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NUTRITIONAL STUDIES ON HIGH LYSINE BARLEY LINES FROM THE ALBERTA AGRICULTURE BREEDING PROGRAM - 1977 HARVEST

by

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ABSTRACT

Recent years have brought a greater awareness of the need for more plentiful as well as more nutritious foods. Discoveries of strains of maize, barley and other cereals having higher levels of essential amino acids have shown that nutritional quality of cereals can be improved through plant breeding. In the experiments reported here, the nutritional value of three cultivars from the Alberta Agriculture high lysine breeding program (Cultivars 1, 2 and 3), hiproly, and a normal commercial variety was studied. Amino acid analysis was done on each barley line to compare the ability of each to supply essential amino acids.

The amino acid composition (g/16 g N) indicated virtually no differences except for cultivar 3 which had a much lower level of total essential amino acids than the rest. Barley proteins were separated by solubility tests to study the proportions in which albumins and globulins, glutelins and hordeins occur and to relate the levels of these protein fractions to the quality of the total protein. Hiproly and cultivars 1 and 2 were found to contain approximately the same levels of albumin plus globulin while cultivar 3 and Galt had lower proportions of these. The latter also had the highest proportion of hordein, an

indication that its protein quality is low.

Chemical determination of available lysine by dye binding difference gave inconclusive results. However, rat growth assays indicated Hiproly and cultivar 2 to be significantly superior in nutritional quality to Galt and cultivars 1 and 3. Nitrogen balance tests showed no significant differences between any of the five barleys. When the barleys were diluted to a standard protein content (12%), the supplementation with soybean meal or lysine plus other essential amino acids made significant improvements in rat performance, but lysine alone had no beneficial effect. This is an indication that the protein of these high lysine barleys is deficient in essential amino acids other than lysine.

Apparent availability of lysine by faecal analysis gave results supporting the fact that high lysine barleys are superior in quality to the normal barley.

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INTRODUCTION

Cereals were once considered to be sources of only poor quality protein, deficient in essential amino acids (EAA), especially lysine (Paulis and Wall, 1974). The growing awareness of the role of protein in world nutrition in the 1960's, stimulated by intense research in the field of nutrition, led to the development of high lysine varieties of maize and other cereals (Munk, 1976). Work on other high lysine cereals started after the discovery of the high lysine maize cultivars opaque-2 and floury-2 in 1965 (Paulis and Wall, 1974). Studies on protein quality and quantity of rice started in 1968, wheat and barley in 1969 and Sorghum in 1972 (Munk, 1972). Rice and barley grains have better amino acid balance than maize and Sorghum (Eggum, 1977b). Lysine content (g/16 g N) of cereal protein has been shown to be negatively correlated with crude protein content (g/100 g DM) (Eggum, 1977a).

Since the discovery of assorted high lysine genes in maize, there has been much research to overcome the depression in yield associated with the high lysine character and also to develop high yielding

strains of other grains that will have improved levels of essential amino acids (Helm, 1972; Paulis and Wall, 1974).

Barley is a major component of the diet of pigs in Canada. When used as the sole grain in the diet, which is commonly the case in Canada, it provides most of the energy and contributes more than half of the total protein. As a protein source to support pig growth it is poor in quality. Like other cereals, barley is seriously deficient in lysine and to a lesser extent in other essential amino acids (EAA) (Fuller, Livingstone, Baird and Atkinson, 1979; Fuller, Mennie, and Crofts, 1979). The addition of suitable protein supplements to barley in the formulation of pig diets not only increases the dietary protein but also corrects imbalances of amino acids. Several sources of high lysine, high protein barley have been developed (Munk, 1969). Presently, there are two major breeding lines of high lysine barley. These two are Hiproly from Svalöf, Sweden (Munk and Wettstein, 1974) and 1508 from Riso, Denmark (Ingversen and Koie, 1973; Doll, Koie and Eggum, 1974).

Hiproly was discovered in the world barley collection in 1968 at the Swedish Seed Association

Research Laboratories, Svalöf, (Munk, Karlsson and Hagberg, 1971). It was selected on the basis of its high content of basic amino acids as measured by the dye-binding capacity (DBC) technique, relative to it protein content by Kjeldahl analysis (Munk and Wettstein, 1974). Mutant 1508 was discovered by Ingversen, Koie and Doll in 1970 in the Danish two-rowed variety Bomi treated with ethyleneimine at Riso (Eggum, 1977a). Both Hiproly and mutant 1508 have a high content of essential amino acids (g/16 g N) especially lysine, methionine and threonine (Munk and Wettstein, 1974). Mutant 1508 has 45% more lysine than the parent variety Bomi, while Hiproly has about 30% more lysine than normal barley (Eggum, 1977a).

High lysine barleys are low in prolamins (hordeins) (Doll, 1977) due to the reduction in their synthesis. It has been shown that in maize, sorghum and barley the genes controlling the synthesis of prolamins are inactivated in the high lysine genotypes (Doll, 1977). Mutant 1508 contains only one third of the normal prolamin content, while the lysine content (g/16 g N) is increased to 150% of the parent variety. The reduction in yield in high lysine genotypes is associated with the reduced prolamin synthesis (Doll,

1977). There is an indication that reduction in prolamin synthesis impairs the accumulation of carbohydrates in the endosperm (Doll, 1977). Increased protein content in high lysine genotypes is due to reduced starch content of the endosperm (Koie and Kreis, 1977). If improving lysine content (g/16 g N) leads to reduced starch synthesis and hence reduced grain yield, one should consider seriously whether protein quality should be improved at the expense of energy yield, bearing in mind that cereals are included in livestock diets mainly for energy. Reduced grain yield is demonstrated as reduced single seed weight but the total number of seeds/unit area is unaffected; so is the total protein yield/unit area.

Breeding cereals for improved protein quality and quantity requires rapid screening methods (Tallberg, 1979). Chemical screening methods are necessary to identify high lysine mutants in barley, but simple visual selection for opacity can be used for high lysine maize and sorghum (Tallberg, 1979). Dye-binding capacity (DBC) has been used extensively as a chemical method for screening high lysine barley (Munk, 1972; Doll et al., 1974; Munk, 1976; and Eggum, 1977a). Most normal barley varieties show a common negative correlation

between the protein level in the seed and the lysine content (g/16 g N) of the protein (Munk, Karlsson and Hagberg, 1971; Doll et al., 1974 and Eggum, 1977a). However, if lysine content is expressed on a dry matter (DM) basis the level of protein and the level of lysine are positively correlated for normal barley (Doll et al., 1974). Within high lysine varieties, the same relationship holds but at a higher level (Doll et al., 1974). Tallberg (1979) has demonstrated these relationships in normal barleys and high lysine varieties. She obtained two regression lines, each with a positive gradient, the higher regression line for high lysine samples and the lower line for normal samples.

