



Nutritional evaluation of 2-rowed barleys selected for high-lysine
by Duane Kip Stutz

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Animal Science

Montana State University

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Abstract:

One hundred twenty female weanling rats were used in each of two duplicate growth trials to compare the nutritive value of 23 different barley varieties. Nineteen of the barleys were selected for potential high-lysine content of their protein. The remaining four barleys were commercial varieties and an isogene of one of the commercial varieties. The barleys were formulated into isonitrogenous, isocaloric 10% protein diets with a casein corn starch diet as control. Five of the barleys produced rat gains that were not significantly different from gains of rats fed the casein control diet. These diets also produced the highest PERs and lowest feed/gain ratios of the barleys compared. The Hiproly (CI 3947) diet was the best of the barleys in terms of gains, feed efficiency and PER. There were no significant differences in feed consumption due to diet. The commercial variety, Compana, (CI 5438), produced the slowest rate of gain and lowest PER and was least efficiently utilized. The percentage of lysine in the barley proteins was significantly correlated to gain, the feed/gain ratio and PER. Feed efficiency was shown to be highly correlated to gain and PER. The nitrogen balance study was conducted with 16 weanling rats fed a nitrogen-free diet to determine metabolic fecal nitrogen and endogenous urine nitrogen excretion. Average metabolic fecal nitrogen was $.023 \pm .001$ g N/rat/5 days and endogenous urine nitrogen was $.020 \pm .004$ g N/rat/5 days. Ten barley varieties were selected from trial I for the determination of biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU). Five barley varieties were grouped together according to their high PER performance and the other five on the basis of their low PER values. When all ten varieties were analyzed individually, the biological value of Hiproly (CI 3947) was significantly higher ($P < .05$) than Imperial (CI 12147) and waxy Compana (CI 294318). True digestibility of Compana was significantly higher ($P < .05$) than Hanna (CI 906), Bolder (CI 7131), CI 10375, Hiproly (CI 3947) and CI 12171. There was no significant difference in net protein utilization of the individual barleys. Composite means of high PER barleys versus low PER barleys showed significant differences ($P < .01$) for biological value and true protein digestibility. Net protein utilization of the high PER barleys was also significant but only at the 10% level of probability.

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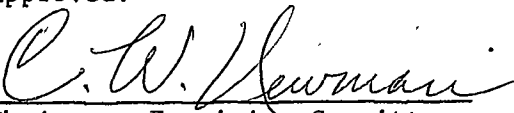
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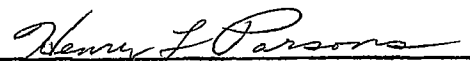
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ABSTRACT

One hundred twenty female weanling rats were used in each of two duplicate growth trials to compare the nutritive value of 23 different barley varieties. Nineteen of the barleys were selected for potential high-lysine content of their protein. The remaining four barleys were commercial varieties and an isogene of one of the commercial varieties. The barleys were formulated into isonitrogenous, isocaloric 10% protein diets with a casein corn starch diet as control. Five of the barleys produced rat gains that were not significantly different from gains of rats fed the casein control diet. These diets also produced the highest PERs and lowest feed/gain ratios of the barleys compared. The Hiproly (CI 3947) diet was the best of the barleys in terms of gains, feed efficiency and PER. There were no significant differences in feed consumption due to diet. The commercial variety, Compana, (CI 5438), produced the slowest rate of gain and lowest PER and was least efficiently utilized. The percentage of lysine in the barley proteins was significantly correlated to gain, the feed/gain ratio and PER. Feed efficiency was shown to be highly correlated to gain and PER. The nitrogen balance study was conducted with 16 weanling rats fed a nitrogen-free diet to determine metabolic fecal nitrogen and endogenous urine nitrogen excretion. Average metabolic fecal nitrogen was $.023 \pm .001$ g N/rat/5 days and endogenous urine nitrogen was $.020 \pm .004$ g N/rat/5 days. Ten barley varieties were selected from trial I for the determination of biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU). Five barley varieties were grouped together according to their high PER performance and the other five on the basis of their low PER values. When all ten varieties were analyzed individually, the biological value of Hiproly (CI 3947) was significantly higher ($P < .05$) than Imperial (CI 12147) and waxy Compana (CI 294318). True digestibility of Compana was significantly higher ($P < .05$) than Hanna (CI 906), Bolder (CI 7131), CI 10375, Hiproly (CI 3947) and CI 12171. There was no significant difference in net protein utilization of the individual barleys. Composite means of high PER barleys versus low PER barleys showed significant differences ($P < .01$) for biological value and true protein digestibility. Net protein utilization of the high PER barleys was also significant but only at the 10% level of probability.

INTRODUCTION

Barley is the major feed grain produced in Montana and ranks fourth only to wheat, rice and corn in total world production of all cereal grains.

As the world's population steadily increases, more and more pressure is put on agricultural production. In many countries, the demand for food and feed grains exceeds the supply. Researchers are continually seeking better methods and improved products which will yield greater quantities of higher quality foods.

With today's pressing world food situation much of the wheat and corn, which previously has been used for livestock production, is now being used for human consumption. This has reduced the supply and increased the cost of these grains, thus putting added pressure on the need for the production of other feed grains.

The broad ecological range of barley and its many varieties and characteristics have stimulated investigators into studying and developing barley's potential nutritive feeding value. Barley can be grown under more extremes of temperature, climate, and soil type than any other cereal grain. Barley can also be seen at more northerly latitudes, at higher elevations, and further into the drylands than any other cereal grain. Furthermore, under the less favorable environments where barley is the most extensively grown cereal, it is doubtful that varieties of any other cereal can be developed that will equal or exceed barley in the production of total available nutrients per unit

of tillable soil.

Considerable variation exists between barley varieties with respect to protein quantity and amino acid balance. These differences are directly related to the nutritive quality of the individual barley varieties and can be used to assess their nutritional value.

There has been considerable interest in recent years in development of new varieties of feed grains that contain higher than normal protein levels and possess a more optimum essential amino acid content. Opaque-2 corn has been one of the most significant discoveries in nutrition and plant genetics in the past fifty years. This mutant strain of corn contains a higher protein level, along with more than twice the level of lysine found in normal varieties. The recent discovery of high protein, high lysine barley varieties has added emphasis to the importance of barley as a high quality feed grain. Lysine is the number one limiting amino acid in normal barley and most other cereal grains. Mutant strains have been found that contain 30 to 40% more lysine in the cereal protein than normal barley. The development of a barley that will furnish high quality protein in addition to a large supply of available energy will undoubtedly have a tremendous influence in the future on nutrition of the human population in certain parts of the world. It is conceivable that barley could become a major dietary component of man in those areas as well as for domestic farm animals.

This study was conducted to evaluate the nutritional value of high lysine barley for nonruminants. This thesis involved testing 23 different 2-rowed barley varieties selected from the world barley collection according to their potential lysine content.

LITERATURE REVIEW

Of the major food crops currently cultivated throughout the world, cereal grains are the most extensively grown. Among all cereal grains, barley ranks fourth in total world production. Even though barley is grown on fewer land units than wheat, rice or corn, respectively, it should be pointed out that barley yields the greatest quantity of protein per unit (R. F. Eslick, personal communication).

Barley is the major feed grain grown in Montana. The average land area planted to barley for the past ten years in Montana has been approximately 607 hectares. In 1970, 693.7 hectares were planted to barley resulting in a crop yield approximately of 1.4 metric tons (Montana Agricultural Basic Facts, 1973).

In past years, barley has had the reputation of having inferior feeding qualities. But through today's improved knowledge in plant genetics, many of the undesirable characteristics of barley have been improved. Certain barley varieties now possess superior chemical and physical properties that are necessary for a high quality feed source.

Barley as a Feed and a Food

In sections of the world where it is too cool or too dry to grow wheat or corn, barley is the major cereal grain produced. It is an excellent feed for growing and finishing swine, beef cattle and poultry. It is not atypical of finishing swine or beef cattle rations to contain as much as 85% barley (Krider and Carroll, 1971). When using barley in swine rations it is a normal practice to grind it to a medium degree

of fineness and may be pelleted. This will reduce wastage and increase its utilization value (C. W. Newman, personal communication).

Carter and Eslick (personal communication) reported that barley is presently the major source of food for millions of people in the Middle East and North Africa. The cooler, semiarid environment of countries in this area of the world enables the people to produce barley whereas other grains are less adapted for production. The increasing human and animal consumption of barley necessitates that it be improved both in productivity and in nutritive quality. The development of the most efficient varieties for any given location must be the objective. To accomplish this nutritionists and plant geneticists must work together to identify and accumulate a genetic pool of those desirable characteristics that will respond favorably to a certain environment.

Barley Proteins and Amino Acids

Since protein is a principal constituent of the animal body, a liberal and continuous supply is needed in the diet throughout an animal's lifetime. The term protein is a broad one which includes an enormous group of closely related but physiologically distinct members. All proteins consist of amino acids linked together in specific sequences by peptide bonds (Lehninger, 1970). From the standpoint of nutrition the important distinguishing feature of the various proteins is their amino acid makeup. Extensive data have shown that proteins having a large percentage of the total protein consisting of

the essential amino acids, produce the greatest and most efficient performance in animals (Howe, Jansen and Gilfillan, 1965).

Barley has been reported to contain an average of 13.0% protein and .52% lysine when expressed on a dry matter basis (National Academy of Sciences, 1971). The percentages of individual amino acids in proteins are frequently used for estimating and comparing their nutritive value. Cereal grain proteins are noted for being deficient in three or four of the essential amino acids. When considering barley, the first limiting amino acid is lysine, followed by methionine, threonine and tryptophan in decreasing order (Howe, Jansen and Gilfillan, 1965).

