	.61
	224	8.5	8.2	2.4	3.6	74.7	6.7	6.7	4.1	.58	.61
Unitan	0	8.4	8.2	4.0	4.7	71.2	7.2	7.0	4.5	.60	.65
	112	8.5	8.4	2.6	4.3	73.5	6.9	6.6	4.2	.58	.62
	224	8.4	8.2	2.3	4.3	74.2	6.7	6.5	4.1	.58	.62
Casein		8.7	9.0	2.9	1.1	76.9	7.3	5.6	3.1	.57	.56

[a]C.P.=crude protein, E.E.=ether extract, C.F.=crude fiber, N.F.E.=nitrogen-free extract, H_2O=moisture, Ca=calcium P=phosphorus.
[b](1)=diet mix 1, (2)=diet mix 2.

RESULTS

There was a varietal effect ($P<.05$) on the yields of the barleys (table 5). Unitan had the highest yield followed by Compana which was not statistically different from Unitan. Hiproly Normal was lower ($P<.05$) than both Compana and Unitan and Hiproly was lower ($P<.05$) than Hiproly Normal. Barleys that received no added nitrogen fertilizer had lower ($P<.05$) yields than barleys receiving 112 or 224 kg/ha added nitrogen (table 6). The difference between the barleys treated with 112 and 224 kg/ha nitrogen was not significant ($P>.05$).

Kernel weight (mg/kernel) was the highest for Compana followed closely by Hiproly Normal (table 5). Unitan was lower ($P<.05$) in kernel weight than Compana and Hiproly Normal. Hiproly had lighter ($P<.05$) kernels than Unitan. Kernel weight decreased ($P<.05$) for each increased level of nitrogen fertilization (table 6). There was a significant cultivar-fertilizer interaction for kernel weight (table 7). This was probably caused by Hiproly barley not changing in kernel weight between 112 and 224 kg/ha nitrogen fertilization.

Compana barley had more ($P<.05$) plump kernels than Unitan or Hiproly Normal which were not different ($P>.05$) (table 5). Hiproly had fewer ($P<.05$) plump kernels than the other three barleys. Percentage plump kernels decreased ($P>.05$) with each increase in nitrogen fertilization (table 6). Table 7 shows a significant interaction for percent plump kernels between variety and nitrogen dressing. The interaction is found in the differences in magnitude of the changes in percent plump kernels for each barley as nitrogen fertilization increased. Percentage thin kernels was nearly as expected, the reverse of percentage plump kernels (tables 5, 6 and 7).

Udy analysis showed that Hiproly had a higher (P<.05) percentage of protein than the other barleys (table 5). Hiproly Normal was lower (P<.05) in Udy protein than Hiproly and higher (P<.05) than Compana and Unitan. Compana and Unitan were nearly equal in Udy protein content. With each increase in nitrogen fertilizer, percentage Udy protein increased (P<.05) (table 6). Udy protein had an interaction (P<.05) between variety and fertilizer level caused by Hiproly not responding to added nitrogen fertilizer (table 7).

Barleys were ranked Hiproly, Hiproly Normal, Compana and Unitan according to their percentage of Kjeldahl protein (table 5). A significant difference occurred between each barley (P<.05). As with Udy protein, Kjeldahl protein increased (P<.05) with increased levels of nitrogen fertilization (table 6). Hiproly barley decreased (P<.05) in Kjeldahl protein when fertilized with 112 kg/ha nitrogen causing a cultivar x fertilizer interaction. In Hiproly barley Udy protein was higher than Kjeldahl due to Hiproly barley protein being higher in basic amino acids than normal barleys. Udy and Kjeldahl protein contents did not differ more than 1% for Hiproly Normal, Compana and Unitan barleys.

Fractionation of the protein (table 8) showed that Hiproly Normal had the highest level of hordein in the protein compared to the other barleys. Compana, Unitan and Hiproly followed in that order in percentage hordein. Hiproly had more salt-soluble (albumins and globulins) and more glutelin proteins than the other barleys. The protein fractions tended to remain fairly constant between fertilizer treatments in both Hiproly and Hiproly Normal barleys. The glutelin fraction remained fairly constant in each of the four barleys for all nitrogen fertilizer levels. In Compana and Unitan an increase in percentage hordeins in the protein and subsequent decrease in salt-soluble proteins was seen between barley receiving 0 and 112 kg/ha nitrogen fertilization. Only slight changes in protein composition were found between 112 and 224 kg/ha nitrogen treated Compana and Unitan barley.