Research for the improvement of the nutritional quality of cereals is justified because cereals are the most important staple food in the world but are deficient in essential amino acids (EAA). An improvement in amino acid balance will supply the EAA at higher levels in the protein without requiring an increase in protein content (g/100 g DM) in the grain. Cereals could then approach and possibly become a

complete balanced food for the growing animal, thereby eliminating the need for the addition of protein supplements. The extensive use of high lysine cereals would thus reduce the sensitivity of the cost of feeding to fluctuations in the price of protein concentrates (Munk, 1976). Improved protein quality is determined by chemical evaluation of early breeding lines and by both chemical and biological evaluation on more advanced lines (PAG, 1975). Selection for characters that are of direct economic importance for the grain farmer such as disease resistance, seed quality and yield are easier to justify in a plant breeding program than quality parameters, e.g., lysine content, which exerts its effect and importance in the latter stages of the production chain (Munk, 1972).

There is a need for the plant breeder to work together with the nutritionist and biochemist who can assist with screening analyses and quality evaluation. Methods that are normally used in the evaluation of protein quality can be divided into (i) chemical, (ii) biological, (iii) microbiological, and (iv) enzymatic.

Microorganisms and enzymes have been widely used for digestibility studies and also in studies on

the availability of amino acids (Ford, 1960, 1962, 1964, 1966; Stott and Smith, 1966; Boyne, Price, Rosen and Stott, 1967; Hsu, Vavak, Satterlee and Miller, 1977; Buchmann, 1979a, 1979b and Ford and Hewitt, 1979). There are a variety of chemical methods used in evaluating protein quality. These include amino acid analysis by ion exchange chromatography after hydrolysis, and dye binding techniques. Chemical methods have been used for measuring available lysine and the availability of other EAA. The FDNB method of Carpenter (1960) is widely used for measuring available lysine in animal products. In plant materials, there is an interference by starch which makes this method unreliable and unsuitable. Biological evaluation of nutritional quality may be done either with laboratory animals or the actual animal for which that protein is to be fed eventually. Laboratory animals most commonly used are rats and mice.

Amino acid composition has been used as one of the criteria for assessing protein quality. Since lysine is the most limiting amino acid in cereals, including barley, lysine level has been used as a measure of nutritional value. Improving the lysine level has been shown to improve nutritional quality (Eggum, 1977a) providing the protein is not heat

damaged before feeding. However, one cannot, on the basis of amino acid composition alone, make a conclusion about the quality of a protein. This is because protein quality is affected both by the digestibility of the protein and the availability of its amino acids. It is also affected by antinutritional factors such as protease inhibitors, saponins and tannins. Barley grain has no saponins or protease inhibitors such as are found in legumes but it can contain low levels of tannins. Tannins are known to impair protein digestibility and the availability of amino acids and thereby reduce protein quality (Ford and Hewitt, 1979). Tannins exert their effect by binding with the proteins in the digesta such that they are precipitated out, or they bind the digestive enzymes so that they are incapable of hydrolysing the dietary proteins (Eggum and Christensen, 1975). These authors found tannin content of their barley samples to range from 0.55 to 1.23%. They observed a significant negative correlation between the tannin level of a barley and its protein digestibility. They also suggested that this correlation may partly explain the relatively low digestibility of barley protein when compared with other cereal proteins.

The quality of a protein also depends on how much albumin and globulin it contains. Albumins and globulins are high quality proteins readily soluble in water, and high in lysine when compared with prolamins, the storage proteins (Folkes and Yemm, 1956 and Ingversen and Koie, 1973). Glutelins are only intermediate in quality when compared with albumins and globulins. It has been shown that albumins and globulins together contribute 44% of the total lysine (Ingversen and Koie, 1973) in Emir barley, a normal variety. The present study involves five lines of barley from the same plot in Southern Alberta. The five lines were Hiproly, Galt (a widely grown commercial variety) and three lines containing the high lysine gene.

LITERATURE REVIEW

Protein Quality

The quality of a protein may be defined as its ability to supply essential amino acids (EAA) in the amounts and proportions required for a given physiological function, e.g., growth. This ability to supply essential amino acids is affected both by amino acid composition and by amino acid availability.

Total amino acids present in a protein are usually measured by chemical methods following hydrolysis. Because amino acids are generally only partially available, total quantity gives only an inaccurate assessment of the amount absorbable from the gastrointestinal tract. However, there are several additional factors that affect protein quality apart from availability of amino acids.

Factors Affecting Protein Quality

1. Amino Acid Composition

Amino acid composition is a basic measure of protein quality. It has been used for computing the

chemical score by comparing with the amino acid composition of a standard protein (Mitchell and Block, 1946). Cereals are known to be deficient in lysine, threonine and tryptophan in varying combinations for different cereals, for the monogastric animal. Lysine, however, is the first limiting amino acid in most cereals (FAO nutritional studies no. 16, 1957; Doll, Koie and Eggum, 1973; Paulis and Wall, 1974; Sauer, Giovannetti and Stothers, 1974; Eggum, 1977a and Batterham, Murrison and Lewis, 1978). Lysine, apart from being the first limiting amino acid in cereal proteins, is also the least available amino acid in cereal proteins (Eggum, 1977a). In practice, due to their deficiency in essential amino acids (EAA), cereal-based diets for animals are supplemented with protein concentrates such as fishmeal, meatmeal and soybean meal (SBM), each of which has an amino acid pattern satisfactory for complementing that of cereals. Synthetic amino acids are also used as supplements for cereal based diets.

Lysine supplementation of grain generally improves animal performance. Addition of other EAA, especially threonine, has also proved beneficial (Pond, Hillier and Benton, 1958 and Chung and Beames, 1974).

In a study with Peace River Barley for growing pigs, Chung and Beames (1974) observed that supplementation of barley with lysine to provide 0.75% improved the performance of growing pigs. Further improvement was observed with the addition of threonine. Bayley and Summers (1968) observed that the response to supplementation of a corn-SBM ration with lysine and methionine depended on the addition of threonine, indicating this amino acid to be deficient in low protein corn-SBM diets. Rosenberg, Culik and Eckert (1959) have found that lysine and threonine supplementation of rice diets caused substantial growth response in weanling rats. Addition of other EAA resulted in further improvement in performance. Waggle, Parrish and Deyoe (1966) also observed improved performance in rats when fed corn or sorghum supplemented with lysine, histidine and arginine.