The early work of Osborne (1907) on solvent fractionation of cereal proteins remains the basis for classification of proteins. Cereal proteins are classified into water soluble proteins, which are the albumins; salt soluble proteins consisting of the globulins; alcohol soluble proteins called prolamines or in the case of barley, hordein, and alkaline soluble glutelin proteins. According to Kollback (El-Negoumy, personal communication), approximately three-fourths of the albumin in barley is located in the endosperm and one-fourth in the germ. About three-fourths of the globulin is located in the germ and one-fourth in the endosperm. The hordein and glutelin proteins appear to be entirely located within the endosperm.

Waldschmidt and Leitz (1961) reported that a portion of barley protein is actually glycoprotein in nature, which means that the protein molecule is conjugated with a substance or substances containing a carbohydrate group (Maynard and Loosli, 1969).

The nature of the salt-soluble proteins (globulins) in barley has been carefully studied by Djurtoft (El-Negoumy, personal communication). The extraction of these proteins is complicated by their dependence on the nature of their protein makeup, and also their carbohydrate and phytic acid content. The globulins of barley have been separated into three fractions by ultracentrifugation (Danielson, 1949). These can be classified as the heavy gamma-globulin (MW 270,000), intermediate beta-globulin (MW 113,000), and the lighter alpha-globulin (MW 24,000). All three globulin fractions have been noted to have different distributions within the kernel (Wall, 1964).

The term prolamine was proposed by Osborne (1907) for the group of plant proteins soluble in alcohol. The name is very appropriate inasmuch as these proteins yield, upon hydrolysis, large amounts of proline and ammonia (Oser, 1965). The prolamines are simple proteins which are insoluble in water and other neutral solvents, but are soluble in 70 to 80% alcohol. They are widely distributed throughout the plant kingdom and are referred to as hordein in barley.

Protein distribution differences also exist between different barley varieties. Much of this variation is due to total protein

content of the kernels and environmental influences during the growing season (Munck, Karlsson, and Hagberg, 1969).

Munck et al. (1969) reported a means of studying amino acid composition of barley kernels by dissection of the aleurone and embryo from the endosperm. Results showed a higher content of essential amino acids, including lysine, to be in the aleurone and embryo portions. Munck suggests that since barley has a multicellular aleurone layer, it should be higher in lysine as a percentage of seed weight. Because the outer layers of barley are highest in protein concentration, more than half of the total proteins of the seed are located in these structures.

Pomeranz et al. (1972) investigated the amino acid composition of high-amylose and Hiproly barleys. Amino acid composition was found to be affected by genetic factors, environmental conditions and by managerial cultural practices. It was noticed that as the protein content of a cultivar is increased, the concentration of the hordein and glutelin fractions increases; and the concentration of the soluble albumins and globulins decreases. This resulted in a decrease in the lysine concentration of the barley protein with increased total protein.

Amino Acid Availability

The development of new grain varieties, with improved amino acid composition and increased protein content, suggests that a greater nutritional value should be obtained from them. However, this may not

always be the case. Nutritive value is not only dependent on the quality and quantity of a protein but also on the biological availability of the individual amino acids and on the fact that the levels of amino acids present in a feed are not necessarily the levels available to the body.

A protein does not contain free amino acids, but instead the condensed units linked together to form peptide chains. Digestion in the gastrointestinal tract proceeds by the hydrolysis of these peptide bonds to release free amino acids. However, a certain proportion of the total amino acids in the dietary constituents may be biologically unavailable, thus the protein is not utilized to its optimum capacity (Eggum, 1973).

Availability of amino acids can be reduced by incomplete digestion and absorption, by the presence of inhibitors of digestive enzymes, by heat damage from processing procedures, or by antagonism between individual amino acids or other dietary components (Eggum, 1973).

The requirement of protein in animal diets was first recognized by Magendie (1816). He later established that not all proteins were of equal value. They differ with respect to their constituent amino acids, both qualitatively and quantitatively.

Determination of nutritional value must involve some means of estimating amino acid availability. Methods of assessing the nutritive value of proteins have been extensively studied by Eggum, (1973).

Four of the main procedures are based upon: (1) nitrogen balance, (2) body growth, (3) tissue regeneration, and (4) amino acid composition of the protein.

Eggum (1973) described the "Fecal Analysis" method for estimating amino acid availability. This method is analogous to the determination of true digestibility of the total protein and consists of measuring the amount excreted in the feces. Metabolic fecal amino acid excretion, which is not of dietary origin, is also taken into consideration, thus a true availability can be obtained.

The fecal analysis method was developed by Kiuken and Lyman (1948). One criticism that has been made about the procedure involves the microbial activity in the intestinal tract of animals. The activity could result in a synthesis or destruction of amino acids, resulting in erroneous fecal amino acid concentrations. To test this factor, Kuiken (1952) added sulfathiazole, a bactericide, to diets in order to reduce any such microbial influence. He found no differences in the availability values due to this treatment. Amino acid availability, as determined by the fecal analysis method, provides a measure of the quantity of amino acid that is released and absorbed during digestion. This method also provides a means of estimating which amino acids are excreted in indigestible insoluble residues from poorly digestible proteins (Eggum, 1973).

Mitchell and Block (1946) developed a method for estimating protein utilization called the "Chemical Score". This calculation is based on the principle that the utilization of the protein is solely dependent upon the limiting essential amino acids. This determination allows a prediction of the possible value of the protein in various dietary combinations. However, this method does not take into account the availability of the amino acids or whether certain amino acids are excessive in relation to the requirements of the organism. These factors probably contribute to a reduction in the relationship between Chemical Score and biological value.

Eggum (1973) conducted in vitro protein digestion studies and concluded that amino acids are liberated from proteins at different rates. The rate of release is affected by the linkage of the amino acids in the protein and their relative ease of absorption through the intestinal wall.

It is generally accepted that protein utilization is higher at low protein levels than at high levels. Armstrong and Mitchell (1955) found that by increasing the intake of protein, pigs responded in a linear increase in the amount of nitrogen in the feces. In experiments with dogs, Allison and Bird (1964) found that the rate of increase of nitrogen eliminated in the feces, with increasing protein intake, was greater for soybean meal, which was moderately digestible, than for highly digestible casein. Bragg et al. (1969) reported excreta from

broiler chicks fed high protein were higher in certain amino acids when compared to excreta from chicks fed low protein diets. These observations indicate that the digestibility and amino acid composition of dietary protein can influence amino acid output in the feces.

Eggum (1973) reported the availability of lysine, isoleucine, threonine and methionine in rats, fed three samples of corn grown under different levels of nitrogen fertilizer applications, to be insignificant. The availability was approximately 90% for lysine, isoleucine, and threonine, and 95% for methionine.

In experiments with mature pigs, Dammers (Eggum, 1973a) found the true digestibility (TD) for individual amino acids of barley to vary from 74% for methionine to 93% for glutamic acid and cystine. Lysine was digested with 89% efficiency. In rat studies using barley, Eggum (1973) reported TD values of 78.3% and 86.4% for lysine and methionine, respectively. These data reflects the considerable variation that exists between TD values of individual amino acids. True digestibility values of amino acids may vary above and below the digestibility of the total nitrogen. Low TD values for lysine are of particular interest as lysine is the first limiting amino acid in barley protein. In Eggum's study, TD values for aspartic acid, glycine, and alanine were lower than that of the total nitrogen, whereas glutamic acid, histidine, and arginine were considerably higher. True digestibility for total nitrogen was about 82 percent. Eggum concluded that the low TD value for lysine was probably due to differences in lysine concentrations in the

different protein fractions of the barley kernel. The lysine content is highest in the aleuron layer and lowest in the endosperm. Since the endosperm possesses the highest digestibility and the lowest lysine content, lower TD values for lysine will be obtained when estimated on total nitrogen. Similarly, high TD values are obtained for glutamic acid which is abundant in the endosperm. It is also possible that microbial protein, rich in lysine, is synthesized in the intestinal tract. This would raise the lysine concentration in the feces and hence give a low TD value. However, a significant effect due to the microflora does not appear to be likely.

Munck (1972a) reported that the amino acid balance of the barley kernel, from the nutritional point of view, is likely to be improved upon extension of the aleuron layer. However, proteins from the multicellular aleuron layer of barley are very difficult for animals to digest. In contrast, wheat and corn have highly available protein and amino acids due to their unicellular aleuron layer. The decrease in amino acid availability, especially that of lysine which is prevalent in barley's aleuron layer, must be given consideration.

Statistical evaluation of amino acid patterns from all types of barley reveals that those amino acids that are easily extracted by the Osborne (1907) extraction series, such as glutamic acid and serine, are also more easily digested, whereas lysine, alanine, and aspartic acid, which are poorly digested, are also difficult to extract. It is

thus reasonable to conclude that relationships between seed morphology, protein extraction property, and amino acid availability are important for the proper nutritional utilization of the cereal seeds (Munck, (1972).

Two-Rowed Barley

Barley is classified as a member of the Gramineae family and the genus Hordeum. The World Barley Collection is comprised of two main types, two-rowed and six-rowed varieties. The distinguishing characteristic is dependent on the physical arrangement of the kernels in the seed head.

In the United States, approximately 90% of malt is made from the midwestern six-rowed Manchurian type barley. These barleys have relatively small kernels, are medium high in protein content, and possess a high enzymatic activity when malted (Kneen and Dickson, 1967). In contrast, two-rowed western malting barleys are relatively low in protein and high in starch. Physically, the two-rowed barleys possess a medium-sized, uniformly plump kernel, with a thin hull. There are also isogenic lines which display the naked or hulless character (Pomeranz et al. 1973).

Wilson (1955) described the central kernels of two-rowed barley varieties as being broadest near the base of the grain. Visual identification of two-rowed and six-rowed barley varieties is most difficult after the barleys have been threshed and cleaned. Barleys are then

recognized mainly by their differences in color and physical characteristics expressed by their endosperms.