Amino acid composition of the barleys are shown in tables 9 and 10 as percentage of the protein and percentage of the whole kernel, respectively. Table 9 shows that Hiproly had more lysine (g/16g N), methionine and triptophan and less cystine and glutamic acid than the Compana, Unitan or Hiproly barleys. Hiproly Normal was highest in glutamic acid and proline and lowest in lysine of the four barleys. Hiproly and Hiproly Normal have more phenylalanine than Compana and Unitan. As nitrogen fertilizer level was increased on Hiproly and Hiproly Normal, no consistent changes in amino acid composition occurred. In Compana and Unitan barley as fertilizer was increased from 0 to 112 kg/ha, alanine, aspartic acid, lysine, methionine and valine decreased while glutamic acid and proline increased. Only a slight decrease was seen for arginine, cystine and glycine for Compana and Unitan barley between the lower two fertilizer treatments. No real changes occur in amino acid composition of the Compana and Unitan barley protein as nitrogen fertilizer increased from 112 to 224 kg/ha. Hiproly barley was highest in lysine (% of whole kernel) followed by Hiproly Normal (table 10).

The effect of cultivar on the performance of rats measured in a 28-day growth trial is shown in table 11. Unitan and Hiproly barley produced the greatest ($P<.05$) gain. Both of these produced gains that were higher ($P<.05$) than Compana; however, rats gained more ($P<.05$) on Compana than on Hiproly Normal. Rats fed Unitan barley diets had the highest feed consumption although the difference was only significant ($P<.05$) compared to rats fed Hiproly Normal and Compana. Rats fed Hiproly, Compana and Hiproly Normal were not different ($P>.05$) in feed consumption. No difference was found between feed/gain ratios for Hiproly, Compana and Unitan fed rats. Rats fed the above three cultivars all had superior ($P<.05$) feed/gain ratios than rats fed Hiproly Normal. The highest PER's were produced on the Unitan diets but they were not higher ($P>.05$) than Hiproly diets. Protein efficiency ratios produced on Compana barley were lower

(P<.05) than those for Unitan but were not different (P>.05) than those for Hiproly fed rats. Hiproly Normal fed rats had lower (P<.05) PER's than the rats fed the other three barleys.

The effect of nitrogen fertilization on the barleys on growth trial measurements is shown in table 12. Barley fertilized with 224 kg nitrogen per hectore produced lower gains (P<.05) than barley fertilized with 0 or 112 kg/ha. Nitrogen fertilization did not effect feed consumption. Poorer feed/gain ratios and PER's were found with each increase in nitrogen fertilization. A significant (P<.05) interaction between cultivar and nitrogen fertilization was detected for all measurements made in the growth trial (table 13). Rats fed Hiproly barley fertilized with 112 and 224 kg/ha performed almost identically and the rats fed Hiproly Normal barley with no fertilizer added performed very poorly. The combination of these probably caused the interaction.

True protein digestibility (TPD) and net protein utilization (NPU) were affected (P<.05) by barley cultivar. The TPD of Compana was higher (P<.05) than Hiproly but was not different from Hiproly Normal. Hiproly and Hiproly Normal diets were not different from one another. Unitan had a lower (P<.05) TPD than all other three barleys. The NPU of Unitan was lowest, also although it was not different (P>.05) from Hiproly Normal. Hiproly and Compana barleys had higher NPU than Unitan (P<.05) but were not different from Hiproly Normal (P>.05)

Unfertilized barley had statistically higher mean biological values (BV) than barley fertilized with 224 kg N/ha ($P<.05$). No difference was detected between unfertilized barley and barley fertilized with 112 or 112 and 224 kg N/ha. Nitrogen fertilization did not effect TPD or NPU. No significant ($P<.05$) interactions between cultivar and fertilizer level were seen for BV, TPD or NPU (table 16).

In table 17, the correlation between percentage protein in the barley and percentage amino acid in the protein are given. With all four cultivars included in the correlation, amino acids that show strong negative correlations between percentage protein and amino acid content of the protein are: cystine, glycine, histidine, leucine, serine and threonine while a strong positive correlation is shown only for phenylalanine. When Hiproly barley is taken out, these correlations change considerably. Lysine, arginine and threonine become very strongly negatively correlated to percentage protein in the three barleys without Hiproly. Glutamic acid and proline become strongly positive-17 correlated to protein percentage. Correlations between protein percentage and amino acid content of the protein change little between those found when Hiproly or both Hiproly and Hiproly Normal are left out of the calculations.