Barley is an important grain in the animal feed industry. Normal barley is deficient in lysine, as are other cereal grains. A number of high lysine barley lines have been developed through plant breeding (Doll et al., 1974; Paulis and Wall, 1974 and Munk et al., 1969). Among the high lysine lines of barley, Hiproly and mutant 1508 have been much studied. Hiproly was isolated from the world barley collection

in Sweden while mutant 1508 was chemically induced in a breeding program at Riso, Denmark. Pomeranz, Robbins, Wessenberg, Hockett and Gilbertson (1973) have shown that the high lysine content (g/16 g N) of Hiproly is associated with high aspartic acid content (g/16 g N). It is known that aspartic acid is a key intermediary in the biosynthesis of lysine in higher plants, bacteria and algae (Pomeranz et al., 1973).

There is a negative correlation between crude protein level in grain and lysine content in protein in most cereals except oats (Munk, 1971). The negative correlation is due to a greater relative synthesis of storage proteins low in lysine and other essential amino acids as the total protein content increases (Munk, 1972). In normal 6-row barley and 2-row barley, the amino acid composition depends on the protein content. In Hiproly, the genes controlling protein level and lysine concentration are not genetically linked so that they segregate independently (Munk, Karlsson, Hagberg and Eggum, 1970 and Pomeranz et al., 1973).

Amino acid composition by itself is not a good measure of protein quality. In proteins that are damaged during processing, the amino acid composition

remains unchanged but biological value is drastically reduced as in heat damaged fish meal (Eggum, 1973).

2. Processing and Storage

Prolonged storage and various procedures used in food processing affect protein quality in several ways (Eggum, 1973). The principal factors involved are temperature level, duration of heat treatment and the presence of moisture and reducing substances. Protein quality may be reduced when a food is exposed to high temperatures during processing. Heat damage of proteins may occur through losses of amino nitrogen by transformation to non-protein nitrogen (NPN) (Eggum, 1973). There may be formation of esters within the protein molecule which decreases the solubility and digestibility of the protein and also cause reduced availability of the amino acids involved in the ester formation (Bjarnason and Carpenter, 1970). Peptides with such linkages are resistant to enzyme action; hence the reduced digestibility and availability.

In heat damaged proteins there could be racimisation, i.e., the L form of amino acids may be converted to the D form which either cannot be used by

the animal or used only partially. A major process that occurs in heat damage of proteins is the Maillard reaction, which is the reaction between lysine and reducing sugars. In this reaction the ε-amino group of lysine forms an enzyme resistant bond with simple sugars and thus becomes nutritionally unavailable. Lysine is the most heat sensitive amino acid (Carpenter, Morgan, Lea and Parr, 1962). Temperature and duration of heating are important in determining the extent of damage, e.g., baking a rat diet at 200°C for 15 to 20 minutes reduced PER from 3.5 to 2.4. A further heating by toasting at 130°C for 40 to 60 minutes reduced PER to 0.8. An addition of lysine corrected the PER to the original value of 3.5, thus indicating that lysine was destroyed by the heat treatments (Eggum, 1973). The extent of heat damage is also affected by the moisture content of the food material (Carpenter et al., 1962). These authors have shown that the loss of available lysine was greatest at 5 to 14% moisture. This means that drying of food materials should not go below 14% moisture. Eggum (1973) reported that sterilization of dry and soaked rat diets (11.2% and 71.6% moisture) caused more damage in the dry feed than in the soaked feed.

3. Availability of Amino Acids

Available amino acids are those that are accessible to the organism for anabolic physiological functions such as protein synthesis. All amino acids that are absorbed and utilized are termed available (Bodwell, 1976 and Erbesdobler, 1976). The availability of amino acids may be impaired by incomplete digestion of the protein and incomplete absorption resulting from indigestible cell walls, a bulky protein structure or many cross-linkages in the protein molecule (Miller, Carpenter and Milner, 1965 and Erbesdobler, 1976). Availability of amino acids may be impaired by the presence of enzyme inhibitors, inhibition of enzyme binding sites in the protein and also the inhibition of amino acid absorption by peptides and peptide-like compounds (Sauer et al., 1974; and Erbesdobler, 1976). Excessive heat treatment during processing tends to reduce amino acid availability by causing interactions between protein side groups and reducing sugars (Erbesdobler, 1976). The sturcture of lysine in the free form has a free ε-amino group, a free α-amino group and a free carboxyl group. In proteins, lysine is linked to other amino acids through peptide linkages

involving the α-amino and α-carboxyl groups. Only the epsilon amino group is free to react with various substances such as sugars. If the ε-amino group is bound through a chemical reaction the affected lysine becomes nutritionally unavailable (Carpenter and Bjarnason, 1969; 1970; Valle-Riestra and Barnes, 1970; Bodwell, 1976 and Erbesdobler, 1976). Lysine may also form cross linkages with other amino acids such as glutamic acid and aspartic acid, thus making them unavailable in heat damaged proteins (Miller et al., 1965 and Erbesdobler, 1976). Cross-linkages hinder the enzyme attack and release of even those amino acids that are not directly involved in the reaction (Miller et al., 1965).

4. Digestibility of Proteins

Digestibility of proteins, i.e., the reduction to dipeptides and amino acids capable of absorption from the gastro-intestinal tract is a primary factor affecting protein quality (Sauer, 1974 and Erbesdobler, 1976). The digestibility of a protein may be reduced by heat damage as was observed by Miller et al. (1965) with heated cod muscle. Such factors as enzyme inhibitors and inacessibility of protein to enzyme

action due to a bulky structure could reduce protein digestibility (Erbesdobler, 1976). Some protein molecules are bulky due to their tertiary structure. In such cases it is difficult for enzymes to cleave the peptide bonds on the inside of this molecule and release the amino acids. Apart from heat damage and protease inhibitors in legumes, the presence of tannins, quinones and saponins could reduce the digestibility of proteins (Cheeke and Myer, 1975). Such antinutritional factors do not occur in barley except for tannins which occur in small amounts of 0.55% to 1.23% (Eggum and Christensen, 1975). Tannins are not a serious problem in barley grains.