Day and Dickson (1957) conducted genetic linkage studies to determine the association of nitrogen content with certain morphological characteristics expressed in barley. They found that nitrogen content was related more to the two-rowed barleys rather than the six-rowed character. In a series of tests, the six-rowed barleys had a mean barley nitrogen percentage significantly lower than that of the two-rowed varieties. This agrees with a survey conducted by the National Barley and Malt Laboratory, which reported an average grain protein of 11.9 and 12.6% in six-rowed and two-rowed barleys, respectively, (Pomeranz, 1973).

Pomeranz et al. (1973) reported work concerning the inherent protein content and amino acid composition of two-rowed and six-rowed barleys. He concluded that the kernels from the two-rowed barleys contained about 3% more total protein than the six-rowed barleys (18.3 vs. 15.1%). The kernels from the two-rowed barleys were also heavier than those from six-rowed barleys. Amino acid analysis showed the protein-rich two-rowed barleys to contain more glutamic acid, proline, and less of most of the other amino acids than the six-rowed barleys. Both types of barley revealed a negative correlation between lysine, the nutritionally limiting amino acid of barley, and the storage protein in the cereals. However, lysine also showed a highly positive correlation

with aspartic acid. This is explained by the fact that aspartic acid is a key intermediate in the biosynthesis of lysine in higher plants.

A survey of two-rowed barleys by Eslick et al. (1973) revealed a significant range in protein content. These differences are attributed to plot location, environment, and genetic variation. This range in protein content was also shown by Pomeranz et al. (1973) to affect the amino acid patterns of two-rowed and six-rowed barleys. Pomeranz determined the amino acids present in proteins of 15 two-rowed and 21 six-rowed barley cultivars which were grown in different locations and which varied widely in protein content. Results showed that the amino acid composition of both the two-rowed and the six-rowed barley types depended more upon the total protein content in the sample rather than the type of barley.

When barley is used in swine rations it primarily serves as an energy source. It is commonly referred to as a fattening feed (Crampton and Harris, 1969). There is controversial data as to the effect of hulled versus hulless barley in growing and finishing diets for swine. Due to the inability of monogastrics to digest cellulose, the hull and fiber content of barley have been labeled as major factors responsible for the inferior performance of swine when fed barley diets. Gill et al. (1966) conducted a feeding trial to evaluate fiber content in growing and finishing swine diets. He compared covered Hannchen barley, Utah hulless barley, Gaines wheat, and corn. Pig gains with the hulless barley diets exceeded those of the covered barley and

equaled those diets of wheat, but gains by pigs fed the corn ration were superior to all others.

Larsen and Oldfield (1961) reported that the increased fiber content of hulled barley decreases the feeding value of covered barley varieties when compared to hulless varieties. They postulated that the increased fiber content, due to the hull, lowers the available energy. Their data also suggested that the barley hull did more than just dilute the available nutrients, as pigs responded differently to fiber from barley hulls than to fiber from purified wood cellulose when added to corn or pearled barley diets. The hull may contain factors which inhibit nutrient digestion, absorption, or utilization. The factors associated with the genetic material controlling the attachment of the hull to the kernel may be involved.

Newman et al. (1968) reported a study comparing hulless and normal barley isogenes of two varieties on the performance of weanling pigs. Data indicated that differences in nutritive value may be due to factors other than just a lower crude fiber content in hulless varieties. A hulless barley developed from Compana was superior to covered Compana, but a hulless Glacier isogene was of no greater value nutritionally than the covered Glacier. This data would support the hypothesis of Larsen and Oldfield (1961), that the barley hull may contain factors other than just fiber that affect nutritional value.

Hiproly Barley

Hiproly barley was first discovered in the world barley collection by Swedish scientists. Its origin has been traced back to Ethiopia (Munck et al., 1969). Hiproly (CI 3947) was first isolated by a screening technique which is based on the dye-binding capacity (DBC) of basic amino acids, among them lysine. The acid dye, Acilane Orange G, combines at pH 2.6 with basic groups of amino acids in the cereal proteins in suspension. The DBC/kg of sample is also correlated to crude protein content (Munck, et al., 1969).

Hiproly is a naked, two-rowed barley with short straw. It is characterized as its name implies; higher in crude protein and higher in lysine than normal barley varieties. A single recessive gene, located on chromosome number seven, has been found to be responsible for increasing the lysine content (gm/16 gm N) by about 30 percent. This gene increases lysine and other essential amino acids in the endosperm independently of protein content. Munck et al. (1969) reported Hiproly as having a 50 and 30% increase of protein and lysine, respectively, over conventional commercial varieties of barley. The lysine was measured as a percentage of the total protein and varieties containing equal protein levels.

Newman et al. (1973) found Hiproly barley to have superior amino acid composition and total protein content as compared to reference barleys. It contained 30 to 36% more lysine and 21 to 38% more

methionine than reported for normal barleys.

The amino acids lysine, methionine, valine, threonine, isoleucine, alanine, glycine, and aspartic acid are higher in Hiproly barley, whereas cysteine, glutamic acid, and proline are lower when compared with other varieties with comparable protein contents (Munck et al., 1970).

One of the most deleterious characteristics of Hiproly is that it has a very low yielding capacity. It has been reported to produce about 30% of that of other commercial varieties (Munck et al., 1972). Hiproly physically exhibits a shriveled, erectoid shaped, hullless seed, which is the major reason for its reduction in yield. It has a 1000 kernel test weight of about 30 to 40 g, which can be compared to 60 and 70 g test weights of other commercial varieties. For germination, Hiproly requires a long-day photoperiod. Due partially to sterility, Hiproly also has a reduced number of seeds per spike. It averages only about 12, whereas other varieties may contain 20 to 36 seeds per spike. (R. F. Eslick, personal communication). Fewer spikes per unit area, because of Hiproly's reduced tillering capacity, is also partially responsible for the lower yields.

The morphological characteristics of the seed associated with the high lysine gene, although undesirable, do not seem to affect the feed quality. However, by changing the gene background of the lysine gene from Hiproly to high yielding varieties, the poor seed type can be

improved without losing the protein quality expression of the gene (Hagberg et al., 1970). A number of genes modify seed structure without interfering with the expression of the high lysine gene as reflected by the amino acid composition. Problems with regard to seed size and yield are also present in Opaque-2 corn. There is, however, no negative relationship between lysine and protein levels and yield in crosses studied, indicating that there are good possibilities to obtain Opaque-2 lines of high productivity. Just as in the case of Opaque-2, it is possible to change the gross morphological character of the Hiproly seed to a more desirable one, while still retaining the favorable amino acid composition (Munck et al., 1972).

The crude fractions of albumins, globulins, glutelins, and insoluble residual proteins of Hiproly differ in amino acid composition as compared to low lysine varieties. Extraction of Hiproly barley endosperms and subsequent amino acid analyses reveal a doubling of the water-soluble albumin fraction. The increase of the lysine rich albumins in Hiproly is reflected in an improved availability of lysine (Munck et al., 1972).

The extractability of protein fractions in Hiproly is improved in comparison to normal barley due to a reduced number of disulfide bridges in the glutelin network. Most Osborne fractions in Hiproly, except the prolamines, display a strongly increased methionine:cysteine ratio due to a decrease of cysteine and increase of methionine

(Munck et al., 1972). The decreased amount of cysteine is a consequence of the high lysine gene and is responsible for the glutelin network being less bridged with disulfide bonds. This in turn changes the binding interactions between different proteins.

Hiproly deviates from the other barleys by having a pronounced variation in the lysine content of the salt-soluble proteins. Only very small amounts of lysine are found in the low molecular weight fractions (alpha-globulins) of Hiproly's salt-soluble protein fractions. However, Ingversen and Koje (1973) by using column chromatography with gel electrophoresis, suggest that the major increase in lysine content of Hiproly could be due to an increase of one or more extremely lysine rich proteins from the salt-soluble proteins. They evidenced salt-soluble globulin proteins of Hiproly to contain 40% of the total lysine and the alkaline-soluble glutelins 50%. These two fractions account for 17% of the total protein content. Ingversen and Koje (1973) concluded, however, that it is unlikely that a fractionation according to solubility can elucidate the changes in the individual proteins which lead to the overall increased lysine content, as both lysine content and concentration in all protein fractions of Hiproly exceed the values found in other varieties.

Data indicate a change in the composition of matrix proteins of Hiproly. Cytological dissection of the high lysine barley endosperm reveals starch grains and protein bodies firmly attached to the matrix

proteins. This is in contrast to normal varieties which possess distinct starch granules separate from the matrix proteins. Hiproly has a high degree of starch-protein adherence. Although the adherence character is inherited from a separate gene, there is a genetic association of starch-protein adherence with the high lysine gene, perhaps involving linkage. The adherence character is believed to be related to the apparent smaller cell size of the Hiproly endosperm (Munck et al., 1970). Tentatively the adherence trait could affect the timing of protein synthesis so that low prolamines are produced earlier while water content is still relatively high in the endosperm, independent of the amount of nitrogen translocated to the seed. The starch-protein adherence character found in Hiproly does not seem to influence the amino acid composition in the endosperm. It does, however, affect the correlation coefficients between different amino acids and protein, so that the usual negative correlation between lysine content and crude protein is now insignificant. Thus, it is possible to select for a changed response with regard to the relationship between protein and the essential amino acids (Munck, 1972b).

Munck et al. (1972) conducted rat nitrogen balance studies to test the nutritional quality of Hiproly in contrast to other high lysine barley varieties which did not possess the starch-protein adherence character. A normal lysine barley was also included as a control. Net protein utilization was 9% higher in the high lysine

lines as compared to the normal control. The starch-protein adherence trait of Hiproly did not affect true digestibility, biological value, or net protein utilization as compared with the other high lysine lines with normal morphology. Consequently, the starch-protein adherence character associated with the high lysine gene in barley does not affect the feed quality.