The correlations between gain, PER, BV and NPU and amino acid composition (g/16g N) and total protein are given in tables 18, 19, 20 and 22, respectively. Since trends within these tables are somewhat similar they will be discussed collectively. Strong positive correlations between the above mentioned measurements and lysine, arginine, alanine and valine were found. Strong negative correlation between glutamic acid and proline and these measurements were found. Table 21 give the correlations between TPD and amino acids (g/16g N) and protein percentage. It is essentially the reverse of the other tables. Large increases in magnitude of all correlations were observed when Hiproly barley was eliminated from the correlation.

TABLE 5. EFFECT OF CULTIVAR ON LEAST SQUARES MEANS OF YIELD, KERNEL WEIGHT, TEST WEIGHT, PERCENTAGE THIN AND PLUMP KERNELS UDY AND KJELDAHL PROTEIN OF BARLEYS

Barley cultivar	Hiproly	Hiproly Normal	Company	Unitan
Yield, kg/plot	2.87^{c}	6.91^{b}	8.74^{a}	9.55^{a}
Kernel wt, mg	36.7^{c}	49.8^{a}	50.8^{a}	41.8^{b}
Test wt, lb/bu	49.1^{b}	55.4^{a}	47.2^{c}	45.5^{d}
Percenage thin	37.9^{a}	6.3^{b}	7.5^{b}	8.6^{b}
Percentage plump	24.4^{c}	81.3^{b}	85.5^{a}	79.0^{b}
Udy protein, %	18.4^{a}	14.5^{b}	10.8^{c}	10.7^{c}
Kjeldahl protein, %	16.6^{a}	15.0^{b}	11.8^{c}	11.0^{d}

[abcd]Means on the same line with the same superscript letter are not statistically different ($P<.05$).

TABLE 6. EFFECT OF NITROGEN FERTILIZER ON LEAST SQUARES MEANS OF YIELD KERNEL WEIGHT, TEST WEIGHT, PERCENTAGE THIN AND PLUMP KERNELS AND UDY AND KJELDAHL PROTEIN OF BARLEYS

Nitrogen fertilizer level, kg/ha	0	112	224
Yield, kg/plot	6.00^{b}	7.27^{a}	7.78^{a}
Kernel wt, mg	47.7^{a}	44.5^{b}	42.0^{c}
Test wt, lb/bu	51.3^{a}	49.1^{b}	47.4^{c}
Percentage thin	11.2^{b}	14.5^{ab}	19.6^{a}
Percentage plump	73.9^{a}	67.7^{b}	61.1^{c}
Udy protein, %	12.0^{c}	13.7^{b}	15.0^{a}
Kjeldahl protein, %	12.3^{c}	13.7^{b}	14.8^{a}

^{abc}Means on the same line with the same superscript letter are not statistically different ($P<.05$).

TABLE 7. EFFECT OF NITROGEN FERTILIZATION AND CULTIVAR ON LEAST SQUARES MEANS OF YIELD, KERNEL WEIGHT, TEST WEIGHT PERCENTAGE PLUMP AND THIN KERNELS AND UDY AND KJELDAHL PROTEIN ANALYSIS OF BARLEY

Barley cultivar	Nitrogen fertilizer level (kg/ha)	Yield (kg/plot)	Kernel weight (mg)	Test weight (lb/bu)	Percentage thin	Percentage plump	Udy protein	Kjeldahl protein
Hiproly	0	1.91	38.9^{h}	50.9^{d}	34.1	28.5^{g}	18.2^{b}	16.8^{a}
	112	2.72	35.5^{i}	48.9^{e}	38.6	23.2^{h}	18.1^{b}	16.1^{b}
	224	3.97	35.6^{i}	47.4^{f}	41.0	21.5^{h}	18.8^{a}	17.0^{a}
Hiproly Normal	0	5.09	52.7^{ab}	58.3^{a}	3.3	90.3^{ab}	13.3^{e}	14.2^{d}
	112	7.10	49.5^{c}	54.5^{b}	6.1	78.9^{de}	14.3^{d}	14.8^{c}
	224	8.54	47.0^{d}	53.4^{c}	9.6	74.8^{ef}	15.7^{c}	15.9^{b}
Compana	0	8.25	54.9^{a}	48.9^{e}	2.4	94.0^{a}	8.1^{g}	9.3^{h}
	112	9.17	51.3^{bc}	47.4^{f}	6.3	86.7^{bc}	11.4^{f}	12.4^{f}
	224	8.80	46.0^{de}	45.3^{g}	13.9	75.7^{ef}	12.7^{e}	13.7^{e}
Unitan	0	8.74	44.0^{ef}	47.2^{f}	5.2	82.8^{cd}	8.5^{g}	9.1^{h}
	112	10.10	41.7^{fg}	45.8^{g}	6.9	81.9^{cd}	10.9^{f}	11.5^{g}
	224	9.80	39.5^{gh}	43.5^{h}	13.7	72.3^{f}	12.8^{e}	12.5^{f}

^{abcdefghi}Means in the same column with the same superscript letters are not statistically different ($P<.05$).