Methods of Measuring Available Lysine

Chemical Determination of Available Lysine

A number of methods have been proposed, the majority of them depending on the reaction of protein with 1-Fluoro-2,4-Dinitrobenzene (Milner and Westgarth, 1973). Of the chemical methods available, the Carpenter (1960) and Silcock (see Roach et al., 1967) methods are most frequently used. Although the Carpenter method is well established for measuring available lysine in

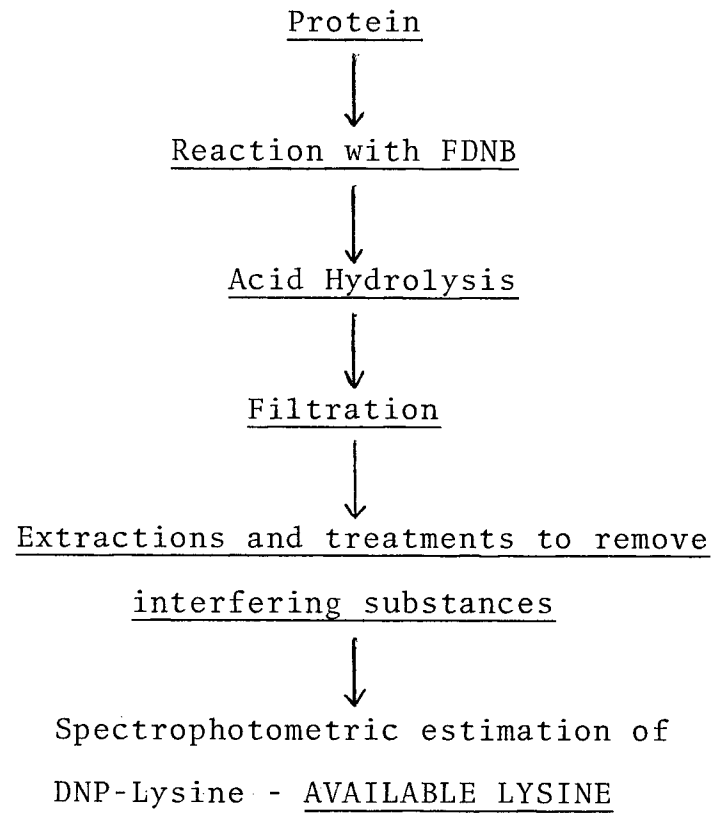
animal products, it has shortcomings when applied to cereal and oil seed meals (Roach et al., 1967). This is because it gives variable results with these feedstuffs and poor recoveries of the lysine derivative ε-DNP-lysine due to their carbohydrates during hydrolysis. Also there is the formation of other yellow products which are not easily separated from the derivative (Roach et al., 1967). The alternative method suggested by these authors is the Silcock method. While the Carpenter method measures available lysine by measuring the amount of FDNB that has reacted with ε-amino groups to form the lysine derivative, the Silcock method measures available lysine by difference, i.e., the difference between total lysine before the reaction with FDNB and the residual lysine after the reaction. The residual lysine is unavailable lysine because it does not react with the FDNB. This difference method has no problem with colour interferences because it does not measure colour as in the Carpenter method but measures lysine in the hydrolysates by ion-exchange chromatography (Roach et al., 1967). However, it does have the shortcoming in requiring an analysis for total lysine in the original material and again in the material

after the reaction. This is more time consuming and expensive than a measurement of the coloured reaction products by spectrophotometry as used in the direct method.

Generally, chemical methods for estimating available lysine are either direct or indirect. Bodwell (1976) described a number of direct methods using various chemicals in the formation of various derivatives. Examples of the direct methods which he mentions include Carpenter's FDNB method and the method of Kakade and Liener (1969) using trinitrobenzene sulfonic acid (TNBS), forming TNP-lysine as a derivative. The value of the direct chemical methods is reduced, due to the fact that the derivatives are partially hydrolysed during acid hydrolysis (Bodwell, 1976). The indirect methods can be done with the same chemicals as used for the direct estimation.

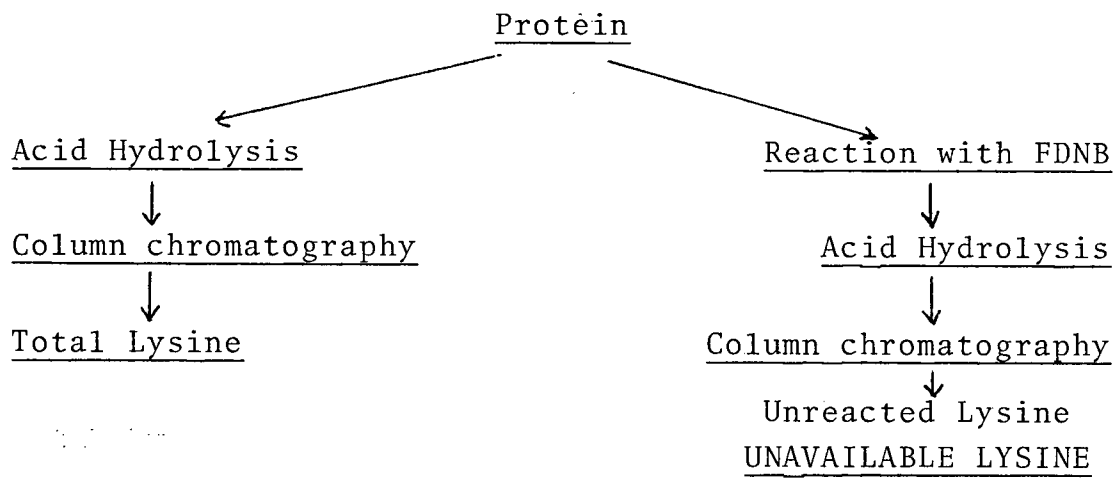
Apart from the Silcock indirect method, dye binding difference is widely used for estimating

(a) FDNB Direct Method (Carpenter Method) A schematic representation (Bodwell, 1976)



(b) Indirect FDNB Method

A schematic representation (Bodwell, 1976)



Available lysine = Total lysine - unreacted lysine

available lysine (Hurrel and Carpenter, 1975, 1976 and Walker, 1979). Dye binding, using acid dyes at a low pH involves the formation of ionic linkages with the basic amino acids lysine, histidine and arginine present in the protein (Walker, 1979). The dye binding method of estimating available lysine involves the blocking of the ε-amino group of lysine with reagents such as ethyl chloroformate (Sandler and Warren, 1974) and propionic anhydride (Hurrel and Carpenter, 1975 and Walker, 1979). The dye binding difference (DBD) was calculated as the difference between the dye binding capacity of the protein before and after blocking the ε-amino group of lysine. The DBD is then used to calculate the percent available lysine (Walker, 1979). Values of reactive lysine measured by DBD compare favourably with values for direct FDNB reactive lysine. This fact is supported by results presented below, obtained by Hurrel and Carpenter (1976). Walker (1979) has observed a correlation coefficient of 0.90 between DBD and FDNB reactive lysine.