Hiproly, although high in protein content, shows a protein and amino acid composition similar to low protein commercial barleys. This means increased amounts of albumins, lysine, aspartic acid, and methionine in percent of Kjeldahl protein and decreased amounts of prolamines, glutamic acid, cysteine, and proline. These effects are due to changes restricted to the endosperm portion of the seed (Munck, 1972b).

Data are in evidence to show that the percentage of lysine in most barleys is negatively correlated with total protein, i.e., the higher the protein level, the lower the lysine percentage of the total protein (Munck et al., 1969). This correlation is due to a greater relative synthesis of storage proteins low in lysine and other essential amino acids at the higher levels of protein in the seed. Hiproly barley protein does not follow this pattern, but contains a consistent amount of lysine, regardless of protein level. This is in the same magnitude as that found in Opaque-2 corn protein. Consequently, a 17% protein Hiproly may contain .6 to .7% total lysine.

Supplementation of Lysine

The first evidence of lysine being a dietary requirement for swine was recognized by Mertz et al. (1949). Extensive research since then has led to our current knowledge that the pig requires 10 essential amino acids plus adequate nitrogen for the synthesis of non-essential amino acids.

As mentioned before, Munck et al. (1969) reported that the percentage of lysine in normal barley protein is negatively correlated with total protein. This factor is extremely important as lysine is already the first limiting amino acid in low protein barleys. The limiting effects are further enhanced as the protein content increases. Regression equations have now been computed which assume a linear relationship between dietary protein level and lysine need (Klay 1964).

Many factors can influence the lysine requirement of swine. Besides protein level other factors include: age, sex and strain of pig, caloric density of the diet, protein source, and physiological state of the animal.

Unlike methionine and phenylalanine, whose requirement levels can be reduced 40 and 30%, respectively, by addition of adequate cystine and tyrosine, lysine has no substitute. There is also little possibility of any antagonism between lysine and other amino acids that would be of any influence in planning a practical swine ration. However,

the adequacy of subsequent limiting amino acids will affect the response obtained from lysine supplementation. Several reports have shown that more than one amino acid is often involved in the limiting effects. Supplementation of the number one limiting amino acid is often not enough. Bayley and Summers (1968) reported no significant improvement in gains or feed efficiency of pigs when lysine or methionine was added to a corn-soybean diet separately but noted a substantial response when they were added together. Pond and Jones (1964) found that the addition of .22% lysine to a 92% corn diet for finishing pigs, resulted in no improved performance. However, when .04% tryptophan was added along with the lysine, performance was notably improved.

Sure (1955) supplemented rat diets comprised of pearled barley with DL-threonine, L-lysine, and DL-methionine. The diets were balanced to supply an 8% protein level. Diets supplemented with .4% L-lysine resulted in a 57.2% increase in growth and 50% increase in protein efficiency ratio (PER) when compared to unsupplemented control diets. Additional supplementation of .5% DL-threonine resulted in further growth increases of 78.6% and higher PER's. The supplementation of pearled barley with .4% L-lysine, .5% DL-threonine, and .5% DL-methionine resulted in additional growth increases of 15.3% and a 56.3% total increase in PER.

Bowland (1962) reported that the addition of .2% L-lysine to a 13.5% protein diet composed of barley, wheat, and soybean meal made the diet equivalent to one containing 16% protein in terms of average daily gain and feed utilization by pigs 36 to 100 pounds. Dinusson et al. (1962) reported that barley diets, supplemented with a protein source and additional lysine, resulted in increased gains and in some cases improved feed efficiency when fed to growing pigs. Nielson et al. (1963) showed that lysine supplementation of 14% protein barley-soybean meal diets resulted in significant increases in average daily gains of pigs during the period from 21 to 57 kilograms.

Soldevila and Meade (1964) found that supplementation of lysine and methionine individually or in combination did not improve a barley diet containing 8.45% soybean meal (40% protein) for growing pigs. The basal barley contained 11.7% protein and diets were balanced to supply 14% total protein. Further testing of lysine supplementation (.2% or .3%) in 14% protein diets composed of a 13.6% protein barley and containing 3.1% soybean meal (44% protein) revealed improved nitrogen retention in growing pigs. Other studies by Soldvila and Meade (1964), utilizing .3% urea in place of soybean meal in barley diets balanced to provide 14% total protein, indicated that a combination of .3% L-lysine and .1% DL-methionine gave the best response in gain and feed efficiency of growing pigs.

Young et al. (1963) and Young and Thomas (1964) reported a series of trials with barley diets supplemented with soybean meal or combinations of soybean meal and safflower meal or cottonseed meal. The addition of lysine to diets supplemented with soybean meal alone had no effect on pig growth or feed efficiency, whereas lysine did improve performance of pigs fed diets containing safflower or cottonseed meal in combination with soybean meal.

Pick and Meade (1971) conducted amino acid supplementation trials with Opaque-2 corn for growing rats. All experimental diets contained 89.5% corn and all essential amino acids that were deficient were added except for the one being studied. Glutamic acid was added to all basal diets to obtain an essential-to-nonessential amino acid nitrogen ratio of approximately 1:1 and to assure that diets would not be limiting in nitrogen for synthesis of nonessential amino acids. Rats fed diets containing .54% lysine grew more rapidly, were more efficient, and retained more nitrogen than rats fed .35% lysine diets. Rats fed diets containing .73% lysine had significantly ($P < .01$) greater gain per feed ratios and retained more nitrogen than those rats fed the .54% lysine diets. Increasing the lysine content to .92% did not improve performance further.

Cromwell, Pickett and Beeson (1967) reported improved gains and feed efficiency of pigs fed Opaque-2 corn as compared to normal corn. The lysine and tryptophan content were 104 and 67% greater, respectively, in Opaque-2 corn than the normal corn. When the normal corn diets

were supplemented with lysine to the level found in Opaque-2 corn, responses were still not improved. However, when lysine and tryptophan were added to the normal corn in combination, greater responses and improved performance were observed. Opaque-2 corn diets still held a slight advantage over the supplemented normal corn.

Hagberg and Karlsson (1969) investigated the possible correlation between total protein content obtained from a Kjeldahl analysis and the dye-binding capacity (DBC) of protein. The DBC measures the basic amino acid content, among them lysine, and gives an estimate of the protein quality. They found a correlation of $r=.93$ between DBC and grams of lysine per 100 g of protein. This method was then used to select barley varieties from the world barley collection which were high in the amino acid lysine.

Although not being an actual supplementation of lysine, it is possible to select barley varieties which contain higher than normal lysine levels. Hiproly (CI 3947) is just one example of a high lysine barley variety and because of its superior feeding value, it has aroused much interest from nutritionists and plant breeders. Hiproly was selected from the world barley collection because of its high DBC. It has now proved its high nutritional value in several growth and metabolism trials.

Munck et al. (1970) conducted feeding trials with mice and rats to compare Hiproly to a reference mixture of four naked barley

varieties. Both diets were balanced to supply 9.4% total protein. In mice, the protein efficiency ratio was higher with the Hiproly diet; and in rats, true digestibility, biological value, and net protein utilization were also superior as compared to the reference diet. True digestibility for lysine in rats, as measured by the fecal analysis method, was significantly increased on the Hiproly diet.

Newman et al. (1974) reported Hiproly to have a superior nutritional value for rats as compared to a sister line isogene (Hiproly Normal) and naked Compana barley. Isonitrogenous-isocaloric diets were formulated by using corn starch and corn oil as varients to equalize all diets to supply 10% protein and 5% ether extract. Rats fed Hiproly barley gained faster, were more efficient, and produced higher PER values than rats fed the Hiproly Normal or naked Compana barleys. Total feed consumption over the 21 day trial was also higher with the Hiproly rats. Hiproly Normal produced a higher PER than the naked Compana. The PER values reported by Newman (1974) in this study agree with those reported by Howe et al. (1965) when normal barley was supplemented with .2% L-lysine or a mixture of .2% L-lysine, .2% DL-methionine, and .5% DL-threonine, respectively. This indicates that the protein of Hiproly barley is similar in nutritional value to normal barley supplemented with free amino acids.

EXPERIMENTAL PROCEDURE

Barleys

Twenty-three barley varieties were selected for this experiment (table 1). Nineteen of these barleys were selected for seed increase on the basis of their high-lysine potential as predicted by their dye-binding-capacity (DBC) (Udy, 1956), and lysine content of their protein by using a Beckman 120C automatic amino acid analyzer (Spackman et al., 1958) (table 2). The remaining four barleys that were included in the feeding trials were waxy Compana and the commercial varieties normal Compana, Unitan and Shabet.

Proximate analyses of the first 23 barleys were obtained according to A.O.A.C. (1970). Calcium percentages were determined by a modified Kramer and Tisdall procedure of Clark and Collip (1925) and phosphorus by the method of Fiske and Subbarow (1925) (table 3). Amino acid composition were obtained as mentioned above (table 4). Table 5 shows the lysine content of each barley calculated as a percentage of the total protein recovered.

Trial I

One hundred-twenty weanling female Holtzman rats, weighing approximately 70 g, were used in each of two duplicate feeding periods for growth studies with each of the 23 barleys. Two feeding periods were necessary due to limited facilities. Twenty-three isonitrogenous, isocaloric, 10.0% protein diets were formulated by diluting each barley with corn starch. A 10% protein control diet formulated from

milk casein (89% protein) and corn starch was also included. All diets contained equal constants of corn oil, vitamin mixture, mineral mixture and calcium carbonate (table 6). Diets were mechanically mixed and screen sieved to ensure uniform mixing of ingredients. Between feedings all diets were stored in a refrigerated environment (-3°C). Chemical analysis of all diets are shown in table 7. Five female weanling rats were assigned to each diet according to initial body weight in each duplicate trial. The total rat weight per diet was adjusted to vary no more than ± 1 g initially. Rats were individually caged and stratified according to cage location with feed and water provided ad libitum. All rats were maintained in an environmentally controlled room with automatic lighting to provide 12 hr of continuous light and 12 hr of continuous darkness. Weight gain and feed consumption were measured weekly for each individual rat and compiled for the entire 21 day trial period. Total gain, average daily gain, total feed consumption, feed efficiency and protein efficiency ratios (PER) were calculated for each individual rat. The PER values were adjusted with a factor obtained from rats fed the casein control diet as suggested by Chapman et al. (1959).