TABLE 8. EFFECT OF CULTIVAR AND NITROGEN FERTILIZATION ON OSBORNE PROTEIN FRACTIONS OF BARLEY

Barley cultivar	Nitrogen Fert. level (kg/ha)	Total protein	Albumins + globulins	Hordein	Glutelin	Residue
Hiproly	0	16.7	30.9	28.5	36.6	3.9
	112	15.9	34.8	26.7	33.4	5.1
	224	16.4	35.1	27.6	32.2	5.2
Hiproly Normal	0	14.0	26.7	38.6	29.3	5.4
	112	14.5	28.2	37.9	28.8	5.2
	224	15.7	27.7	39.0	28.2	5.2
Compana	0	9.0	33.6	31.3	28.3	6.8
	112	11.6	30.3	36.5	27.7	5.5
	224	13.4	29.8	36.2	28.7	5.3
Unitan	0	8.9	33.4	25.5	31.9	9.2
	112	11.1	31.1	29.7	32.0	7.2
	224	12.3	30.5	30.7	32.0	6.9

TABLE 9. AMINO ACID COMPOSITION OF BARLEY COMPOSITES EXPRESSED AS A PERCENT OF THE PROTEIN (g AMINO ACID/16 g N)

Barley cultivar	Hiproly			Hiproly Normal			Compana			Unitan		
Fertilizer level (kg/ha)	0	112	224	0	112	224	0	112	224	0	112	224
Amino acid												
Alanine	3.99	4.35	4.11	3.61	3.68	3.42	4.12	3.69	3.74	4.04	3.88	3.72
Arginine	4.94	5.09	4.96	4.56	4.36	4.42	5.01	4.86	4.77	5.19	4.89	4.89
Aspartic acid	6.71	6.99	6.88	5.64	6.00	5.60	6.27	5.82	5.82	6.36	6.17	6.00
Cystine/2	1.51	1.80	1.68	2.10	2.20	1.85	2.60	2.41	2.07	2.46	2.65	2.39
Glutamic acid	24.71	23.52	24.35	27.37	26.84	27.98	24.79	26.60	27.03	25.07	26.31	26.60
Glycine	3.32	3.54	3.38	3.32	3.34	3.20	3.62	3.29	3.31	3.65	3.48	3.37
Histidine	2.31	2.38	2.28	2.21	2.30	2.23	2.41	2.36	2.35	2.42	2.37	2.33
Isoleucine	3.74	3.81	3.79	3.62	3.61	3.60	3.78	3.72	3.79	3.71	3.68	3.69
Leucine	6.93	7.02	6.92	6.95	6.84	6.86	7.12	7.06	6.98	7.10	6.96	6.95
Lysine	4.05	4.33	4.11	3.06	3.42	3.20	3.78	3.33	3.31	3.74	3.51	3.44
Methionine	1.95	1.98	1.98	1.74	1.67	1.69	1.85	1.71	1.75	1.78	1.74	1.74
Phenylalanine	5.84	5.66	5.69	5.69	5.72	5.88	5.00	5.17	5.26	4.87	4.90	5.08
Proline	11.86	11.25	11.53	12.54	12.54	13.15	10.89	11.90	11.71	10.87	11.38	11.70
Serine	4.52	4.50	4.56	4.56	4.42	4.35	4.77	4.46	4.58	4.81	4.69	4.56
Threonine	3.54	3.63	3.56	3.39	3.50	3.38	3.78	3.61	3.59	3.72	3.64	3.61
Tryptophan	1.78	1.90	2.10	1.86	1.68	1.71	1.93	2.02	1.86	2.09	1.51	1.63
Tyrosine	3.30	3.16	3.20	3.18	2.94	3.21	3.11	3.23	2.98	2.93	2.98	2.94
Valine	5.01	5.10	5.10	4.65	4.74	4.52	5.21	4.80	4.96	5.38	5.19	5.17