Relative lysine concentrations (mmol lysine/kg crude protein) in vegetable and pure proteins determined by dye binding difference and by reaction with FDNB.

	DBD-reactive lysine	FDNB-reactive lysine
Wheat gluten	110	100
Whole wheat	190	180
Rice	200	200
Sweet corn	220	220
Groundnut flour	230	220
Broad bean	330	340
Soya bean	370	390
Chick pea	440	410
Wing bean	500	470
Bovine plasma albumin	810	830

Biological Determination of Available Lysine

This may be done by the use of laboratory animals or farm animals. Lysine is normally the first limiting amino acid in cereal-based diets for growing pigs. This limitation is usually overcome by

supplementing with protein concentrates. Availability of lysine in most protein concentrates that have not been heat damaged is high, e.g., fish meal and soybean meal have high lysine levels of 7.60 g/16 g N and 5.98 g/16 g N respectively with availabilities of 96.8% and 91.6% as determined by rat experiments (Eggum, 1973). Other protein concentrates such as groundnut meal and sunflower seed meal that are used as supplements have lysine availabilities of 86.9% and 88.0% respectively (Eggum, 1973) as determined in rat experiments. However, Milner and Westgarth (1973) using Silcock difference method obtained lysine availabilities of 94.6%, 92.7%, 96.7% and 94.7% for fish meal, groundnut meal, soybean meal and sunflower seed meal. Batterham, Murison and Lewis (1978) using wheat-based diets supplemented with various protein concentrates (fish meal, meat meal skim milk, soybean meal, etc.) and lysine, and a control diet with no supplement, showed the differences in growth response to be due to differences in available lysine. The available lysine contents of the different protein concentrates were measured by rat bioassay, and these were said to correspond to the values estimated by the Silcock difference method,

although the authors showed no figures for this comparison.

Biological availability of amino acids has been determined from the difference between the amino acid content of the feed consumed and the amino acid content of the faeces (Ousterhout, Grau and Lundholm, 1959; DeMuelenaere and Feldman, 1960 and Eggum, 1973). This method has two disadvantages, which are:

(a) The action of intestinal microflora on food

(b) The fact that the diet can influence the secretion of protein rich materials into the gastro-intestinal tract.

(Lymman, 1957 and Sauer, Stothers and Parker, 1977). These factors affect the magnitude of the metabolic faecal and endogenous urinary nitrogen so that true availability of amino acids obtained by faecal analysis is inaccurate. Another biological method that has been used involves the comparison between growth rates obtained on diets low in EAA and growth rates obtained on similar diets to which known amounts of test protein have been added. By supplementing the basal diet with several levels of the limiting amino

acids a growth response curve can be obtained and the amount of the amino acid in the test protein determined (Ousterhout et al., 1959). Researchers using this method often end up with different results for the same amino acid (Schweigert and Guthneck, 1953; Guthneck, Bennet and Schweigert, 1953, and Gupta, Dakrouy, Harper and Elvehjem, 1958). Sauer et al. (1974) fed various cereals (barley, wheat, triticale) and soybean meal to barrows and determined available lysine by faecal analysis. The metabolic and endogenous amino acids were determined with nitrogen free diets. In this study, they observed that lysine availability was 77% in normal barley, 80.8% in wheat, 94.2% in triticale and 94.9% in soybean meal with 10 kg pigs. The same diet fed to 30 kg pigs produced availabilities of 65%, 67%, 77.5% and 91.2% in barley, wheat, triticale and soybean meal respectively. No explanation was given for the decreased availability in the older animals.

Microbiological Estimation of Available Lysine

A number of microorganisms have been used to estimate protein quality in feed materials. Tetrahymena pyriformis, W; Streptococcus zymogenes and Streptococcus faecalis have been used in estimating gross protein value, net protein utilization and net protein retention. Useful correlations have been found between protein quality for rats and chicks and the results from assays with T. pyriformis, S. zymogenes and S. faecalis (Boyne, Price, Rosen and Stott, 1967). T. pyriformis is similar to higher animals in the response to protein quality, especially available lysine (Boyne et al., 1967). Several microbiological methods have been recommended for use in the study of protein quality (Ford, 1960). Indices of protein value found with microbiological methods agree with the findings from rat growth tests; T. pyriformis simulates more closely the circumstances of biological tests (Ford, 1960). It has the same amino acid requirements similar to the growing rat and is able to use intact protein. Ford (1960) found a good positive correlation between the relative nutritional values for S. zymogenes on a number of proteins and their net protein utilization (NPU) as determined with

rats. Stott and Smith (1966) have observed that Tetrahymena estimates of available lysine are similar to those obtained by the FDNB method of Carpenter for samples high in available lysine. For samples of lower available lysine content Tetrahymena estimates were higher than those with FDNB. The authors attempted to explain this difference by saying that factors other than binding of the ε-amino group appear to have influenced the Tetrahymena assays.

Supplementation of Cereals with Essential Amino Acids

Supplementation of protein with the first limiting EAA is highly beneficial if it brings the total amount of this amino acid present in the protein and available to the organism into balance with the second limiting amino acid (Rosenberg, Culik and Eckert, 1959). When the proper balance has been achieved between the first two limiting amino acids, further improvement can be achieved if both are added simultaneously in the proportions of the body's requirement (Rosenberg et al., 1959). There are a number of reports in the literature that combination of lysine and threonine

causes substantial growth responses in weanling rats fed cereal-based diets. Rosenberg et al. (1959) have observed that addition of other EAA to lysine-threonine supplemented diets caused further improvement in rat performance. Chung and Beames (1974) observed improvement in the performance of growing pigs when fed barley supplemented to a level of 0.75% lysine. An additional improvement was observed with the addition of threonine. Other workers have made similar observations with other cereals (Pond, Hillier and Benton, 1958 and Bayley and Summers, 1968). Margruder, Sherman and Reynolds (1961) observed a significant increase in the rate of gain of baby pigs by adding 0.1% lysine to a 14% crude protein diet containing 0.74% lysine. The rate of gain with supplementation was equal to that on a 16% protein starter, not supplemented with lysine. No significant increase in feed efficiency was observed with lysine supplementation. They also observed a tendency toward improvement of some carcass characteristics in lysine fed pigs. Bayley and Summers (1968) observed no beneficial effect on growth rate and feed efficiency when practical corn-SBM rations were supplemented with 0.1% lysine or 0.05% methionine. However, when both were