Data from the duplicate trials were combined and analyzed by least squares analysis of variance (Harvey, 1960) as a preliminary analysis indicated no interaction between trial and barley.

Trial II

A nitrogen balance trial was conducted with 16 female Holtzman weanling rats weighing approximately 75 g to determine metabolic nitrogen (MN) and endogenous nitrogen (EN) excretion. Metabolic nitrogen represents nitrogen losses through the feces while EN measures nitrogen losses in the urine. A nitrogen-free diet was formulated by using corn starch, purified wood cellulose, corn oil, vitamins, minerals, calcium carbonate, and antibiotics (table 8). A Kjeldahl nitrogen analysis was performed to assure the nitrogen-free status. All rats were caged individually and fed the nitrogen-free diet ad libitum for a 4 day adjustment period. Water was also available at all times. After the adaption period, each rat was weighed and transferred to a separate metabolism cage for a 5 day collection period. During collection, each rat received 15 g of feed once daily.

Procedures for urine and fecal collection were patterned after those of Eggum (1973b). Methods were slightly modified due to differences in cage construction. Urine was automatically collected for all 5 days in a flask containing 50 ml of 5% sulfuric acid. All urine passed through a glass wool filter before entering the flask. The glass wool was rinsed several times daily with distilled water to ensure that all nitrogen was collected. At the conclusion of the collection period all parts which might have come into contact with urine were rinsed thoroughly with distilled water and a soft brush

and collected in the urine flask. Feces fell directly to a screen beneath each cage and were collected 4 times daily and placed into separate beakers containing 100 ml of 5% sulfuric acid.

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 urine and fecal sample and an average nitrogen excretion was determined from the duplicate results. Procedures for determining endogenous nitrogen (EN) involved diluting the individual urine collections to 200 ml with water and taking a 15 ml aliquot for a macro-Kjeldahl. Feces were prepared for metabolic nitrogen (MN) analysis by first macerating the collection and then adding 4 volumes of 25 ml concentrated sulfuric acid. After each addition the mixture was stirred thoroughly with a spatula and left to cool to room temperature. After the fourth addition of acid, a homogenous fecal solution remained. This was then diluted to 500 ml with water and a 100 ml aliquot was taken for macro-Kjeldahl analysis. Endogenous nitrogen and metabolic nitrogen were calculated for each rat to express grams of nitrogen excreted per rat per 5 days. Average values for EN and MN were then determined from the total of all 16 rats. These values were then used as constants in trials III and IV for the calculations of biological value (BV), true digestibility (TD), and net protein utilization (NPU).

Trial III

A nitrogen balance trial was conducted with five diets selected from trial I (table 6). Criteria for selection was based upon protein efficiency ratios (PER) obtained from barleys in trial I. The five barleys showing the highest average PER values were selected (diets: 1, 5, 10, 13 and 18). Procedures for formulating diets were identical to those of trial I where isonitrogenous, isocaloric diets of 10.0% protein were used. Procedures for chemical analyses and calcium and phosphorus determinations were the same as in trial I (table 9).

Six weanling female Holtzman rats weighing approximately 78 g were placed on each diet. Rats were assigned to diets according to initial body weight and total rat weight per diet was adjusted to within \pm 1 g. Rats were individually caged and stratified according to cage location for a 4 day adjustment period. Individual supplies of feed and water were available ad libitum throughout the adjustment period. Body weight was recorded at the conclusion of the 4 day adaption period and rats were transferred to stratified metabolism cages for a 5 day collection period. Feed was limited to 15 g once a day during the collection period.

Procedures for urine and fecal collection were those of Eggum (1973b) as described in trial II. Duplicate Kjeldahl nitrogen analyses were performed on each rat's urinary and fecal excretion as explained

in trial II. Determinations of urinary nitrogen (UN) and fecal nitrogen (FN) excretions were analogous to calculations of EN and MN of trial II, respectively. Nitrogen intake (N-intake) during the collection period was calculated from total feed consumption, assuming a factor of 16.0% nitrogen for the diets' protein content. Values for EN and MN obtained in trial II as constants, were then incorporated with N-intake, FN, and UN into the equations of Thomas and Mitchell (Eggum, 1973b) for the determination of biological value (BV), true digestibility (TD), and net protein utilization (NPU). Diet averages for each calculation were then obtained by the sum of all rats on each ration.

Trial IV

Thirty female weanling rats weighing approximately 75 g were selected for a third nitrogen balance trial. Five barleys were selected from trial I on the basis of protein efficiency ratios (PER). The barleys with the five lowest average PER values in trial I were selected (diets: 16, 17, 20, 21 and 23). Diets were again formulated to supply 10.0% protein. Proximate analysis, calcium and phosphorus determinations for trial IV diets are listed in table 9. The number of rats per diet, the methods for adaption and collection, analyses of urine and feces, and the parameters measured were the same as in trial III. The only factor differing was the amount of feed allowed per day. It was learned in trial III that some feed wastage occurred when 15 g per day was fed. To prevent this in trial IV daily feed

allowance was reduced to 10 g once daily.

Data from trial III and trial IV were analyzed by least squares analysis of variance (Harvey, 1960) and a regression analysis was included to take out the effect of different nitrogen intakes.

TABLE 1. IDENTIFICATION, DESCRIPTION AND ORIGIN OF BARLEYS

Diet No.	CI No.	Variety	Hull ^a	Color ^b	Origin
1	906	Hanna	C	W/A	Austria
2	3383	Bargiers	C	W/A	Algeria
3	6400	--	C	W/A	Poland
4	6407	Dornberger Heil Franke	C	W/A	Poland
5	7131	Bolder	C	W/A	Sweden
6	7622	Lenta	C	W/A	Denmark
7	8192	--	C	W/A	Turkey
8	10236	--	C	W/A	Germany
9	10328	Italian	C	W/A	Jordan
10	10375	--	C	W/A	Germany
11	11201	Wiebull's 5573	C	W/A	Sweden
12	11308	Bonus	C	W/A	Sweden
13	11310	Brage	C	W/A	Sweden
14	11315	Primus II	C	W/A	Sweden
15	12099	--	C	W/A	Ethiopia
16	12147	Imperial	C	W/A	USA ND
17	12171	--	C	W/A	--
18	3947	Hiproly	N	W/A	UAR
19	12103	--	C	W/A	Ethiopia
20	5438	Compana	C	W/A	USA MT
21	294318 ^c	Waxy Compana	C	W/A	USA MT
22	10421	Unitan	C	W/A	USA MT
23	13827	Shabet	C	W/A	USA MT

^a C = covered

N = naked

^b W/A = white/amber

^c Montana

TABLE 2. KJELDAHL PROTEIN, LYSINE PERCENTAGE AND PERCENTAGE TRANSMITTANCE OF THE 19 SELECTED BARLEYS

Diet No.	CI No.	Protein (%)	Lysine (%)	Transmittance (%)	
1	906	15.0 ^a	.41 ^b	3.83 ^c	47.8
2	3383	15.1	.46	3.67	50.2
3	6400	15.0	.50	3.79	47.2
4	6407	14.5	.53	3.87	51.5
5	7131	13.3	.44	3.90	50.0
6	7622	13.9	.50	4.01	48.5
7	8192	15.8	.57	3.83	48.0
8	10236	13.4	.40	3.82	50.5
9	10328	14.2	.47	3.78	51.5
10	10375	13.6	.37	3.81	57.2
11	11201	11.9	.44	4.03	50.5
12	11308	13.2	.45	3.76	49.0
13	11310	14.9	.55	3.77	48.5
14	11315	14.5	.54	4.04	49.5
15	12099	15.1	.52	3.79	49.0
16	12147	15.4	.50	3.70	46.2
17	12171	15.9	.49	3.82	48.5
18	3947	17.8	.78	4.66	51.2
19	12103	14.5	.52	3.81	50.5

^a N x 6.25.

^b Lysine as a percentage of the whole grain.

^c Lysine as a percentage of the protein recovered in the amino acid analysis.