given simultaneously there was a positive effect on gain. It is known that corn is deficient in lysine and soybean meal is deficient in methionine. Berry, Combs, Wallace and Robbins (1966) observed that with corn-soybean diets, the response to supplementation with lysine and methionine depended on the addition of threonine. This could be because threonine is the second limiting amino acid in the diet. It is necessary to obtain a balance between the first and second limiting amino acids to see the beneficial effect of supplementation. Fuller et al. (1979) have observed that growth rate of pigs increased from .36 kg/day to a maximum of 0.65 kg/day with the addition of 3.8 g lysine/kg and 1.8 g threonine/kg to their barley-based diet. Carcass quality was also found to be improved with addition of lysine (Fuller et al., 1979). They also observed that back fat thickness decreased progressively with increasing addition of lysine and threonine. It has been established that threonine is the second limiting amino acid in barley for growing pigs (Chung and Beames, 1974). Fuller et al. (1979) have proved that much improvement in the utilization of cereal protein can be achieved by the simultaneous addition of just lysine and threonine.

Supplementation of a diet with amino acids for animals require a knowledge of the amino acid requirements of the particular animal. Amino acids utilized by animals may be essential or non-essential. Essential amino acids (EAA) are those that either cannot be synthesized by the animal or are not synthesized in adequate quantities to meet requirements. Arginine, histidine, isoleucone, leucine, lysine, methionine, tryptophan and valine are the essential amino acids for the growth of rats, pigs and man. Non-essential amino acids can be synthesized by animals, however, they need an adequate nitrogen supply to achieve this. Some amino acids that may be essential for the young animal may not be essential for the adult animal. Amino acid requirements are affected by genetic factors, age and physiology, food intake and dietary protein and energy level.

Different species of animals of the same physiological age require different amounts of EAA, e.g., growing chicks 0-6 weeks old require 1.25% lysine in their diet while growing pigs 5-10 kg liveweight require 0.96% lysine (NRC, 1971, 1973). Different species have different nutrient requirements

and even different strains of the same breed of animals have different nutrient requirements.

There is a significant interaction between age and amino acid levels in the diet (Hays, Speer, Hartman and Catron, 1959). Generally, amino acid requirements decrease as the animal gets older. The rate of decline of amino acid requirement with age varies for different amino acids and for different species for the same amino acids (Homb, 1976). In the growing pig, lysine requirement declines faster than methionine plus cystine requirements (Homb, 1976).

Amino acid requirements also vary for different physiological states or function of animal. Protein depleted animals have higher amino acid requirements than undepleted animals due to increased protein requirements (Fisher, Griminger and Leveille, 1959). Growing animals have higher requirements than mature animals because of their need to form new tissues. It has been demonstrated by Kielanowski (1972) using nitrogen balance studies, that boars, castrates and gilts have different amino acid requirements because they vary in their rate of protein deposition. It is general knowledge that amino acid

requirements increase during pregnancy, lactation and egg laying. Stresses such as injury or infection cause an increase in amino acid requirements (Leveille et al., 1959).

Amino acid requirement increases with level of intake if requirement is expressed on per day basis. Thus, it is affected by all the factors that generally affect food intake (March and Biely, 1972). Factors such as age, health and function affect the intake of an animal. Also sex affects level of intake. There is evidence to support the fact that the requirement for the most limiting amino acid in a diet rises as the dietary protein level is increased (Munaver and Harper, 1959). These workers reported that increasing the level of a protein deficient in a certain amino acid increases the requirement for that amino acid. Lysine requirement of rats for maximum growth increased from 0.9% of the diet containing 30% wheat gluten to 1.2% of a diet containing 60% wheat gluten (Munaver and Harper, 1959). This they explained as being due to the poor amino acid balance of wheat gluten. Imbalance is due to excess levels of other amino acids. In an attempt to provide an adequate amount of the most limiting amino

acid by increasing protein content, the requirement for this amino acid increases because of the high levels of the other EAA. The growth retarding effects of excess amino acids can be corrected by adding the limiting amino acid instead of increasing the protein level. This is the basis of amino acid supplementation of poor quality proteins.

March and Biely (1972) have shown that energy content of the diet affects the requirement of amino acid by chicks. Animals on high energy diets tend to have a higher amino acid requirement (g/100 g DM diet) due to the fact that food consumption generally decreases as energy content increases, i.e., there is a need to increase the amino acid concentration in high energy diets. In supplementing any diet with amino acids, one has to consider the protein level, energy level, the age and physiological state and function of the animal for which such a diet is going to be given.

Protein : Calorie Ratio

The importance of dietary protein : calorie ratio has long been recognized for achieving maximum

growth rate and efficiency of feed conversion with animals (March and Biely, 1972). The efficiency of feed utilization increases as the energy content is increased (Rosenberg, 1957). Animals eat to satisfy their energy requirement so that the higher the energy content the lower the amount consumed per unit weight gain. But this lower amount must also contain all the nutrient requirement of the animal. Energy intake has been shown to be one of the factors controlling amino acid requirement (Rosenberg, 1957).

In evaluating protein quality it is necessary to provide adequate energy in the diet in the N-free fraction. This ensures that protein will not be used as a source of energy. Forbes and Yohe (1954) investigated the effect of energy consumption on BV using rats. They showed that at a low feed intake, BV was low because not enough energy is consumed and protein is used as energy source.

Eggum (1973) cites a number of authors who have suggested some effects of energy intake on protein utilization. However, Eggum himself (1973) observed no beneficial effect of high energy content of diet on protein utilization. When the energy content of diet

is adequate such that no protein is used for energy purposes, extra energy will not have any effect on protein utilization. However, if energy is low and protein is being deaminated, added energy will improve BV.

Biological Methods of Measuring Protein Quality

Nutritional quality of proteins has been measured by chemical, biological, enzymatic and microbiological methods. Biological methods of assessing protein quality, directly or indirectly, measure body protein changes in response to the test protein. Direct measurements of body protein is complicated but changes in body weight are easy to measure. Changes in body weight are well correlated with changes in body protein but are not an accurate measure of body protein changes. Methods that have been used in biological estimation of protein quality include protein efficiency ratio (PER), net protein utilization (NPU), net protein retention (NPR) and biological value (BV).

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$NPR = \frac{\text{Weight gain of test protein group (g) - Weight loss of protein free group (g)}}{\text{Protein intake (g)}}$$

TD = True digestibility of nitrogen

$$BV = \frac{\text{Nitrogen intake - (faecal nitrogen - metabolic faecal nitrogen) - (urinary nitrogen - endogenous urinary nitrogen)}}{\text{Nitrogen intake - (faecal nitrogen - metabolic faecal nitrogen)}}$$

$$NPU = \frac{TD \times BV}{100}$$

These definitions of the biological measures are as given by Eggum (1973).

According to McLaughlan and Keith (1974), PER is not a good measure of protein quality. It is highly correlated with weight gain but is not characteristic of the protein (Hegsted and Chang, 1965). It is not a true measure of protein efficiency since not all the protein goes into weight gain (Hegsted et al., 1965). PER varies with the sex of the animal (Block and Mitchell,

1946). NPU, on the other hand, is affected by amino acid composition, protein:calorie ratio, adequacy of calorie intake and vitamin and mineral balance (Miller and Payne, 1961). According to Platt and Miller (1958), NPU is not directly related to amino acid levels in the diet but to the amino acid balance. NPU is a function of digestibility and biological value so that all factors affecting TD and BV will affect NPU.

Biological tests for protein quality can be put into three categories:

(1) Tests in which the test material serves as the sole source of protein. The level of this protein is usually fixed at 10%. Tests using this category include BV, NPU and PER. NPU and BV are best determined on restricted feeding while PER is done on ad libitum feeding.

(2) Tests which measure the supplementary effect of protein when used in combination with a test diet which is deficient in a particular amino acid, usually lysine.

(3). Tests using the supplement at practical levels such as those used in animal husbandry (Olley and Payne, 1967).

Hegsted and Yet-Oy Chang (1965) examined the application of standard bioassay procedures for the evaluation of nutritive value of protein for rats. The slope ratio technique or relative protein value appeared to them to be the most satisfactory. This method uses gain as the response and nitrogen intake as a measure of dose. In this slope ratio method, diets of varying protein levels are used. At low levels of protein such as 2% or 5% one should critically ponder how meaningful the changes in body weight are in relation to nitrogen intake. At such levels, far below the requirement of the animal, the animal will be in a poor physiological state and weight changes may not be the direct response to nitrogen intake but to other physiological factors.

For some time BV and PER had been the two biological procedures for evaluating the nutritive value of proteins (Hegsted and Yet-Oy Chang, 1965). According to these authors, BV can be determined only when the animal is in negative nitrogen balance or

near balance; so, low protein diets are fed for this determination.

The protein efficiency ratio was originally proposed by Osborne and coworkers in 1919. In this method, the gain per gram of protein eaten by young rats on low protein diets (9-10%) is calculated (Hegsted and Yet-OY Chang, 1965). This method has been criticized because PER is not characteristic of the protein but of the rate of gain of the animals consuming the diet. It is also not a measure of efficiency since not all the protein is used for growth (Hegsted and Yet-OY Chang, 1965).

NPU as a measure of protein quality is a relatively recent procedure when compared to BV and PER. It has long been recognized that the BV appears to over-estimate the nutritive value of poor quality proteins. Mitchell et al. (1952) and Dobbins, Krider, Hamilton and Terrill (1950) reported that the BV of corn protein decreased as the protein content increased. It has been established that BV is independent of food intake but decreases as the protein content of the diet increases above a certain level (usually 10%) (Eggum, 1973).

NPU can be determined by carcass analysis or by nitrogen balance techniques (Chalupa and Fisher, 1963) NPU determination was developed by Miller and Bender (1955) who used total carcass analysis. They fed diets with standardized amounts of protein, fat, minerals and vitamins. In their study, it was suggested that a protein-free diet or a diet containing 4 to 4.5% egg protein be fed to the control group from which endogenous and metabolic nitrogen was determined. Eggum (1973) recommends the use of a 4% egg protein and not a nitrogen-free diet. Egg protein is completely digested and utilized (Eggum, 1973) so that it makes no contribution to metabolic or endogenous nitrogen, and the animal is in a better physiological state than it would be on a nitrogen-free diet. A modified form of NPU referred to as net protein value (NPV) has been used by some workers who have observed a close correlation between sulfur content of foods and their NPV (Morrison, Sabry and Campbell (1962). Lysine level of diets have been found to affect NPU values. Morrison et al. (1962) observed that when lysine concentration in rat diets was raised from 0.32% to 0.72% NPU increased from 21 to 48 and they observed a high correlation ($r = 0.85$) between NPU and lysine level of the diet, indicating that NPU is

highly dependent on lysine. Other measures of protein quality such as PER and net protein retention (NPR) have also been found to be well correlated with lysine level in diet.

Morrison et al. (1962) have observed that NPU measured by carcass analysis is sensitive to dietary lysine level. Chalupa and Fisher (1963) observed that NPU determined by carcass analysis showed much variation when animals were fed ad libitum. However, the nitrogen balance method gave consistent results. Based on this observation these authors suggested the use of a nitrogen balance method for measuring NPU when animals are on ad libitum feeding. Eggum (1973) determined NPU using both carcass analysis and a nitrogen balance method with restricted feeding, so that the effect of variable feed intake is removed.

It has been reported that NPU and BV estimated at relatively low levels of protein may be expected to be erroneously high for foods in which lysine is the limiting amino acid (Eggum, 1973). It is known that BV overestimates the nutritive value of poor quality proteins, thus the lysine deficient protein will be overestimated in nutritional value. Also, at low

levels of protein one tends to get high BV. These factors have necessitated the need for standard conditions being used in such determinations. However, BV and NPU are generally regarded as specific characteristics of food proteins. According to Eggum (1973) nitrogen retention may be measured by the nitrogen balance technique or by total body analysis. The two methods have given results that show close agreement by some workers but others have reported the carcass analysis method to be more accurate. These differences in opinion could be due to differences in accuracy of the different researchers. In nitrogen balance methods, metabolic and endogenous nitrogen are important factors. It is noted that heavier rats excrete more nitrogen in their faeces than lighter ones (Eggum, 1973). This is only important when large differences in body weight are involved. In biological evaluation of protein quality, animals of uniform weights are used. This is usually achieved by using animals of the same age.