TABLE 3. PROXIMATE COMPOSITION AND CALCIUM AND PHOSPHORUS COMPOSITION OF SELECTED BARLEY VARIETIES

Diet No.	CI No.	Protein	Ether extract	Crude fiber	NFE	Moisture	Ash	Ca	P
1	906	16.1	1.7	4.6	66.0	5.9	5.7	.01	.40
2	3383	16.4	1.7	4.7	63.3	5.9	8.0	.01	.36
3	6400	16.2	2.2	5.3	61.6	5.8	8.9	.01	.36
4	6407	15.4	1.1	4.3	65.3	6.5	7.4	.01	.32
5	7131	13.8	1.3	5.2	67.6	6.2	5.9	.02	.33
6	7622	15.1	1.2	4.8	68.1	5.9	4.9	.02	.36
7	8192	15.2	1.9	5.0	69.2	5.6	3.1	.01	.43
8	10236	13.8	1.4	4.2	71.5	6.6	2.5	.01	.38
9	10328	16.6	1.7	5.3	67.2	6.1	3.1	.03	.47
10	10375	15.1	1.3	3.7	71.4	5.8	2.7	.01	.42
11	11201	13.4	1.3	4.5	72.3	5.9	2.6	.02	.35
12	11308	14.4	1.5	4.5	71.0	5.7	2.9	.01	.38
13	11310	15.7	2.0	4.9	68.2	6.2	3.0	.04	.45
14	11315	15.2	1.9	4.2	70.0	6.3	2.4	.03	.41
15	12099	13.9	1.9	4.3	71.0	6.4	2.5	.03	.42
16	12147	15.8	2.1	4.0	69.6	5.6	2.9	.04	.46
17	12171	17.0	1.3	4.2	69.2	5.8	2.5	.03	.44
18	3947	19.7	1.9	2.1	67.9	6.1	2.3	.00	.53
19	12103	13.5	1.7	3.7	72.7	6.1	2.3	.02	.36
20	5438	15.4	1.4	3.8	71.2	5.6	2.6	.03	.41
21	294318 ^a	16.4	1.6	4.2	69.4	6.0	2.4	.02	.43
22	10421	13.2	1.7	4.2	71.7	7.0	2.2	.04	.36
23	13827	14.7	1.9	3.7	70.1	7.2	2.4	.01	.37

^a Montana

TABLE 4. AMINO ACID ANALYSIS OF SEED INCREASES

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
CI No.	906	3383	6400	6407	7131	7622	8192	10236	10328	10375	11201	11308	11310	11315	12099	12147	12171	3947	12103	5438	284318 ^b	10421	13827
Amino acid^a																							
Alanine	.51	.46	.47	.44	.42	.46	.46	.44	.50	.45	.42	.44	.49	.45	.42	.50	.51	.64	.41	.46	.49	.39	.46
Arginine	.70	.65	.66	.57	.60	.65	.61	.59	.76	.64	.58	.64	.69	.64	.59	.70	.77	.85	.59	.64	.67	.57	.63
Aspartic acid	.83	.73	.76	.71	.70	.74	.76	.73	.79	.78	.67	.72	.83	.75	.71	.83	.83	1.16	.70	.73	.78	.64	.75
Glutamic acid	3.83	3.42	3.46	3.19	3.06	3.19	3.16	3.05	3.68	3.65	2.80	3.09	3.59	3.21	2.81	3.46	4.08	4.27	2.87	3.86	4.13	2.88	3.50
Glycine	.49	.43	.45	.41	.42	.43	.42	.40	.45	.44	.39	.42	.49	.41	.39	.45	.49	.59	.38	.47	.44	.37	.44
Histidine	.32	.28	.29	.24	.26	.28	.28	.26	.32	.30	.25	.26	.31	.27	.26	.30	.33	.35	.24	.31	.31	.24	.29
Isoleucine	.51	.47	.48	.45	.42	.46	.45	.42	.53	.46	.41	.44	.50	.46	.41	.50	.54	.63	.40	.50	.53	.40	.47
Leucine	.99	.91	.94	.87	.85	.89	.85	.81	.97	.90	.78	.87	.96	.87	.78	.92	1.03	1.15	.80	.92	.98	.75	.89
Lysine	.50	.44	.46	.42	.45	.44	.43	.42	.48	.46	.40	.43	.52	.43	.41	.46	.49	.72	.40	.41	.45	.36	.44
Methionine	.23	.22	.22	.22	.19	.21	.22	.21	.24	.21	.20	.20	.23	.22	.20	.22	.24	.30	.19	.22	.23	.19	.22
Phenylalanine	.83	.75	.76	.68	.68	.72	.68	.67	.79	.79	.62	.72	.83	.73	.63	.75	.85	1.05	.64	.80	.84	.59	.74
Proline	1.78	1.60	1.66	1.42	1.43	1.51	1.48	1.37	1.68	1.59	1.30	1.48	1.53	1.48	1.26	1.56	1.86	2.12	1.35	1.78	1.92	1.31	1.61
Serine	.68	.58	.59	.56	.55	.58	.59	.56	.65	.63	.52	.54	.64	.56	.53	.63	.72	.78	.51	.63	.65	.52	.62
Threonine	.51	.45	.45	.45	.41	.44	.45	.43	.48	.46	.40	.42	.50	.42	.42	.47	.49	.61	.40	.47	.48	.38	.45
Tyrosine	.41	.40	.38	.36	.34	.39	.40	.37	.46	.37	.36	.41	.39	.41	.38	.38	.43	.48	.38	.41	.43	.37	.38
Valine	.66	.61	.63	.57	.56	.59	.59	.55	.68	.60	.54	.57	.64	.60	.52	.63	.70	.83	.53	.62	.64	.53	.61

^a Amino acid as a percentage of the whole grain.

^b Montana.

TABLE 5. LYSINE PERCENTAGE IN THE PROTEIN OF BARLEYS

Diet No.	CI No.	Lysine, %
1	906	3.63
2	3383	3.55
3	6400	3.63
4	6407	3.62
5	7131	3.97
6	7622	3.67
7	8192	3.63
8	10236	3.72
9	10328	3.57
10	10375	3.61
11	11201	3.76
12	11308	3.69
13	11310	3.96
14	11315	3.61
15	12099	3.82
16	12147	3.61
17	12171	3.41
18	3947	4.36
19	12103	3.71
20	5438	3.10
21	294318 ^a	3.22
22	10421	3.43
23	13827	3.52

^a Montana

TABLE 6. PERCENTAGE COMPOSITION OF BARLEY AND CASEIN DIETS

Diet No.	Barley	Corn starch	Corn oil	Mineral mixture ^a	Vitamin mixture ^a	Calcium carbonate ^b	Casein ^c
1	62.11	29.19	3.50	2.00	2.20	1.00	--
2	60.97	30.33	3.50	2.00	2.20	1.00	--
3	61.73	29.57	3.50	2.00	2.20	1.00	--
4	64.94	26.36	3.50	2.00	2.20	1.00	--
5	72.46	18.84	3.50	2.00	2.20	1.00	--
6	66.23	25.07	3.50	2.00	2.20	1.00	--
7	65.79	25.51	3.50	2.00	2.20	1.00	--
8	72.46	18.84	3.50	2.00	2.20	1.00	--
9	60.24	31.06	3.50	2.00	2.20	1.00	--
10	66.23	25.07	3.50	2.00	2.20	1.00	--
11	74.63	16.67	3.50	2.00	2.20	1.00	--
12	69.44	21.86	3.50	2.00	2.20	1.00	--
13	63.69	27.61	3.50	2.00	2.20	1.00	--
14	65.79	25.51	3.50	2.00	2.20	1.00	--
15	71.94	19.36	3.50	2.00	2.20	1.00	--
16	63.29	28.01	3.50	2.00	2.20	1.00	--
17	58.82	32.48	3.50	2.00	2.20	1.00	--
18	50.76	40.54	3.50	2.00	2.20	1.00	--
19	74.07	17.23	3.50	2.00	2.20	1.00	--
20	64.94	26.36	3.50	2.00	2.20	1.00	--
21	60.98	30.32	3.50	2.00	2.20	1.00	--
22	75.76	15.54	3.50	2.00	2.20	1.00	--
23	68.03	23.27	3.50	2.00	2.20	1.00	--
24	--	80.06	3.50	2.00	2.20	1.00	11.24

^a ICN Nutritional Biochemicals; Bernhart-Tomavelli salt mixture, modified 1135-B and vitamin diet fortification mixture.

^b Reagent grade.

^c ICN Nutritional Biochemicals; casein, purified, high nitrogen.

TABLE 7. PROXIMATE ANALYSES AND CALCIUM AND PHOSPHORUS CONTENT OF DIETS FED IN GROWTH TRIAL

Diet No.	Protein	Ether extract	Crude fiber	NFE	Moisture	Ash	Ca	P
1	10.2	5.8	2.6	69.5	5.9	6.1	.66	.67
2	10.2	5.2	2.5	67.1	5.9	9.1	.62	.58
3	10.2	4.9	2.5	66.4	6.0	10.0	.66	.59
4	9.8	4.8	2.6	66.2	6.0	10.6	.66	.61
5	9.9	4.8	3.6	66.8	6.0	8.9	.64	.61
6	10.1	4.6	3.0	69.2	5.7	7.4	.66	.65
7	10.1	5.2	3.0	71.9	5.3	4.5	.66	.72
8	10.4	5.3	2.9	71.1	5.9	4.4	.64	.72
9	10.4	5.1	2.8	71.9	5.5	4.3	.65	.73
10	10.1	4.7	2.3	73.7	5.2	4.0	.63	.66
11	10.3	4.9	2.8	72.4	5.3	4.3	.65	.70
12	10.1	4.8	2.6	73.0	5.2	4.3	.67	.72
13	10.2	4.9	2.9	72.2	5.4	4.4	.64	.74
14	10.3	4.9	2.8	72.5	5.5	4.0	.63	.73
15	10.1	5.0	2.8	72.4	5.5	4.2	.66	.75
16	10.3	4.8	2.2	73.6	5.0	4.1	.67	.58
17	10.0	4.6	2.6	71.2	4.9	4.1	.68	.74
18	10.3	4.6	1.1	75.3	5.0	3.7	.66	.75
19	10.2	5.2	2.7	72.6	5.0	4.3	.66	.74
20	10.1	2.7	2.7	75.1	5.2	4.2	.67	.77
21	10.0	4.2	2.5	73.9	5.3	4.1	.69	.68
22	10.3	2.7	2.7	74.8	5.3	4.2	.65	.70
23	10.2	3.0	3.6	73.6	5.5	4.1	.66	.69
24	10.0	2.9	.1	79.2	5.1	2.7	.62	.52

TABLE 8. PERCENTAGE COMPOSITION OF THE NITROGEN-FREE DIET

<u>Ingredients, %</u>	
Corn starch	84.55
Corn oil	5.00
Alphacel ^a	5.00
Vitamin mixture ^a	2.20
Mineral mixture ^a	2.00
Calcium carbonate ^b	1.00
Antibiotic ^c	0.25
	<hr/> 100.00

^a ICN Nutritional Biochemicals, nonnutritive cellulose, vitamin diet fortification mixture and Bernhart-Tomarelli salt mixture, modified 1135-B, respectively.

^b Reagent grade.

^c Aureomycin, penicillin and sulfamethazine, 220.5, 110.2 and 220.5 g per kilogram of antibiotic mixture, respectively.

TABLE 9. PROXIMATE ANALYSES AND CALCIUM AND PHOSPHORUS CONTENT OF DIETS FED IN NITROGEN BALANCE TRIALS

Diet No.	Protein	Ether extract	Crude fiber	NFE	Moisture	Ash	Ca	P
1	9.6	5.2	2.5	72.9	5.5	4.3	.78	.72
5	9.6	4.8	2.8	72.8	5.6	4.4	.81	.71
10	10.3	4.7	2.4	73.3	5.2	4.1	.79	.73
13	10.1	4.8	3.1	71.8	6.1	4.1	.77	.71
18	9.9	4.7	1.3	75.7	4.9	3.5	.77	.71
16	10.0	4.8	2.7	74.5	3.7	4.3	.79	.67
17	10.0	4.5	2.9	75.0	3.5	4.1	.74	.67
20	10.5	3.3	3.0	76.1	2.9	4.2	.77	.67
21	10.3	3.4	2.7	75.5	3.9	4.2	.77	.69
23	10.1	4.3	2.8	75.6	3.1	4.1	.76	.66

RESULTS AND DISCUSSION

Trial I. Performance of rats in the growth trial are shown in table 10. Rats fed the casein control diet tended to gain faster and more efficiently and had a significantly higher ($P < .05$) PER than rats fed any of the barley diets. Five of the barleys produced rat gains that were not significantly different from those on the casein control diet (59.7 g). These were Hiproly (CI 3947) (58.4 g), Hanna (CI 906) (58.2 g), CI 10375 (54.9 g), CI 12099 (53.8 g) and Bolder (CI 7131) (53.1 g). These diets also produced the highest PER and lowest feed/gain ratios of the barleys in the test.

The Hiproly diet was the best of the barleys in terms of gain, feed efficiency and PER but all differences were not statistically significant as indicated in table 10. The casein diet was more efficiently utilized ($P < .05$) when compared to 22 of the barleys but was not significantly different in this regard from the Hiproly diet. There were numerical differences in feed consumption but the large variation in this parameter between rats within diet made these differences nonsignificant.

The commercial variety, Compana (CI 5438), produced the slowest rate of gain (38.3 g) which was significantly different ($P < .05$) from gains of 53.8 g or greater. Rats fed Compana also tended to be the least efficient (feed/gain = 6.31) and had the lowest PER (1.59). Waxy Compana (CI 294318), an isogene of Compana, was similar in all parameters to the latter barley. The other commercial varieties,

Unitan (CI 10421) and Shabet (CI 13827), appeared to be intermediate in comparison to the other barleys. Barleys producing low gains comparable to Compana and waxy Compana were CI 12171 (40.0 g), CI 8192 (41.0 g) and Bonus (CI 11308) (44.4 g). With two exceptions, these three barleys and the Compana isogenes also produced the lowest PER (1.59 to 1.76) and the highest feed gain ratio (6.31 to 5.78). Rats fed the variety Imperial (CI 12147), gained somewhat more (47.2 g) than the five low PER barleys, but produced a low PER (1.67). Shabet produced a gain of 48.0 g with a PER and feed/gain ratio of 1.71 and 5.85, respectively.

Correlation coefficients for the parameters measured in trial I are shown in table 11. Gain was highly correlated ($P < .01$) with PER, feed/gain ratio and feed consumption ($r = .84, -.81$ and $.88$, respectively). Feed consumption was less correlated to PER and the feed/gain ratio ($r = .54$ and $.51$, respectively) than to gain, however the r values were significant ($P < .01$). Feed efficiency was highly correlated to PER ($r = -.94$).

The percentage of lysine in the barley proteins was highly related ($P < .01$) to gain, the feed/gain ratio and PER ($r = .73, -.82$ and $.84$, respectively) but was less correlated with feed consumption ($r = .46$) (table 12). The PER value appeared to be the best indicator of the percentage of lysine in the barley protein since the latter was responsible for 71% of the variation in the PER.

Trial II. The results of the determinations for metabolic fecal nitrogen (MN) and endogenous urine nitrogen (EN) are shown in table 13. The average MN and EN were $0.023 \pm .001$ and $0.020 \pm .004$ g per rat per five days. There was considerably more variation in EN than in MN in this trial. All rats lost body weight while on the nitrogen-free diets.

Trials III and IV. The biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU) of the five high and five low PER barleys are shown in table 14. Hiproly had the highest BV (72.6%) but was only significantly greater ($P < .05$) than two other varieties in this respect, Imperial (59.1%) and waxy Compana (57.0%). The barleys with the high PER values tended to be higher in BV but lowest in TPD. The TPD of Hiproly barley protein was lowest of the barleys compared and was significantly less ($P < .05$) than the TPD of Compana. The latter barley had the highest TPD of the ten barleys compared. There were no significant differences in NPU between the ten varieties.

Table 15 shows the means of the high and low PER barleys. The BV, TPD and NPU of the two groups were significantly different in the combined analysis. The high PER barleys had the greater ($P < .01$) BV when compared to the five low PER barleys as a group, however they were lowest in TPD ($P < .01$). Even though the TPD was lower for the high PER group, the NPU favored the latter group ($P < .10$).

Protein efficiency ratios appeared to be the best indicators of barley protein quality (table 16). Protein efficiency ratios obtained in trial I were highly and positively correlated ($P < .01$) with BV ($r = .81$) but were negatively correlated ($P < .01$) with TPD ($r = -.73$). The negative relationship of PER and BV with TPD is difficult to explain on the basis for this data. This relationship may have been due to differences in total amino acid balance in the barley protein, the presence of non-protein nitrogen, the availability of the amino acids or interactions of amino acids and/or protein with other nutrients such as starch or cellulose. Further study is indicated for a complete understanding of the characteristics of barleys compared in this study.

TABLE 10. GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS, ISONITROGENOUS BARLEY AND CASEIN FOR 21 DAYS, TRIAL I

Diet No.	CI No.	Variety	No. rats	Gain	Feed consumed	Feed/gain ratio	Adjusted P.E.R. ^g
1	906	Hanna	10	58.2 ^{ab}	288.5 ^a	5.06 ^{cd}	1.96 ^{bc}
2	3383	Bargiers	8	46.4 ^{abcde}	258.5 ^a	5.65 ^{abc}	1.77 ^{cdef}
3	6400	---	9	45.8 ^{bcde}	250.1 ^a	5.67 ^{abc}	1.78 ^{cdef}
4	6407	Dornberger Heil Franke	9	47.2 ^{abcd}	256.1 ^a	2.65 ^{abc}	1.85 ^{bcdef}
5	7131	Bolder	9	53.1 ^{abcde}	278.6 ^a	5.28 ^{bcd}	1.92 ^{bcd}
6	7622	Lenta	10	48.6 ^{abcde}	253.0 ^a	5.37 ^{bcd}	1.87 ^{bcde}
7	8192	---	10	41.0 ^{de}	235.1 ^a	5.86 ^{abc}	1.72 ^{cdef}
8	10236	---	10	49.2 ^{abcde}	253.7 ^a	5.55 ^{abc}	1.82 ^{cdef}
9	10328	Italian	10	47.4 ^{abcde}	256.7 ^a	5.55 ^{abc}	1.78 ^{cdef}
10	10375	---	9	54.9 ^{abc}	275.7 ^a	5.12 ^{cd}	1.94 ^{bcd}
11	11201	Wiebull's 5573	9	50.2 ^{abcde}	265.4 ^a	5.46 ^{bcd}	1.83 ^{cdef}
12	11308	Bonus	10	44.4 ^{cde}	248.5 ^a	5.78 ^{abc}	1.76 ^{cdef}
13	11310	Brage	10	51.4 ^{abcde}	265.7 ^a	5.41 ^{bcd}	1.88 ^{bcde}
14	11315	Primus II	10	45.3 ^{bcde}	244.4 ^a	5.52 ^{abc}	1.80 ^{cdef}
15	12099	---	9	53.8 ^{abcd}	275.6 ^a	5.27 ^{bcd}	1.91 ^{bcd}
16	12147	Imperial	10	47.2 ^{abcde}	263.6 ^a	5.80 ^{abc}	1.67 ^{def}
17	12171	---	10	40.0 ^e	233.6 ^a	5.99 ^{ab}	1.68 ^{def}
18	3947	Hiproly	10	59.4 ^a	274.8 ^a	4.67 ^{de}	2.09 ^b
19	12103	---	10	52.1 ^{abcde}	280.5 ^a	5.33 ^{bcd}	1.85 ^{bcdef}
20	5438	Compana	10	39.3 ^e	243.4 ^a	6.31 ^a	1.59 ^f
21	294318 ^h	Waxy Compana	10	40.9 ^{de}	254.4 ^a	6.07 ^{ab}	1.64 ^{ef}
22	10421	Unitan	10	49.4 ^{abcde}	276.0 ^a	5.62 ^{abc}	1.77 ^{cdef}
23	13827	Shabet	9	48.0 ^{abcde}	272.7 ^a	5.85 ^{abc}	1.71 ^{cdef}
24	---	Casein	10	59.7 ^a	246.2 ^a	4.17 ^e	2.41 ^a

abcdef Means in the same column with different superscript letters, differ significantly, P_{0.05}.

^g Protein efficiency ratio.

^h Montana

TABLE 11. CORRELATION COEFFICIENTS FOR PERFORMANCE DATA IN THE GROWTH TRIAL, TRIAL I

x	y	r	r ²
Gain	PER	.84 ^a	.71
	Feed/gain	-.81 ^a	.66
	Feed consumed	.88 ^a	.77
Feed consumed	PER	.54 ^a	.29
	Feed/gain	-.51 ^a	.26
Feed/gain	PER	-.94 ^a	.88

a P<.01.

TABLE 12. CORRELATION OF THE PERCENTAGE LYSINE IN BARLEY PROTEIN OF 23 VARIETIES WITH RAT GAIN, FEED CONSUMPTION, FEED/GAIN RATIO AND PER VALUES OBTAINED IN TRIAL I.

x	y	r	r ²
% Lysine	Gain	.73 ^a	.53
	Feed consumption	.46 ^b	.21
	Feed/gain ratio	-.82 ^a	.67
	PER	.84 ^a	.71

^a P<.01.

^b P<.05.

TABLE 13. METABOLIC NITROGEN AND ENDOGENOUS NITROGEN EXCRETION BY RATS FED A NITROGEN-FREE DIET, TRIAL II

Rat No.	Metabolic fecal nitrogen (grams/rat/5 days)	Endogenous urine nitrogen (grams/rat/5 days)
1	.017	.030
2	.025	.038
3	.024	.055
4	.029	.021
5	.020	.015
6	.024	.006
7	.022	.004
8	.015	.010
9	.023	.004
10	.022	.034
11	.017	.029
12	.034	.032
13	.020	.007
14	.030	.004
15	.028	.024
16	.017	.009
Average	.023±.001 ^a	.020±.004 ^a

^a Standard error of the mean (\bar{s}_x).

TABLE 14. LEAST SQUARES MEANS OF BIOLOGICAL VALUE, TRUE DIGESTIBILITY AND NET PROTEIN UTILIZATION OF RATS FED HIGH AND LOW PER BARLEYS, TRIALS III AND IV

Diet No.	CI. No.	Variety	Classification	No. Obs.	Biological value	True protein digestibility	Net protein utilization
1	906	Hanna	High PER ^b	6	66.0 ^{de}	74.5 ^e	49.2 ^d
5	7131	Bolder	High PER	6	68.5 ^{de}	74.9 ^e	51.4 ^d
10	10375	---	High PER	6	67.9 ^{de}	75.4 ^e	51.2 ^d
13	11310	Brage	High PER	6	67.1 ^{de}	80.0 ^{de}	53.6 ^d
18	3947	Hiproly	High PER	6	72.6 ^d	74.1 ^e	53.8 ^d
16	12147	Imperial	Low PER ^c	6	59.1 ^e	78.2 ^{de}	46.2 ^d
17	12171	---	Low PER	6	63.8 ^{de}	74.9 ^e	47.5 ^d
20	5438	Compana	Low PER	6	65.6 ^{de}	82.0 ^d	53.9 ^d
21	294318 ^a	Waxy Compana	Low PER	6	57.0 ^e	81.2 ^{de}	46.2 ^d
23	13827	Shabet	Low PER	6	61.6 ^{de}	80.1 ^{de}	49.4 ^d

^a Montana

^b Barleys selected from trial I on basis of highest PER performance

^c Barleys selected from trial I on basis of lowest PER performance.

^{de} Means in the same column with different superscript letters differ significantly, P<.05.

TABLE 15. COMPOSITE LEAST SQUARES MEANS OF BIOLOGICAL VALUE, TRUE DIGESTIBILITY AND NET PROTEIN UTILIZATION OF RATS FED HIGH AND LOW PER BARLEYS

Barleys	Number observations	Biological value	True protein digestibility	Net protein utilization
High PER ^a	29	68.3 ^c	75.7 ^c	51.7 ^e
Low PER ^b	30	61.4 ^d	79.3 ^d	48.7 ^f

^a Five barleys selected from trial I on basis of highest PER performance.

^b Five barleys selected from trial I on basis of lowest PER performance.

^{cd} Means in same column with different superscript letters differ significantly $P < .01$.

^{ef} Means in same column with different superscript letters differ significantly $P < .10 > .05$.

TABLE 16. CORRELATION OF PER OBTAINED IN TRIAL I WITH BIOLOGICAL VALUE AND TRUE PROTEIN DIGESTIBILITY AND NET PROTEIN UTILIZATION OF TEN BARLEYS

x	y	r	r ²
PER	Biological value	.81 ^a	.66
	True protein digestibility	-.73 ^a	.53
	Net protein utilization	.48	.24

a P<.01.

SUMMARY

One hundred twenty female weanling rats were used in each of two duplicate growth trials to compare the nutritive value of 23 different barley varieties. Nineteen of the barleys were selected for potential high-lysine content of their protein. The remaining four barleys were commercial varieties and an isogene of one of the commercial varieties. The barleys were formulated into isonitrogenous, isocaloric 10% protein diets with a casein corn starch diet as control. Five of the barleys produced rat gains that were not significantly different from gains of rats fed the casein control diet. These diets also produced the highest PERs and lowest feed/gain ratios of the barleys compared. The Hiproly (CI 3947) diet was the best of the barleys in terms of gains, feed efficiency and PER. There were no significant differences in feed consumption due to diet. The commercial variety, Compana, (CI 5438), produced the slowest rate of gain and lowest PER and was least efficiently utilized. The percentage of lysine in the barley proteins was significantly correlated to gain, the feed/gain ratio and PER. Feed efficiency was shown to be highly correlated to gain and PER. The nitrogen balance study was conducted with 16 weanling rats fed a nitrogen-free diet to determine metabolic fecal nitrogen and endogenous urine nitrogen excretion. Average metabolic fecal nitrogen was $.023 \pm .001$ g N/rat/5 days and endogenous urine nitrogen was $.020 \pm .004$ g N/rat/5 days. Ten barley varieties were selected from trial I for the determination of biological value (BV), true

protein digestibility (TPD) and net protein utilization (NPU). Five barley varieties were grouped together according to their high PER performance and the other five on the basis of their low PER values. When all ten varieties were analyzed individually, the biological value of Hiproly (CI 3947) was significantly higher ($P < .05$) than Imperial (CI 12147) and waxy Compana (CI 294318). True digestibility of Compana was significantly higher ($P < .05$) than Hanna (CI 906), Bolder (CI 7131), CI 10375, Hiproly (CI 3947) and CI 12171. There was no significant difference in net protein utilization of the individual barleys. Composite means of high PER barleys versus low PER barleys showed significant differences ($P < .01$) for biological value and true protein digestibility. Net protein utilization of the high PER barleys was also significant but only at the 10% level of probability.

APPENDIX

APPENDIX TABLE 17. LEAST SQUARES ANALYSIS OF VARIANCE OF RAT GROWTH, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY DATA OF RATS FED ISONITROGENOUS BARLEY AND CASEIN DIETS FOR 21 DAYS

Source of variation	d.f.	Gain	Feed consumed	Feed/gain ratio	Adjusted PER
Total	231				
Total reduction	24	327.3	3120.6	1.929	1.334
MU-Y	1	294.0	23343.8	.001	25.552
Diet	23	328.1 ^a	2281.2	2.013 ^a	.277 ^a
Error	207	152.1	1914.0	.592	.058

^a P<.01

APPENDIX TABLE 18. LEAST SQUARES ANALYSIS OF VARIANCE OF BIOLOGICAL VALUE, TRUE DIGESTIBILITY AND NET PROTEIN UTILIZATION OF RATS FED HIGH AND LOW PER BARLEYS

Source of variation	d.f.	Biological value	True protein digestibility	Net protein utilization
Total	60			
Total reduction	10	272.394	51.813	176.022
MU-Y	1	1549.305	3.096	1275.226
Barley	9	130.515 ^a	57.226 ^a	53.888
Error	50	53.696	16.093	37.660

a P<.05

APPENDIX TABLE 19. COMPOSITE^a LEAST SQUARES ANALYSIS OF VARIANCE OF BIOLOGICAL VALUE, TRUE DIGESTIBILITY AND NET PROTEIN UTILIZATION OF RATS FED HIGH AND LOW PER BARLEYS

Source of variation	d.f.	Biological value	True protein digestibility	Net protein utilization
Total	59			
Total reduction	2	1143.35	97.01	717.97
MU-Y	1	1545.20	4.02	1281.92
PER	1	705.44 ^c	190.89 ^c	139.29 ^b
Error	57	54.77	19.80	38.72

^a All five high PER barleys were grouped together and all five low PER barleys were grouped together.

^b P<.01..

^c P<.10>.05.

APPENDIX TABLE 20. SUM OF SQUARES AND CROSSPRODUCTS OF PER (x) AND BIOLOGICAL VALUE (y), TRUE DIGESTIBILITY (y) AND NET PROTEIN UTILIZATION (y)

<u>x</u>		<u>y</u>		
PER		Biological value	True protein digestibility	Net protein utilization
u = 10	u =	10	10	10
$\bar{u} = 1.81$	$\bar{y} =$	64.9	77.5	50.2
$\Sigma x^2 = .26$	$\Sigma y^2 =$	195.30	85.90	81.56
	$\Sigma xy =$	5.73	-3.42	2.22
$s\bar{x} = .16$	$s\bar{y} =$	4.4	2.9	2.9

APPENDIX TABLE 21. SUM OF SQUARES AND CROSSPRODUCTS OF PERCENTAGE OF LYSINE (x) IN BARLEY PROTEIN AND GAIN (y), FEED CONSUMPTION (y) FEED PER GAIN RATIO (y) AND PER (y)

<u>x</u>		<u>y</u>			
Lysine		Gain	Feed consumed	Feed/gain ratio	PER
u = 23	u =	23	23	23	23
$\bar{x} = 3.64$	$\bar{y} =$	48.4	260.7	5.56	1.81
$\Sigma x^2 = 1.39$	$\Sigma y^2 =$	642.94	5165.02	2.84	.29
	$\Sigma xy =$	21.79	38.85	1.62	.53
$s\bar{x} = .25$	$x\bar{y} =$	5.3	15.0	.35	.11

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