In the investigations on Hiproly, the early experiments with rats and mice have shown that animals on this high lysine barley show better nitrogen retention and net body nitrogen gain than those on normal

barleys with high protein (Munk, 1972). The availability of lysine in Hiproly was higher than in normal barley with an increase in nitrogen content (Munk, 1972). An improved availability pattern reflects the enhancement of water soluble, lysine rich proteins that are easily digestible. Since availability depends on the protein digestibility, the increased availability in Hiproly is due to the fact that high lysine barleys contain higher levels of albumins and globulins than normal barley and have a reduced prolamin level (Doll, 1977; Anderson, 1977 and Koie and Kreis, 1977). Albumins and globulins are readily digestible and high in lysine, so it is no wonder that the increased levels of these proteins in barley grain causes an increase in available lysine. Eggum (1969) has demonstrated that the stage of ripeness at harvesing affects the protein quality. He has observed that lysine content decreases when barley is harvested overripe, with the BV and NPU also reduced. Unfortunately, he does not tell us what stage of ripeness is overripe.

EXPERIMENTAL

This project was designed to evaluate high lysine lines of barley from the Alberta Agriculture breeding program using chemical and biological methods. The chemical procedures used in this research include proximate analysis, separation and quantitation of barley proteins, amino acid analysis and available lysine estimation by the dye-binding technique.

The biological determinations involved growth assays, measurements of true nitrogen digestibility (TD), true biological value (BV), net protein utilization (NPU) and relative protein value (RPV).

CHEMICAL EVALUATION OF PROTEIN QUALITY

Proximate Composition

Introduction

Proximate analysis of feedstuffs is the most basic chemical method applied in nutritional studies. It gives a crude estimate of the composition of a feedstuff.

However, it has marked shortcomings in that it gives only estimates of nutrient content. Its value is in the fact that it is a simple easily reproducible system for categorizing a food. Moisture determination allows an assessment to be made on suitability for storage; also the dry matter content sets an upper limit on nutrient level. Crude protein, although measuring only total nitrogen content, permits assumptions to be made on amino acid content, inaccurate as they may be, from standard tables such as those published by the National Research Council (NAS-NRC-1971, 1973). For monogastric animals, energy content would be increased with increasing levels of ether extract; decreased with increasing levels of ash and fibre. Consequently, in terms of broadly defining a food, the proximate analysis still serves a useful purpose.

Materials and Methods

Five barley samples, Hiproly, Galt and three cultivars, H69019109000, H69024038000 and H690240004191 were obtained from the 1977 harvest of the Alberta Agriculture breeding program.

Hiproly, a mutant discovered in Sweden (Munk, Karlson and Hagberg, 1971) with high protein, high lysine content, is low yielding and deficient in several agronomic traits (flinty, shrivilled seed, small spikes and uneven tillering pattern). Galt is a widely used commercial variety. Cultivar H69019109000 (Cultivar 1) is a selection from the F_{10} generation of a Hiproly x Orange Lemma cross. The Orange Lemma carries a marker for lemma colour plus a factor for high α-amylase activity. It is a 6-row barley with covered seed, high yield and high number of seeds per culm. Both cultivars H69024038000 (cultivar 2) and H690240004191 (cultivar 3) are selections from the F_{10} generation of a Hiproly x cultivar OR61-2141-9. This latter cultivar was selected from the Oregon breeding program as a potential high-yielding 2-row malting barley. It has good agronomic characteristics and yielding ability. Further information on the parent cultivars has been presented by Helm (1972).

All grain was combine harvested when less than 20% moisture content and dried in a dryer operating at 32 to 38°C.

Moisture content, crude protein, ether extract and ash were determined according to AOAC (1970). Acid

detergent fibre (ADF) was determined according to the method of Waldern (1971).

Results

Table 1. Proximate composition of barleys - % DM basis

Sample	Dry Matter	Crude Protein N x 6.25	Ether Extract	Ash	Acid Detergent Fibre	NFE*
Hiproly	92.62	18.57	1.94	2.04	3.06	74.39
Galt	92.69	15.16	1.78	2.14	4.66	76.26
Cultivar 1	91.92	17.45	1.65	2.46	5.42	73.02
Cultivar 2	92.55	18.10	2.13	2.28	4.34	73.15
Cultivar 3	92.64	16.81	1.69	2.47	4.38	74.65

Cultivar 1 = H69019109000

Cultivar 2 = H69024038000

Cultivar 3 = H69024004191

NFE* = Modified NFE due to the use of ADF. Modified NFE has been used by Morgan, Cole and Lewis (1975).

Discussion

From Table 1 one can see the high protein contents of the five barleys being studied. Canadian commercial barley generally contain 10 to 14% crude protein (Coates, Slinger, Summers and Bayley, 1977). All the barleys including Galt, the commercial control, have a higher protein content than this range of 10 to 14%. It is possible that the environment for cultivation contributed to this relatively high crude protein content in Galt.

Hiproly contains the highest level of protein and the lowest level of fibre and ash, the former being associated with the fact that Hiproly is hull-less. In normal barley in Canada, the ether extract is low, ranging from 1.96 to 3.16% (Coates et al., 1977) with an average of 2.43%. The barley cultivars being studied in this work have ether extracts ranging from 1.65 to 2.13%. Coates et al. (1977) reported ADF values for 16 normal barley samples from a number of countries. The values ranged from 5.64 to 8.81%. The ADF values of the 5 barleys under investigation range from 3.06 to 5.42%. Ash in this study ranged from

2.04 to 2.47% while values for the 16 barleys of Coates et al. (1977) ranged from 2.25 to 3.82%.

Separation of Barley Proteins by Solubility

Introduction

There are four different types of plant proteins. These have characteristic solubilities and, based on these solubilities, proteins are classified into four categories namely:

Albumins
Globulins
Glutelins
Prolamins

Albumins are soluble in water, globulins in salt solution, prolamins in alcohol and glutelins in dilute acid or alkali. This method of separation was first used by Osborne in 1895 for barley proteins.

Structure of the Barley Grain

In most cultivated barleys, the husk adheres to the grain (e.g., Bomi) but some varieties are naked

(Kreis, 1979). The embryo occupies only a small part of the kernnel, i.e., about 2 to 5% of the dry weight, and it controls the mobilization of stored nutrients. Eighty percent of the grain protein and most of the carbohydrates are located in the endosperm (Kreis, 1979). In normal barley albumins plus globulins form 18% of the crude protein, non-protein nitrogen forms 12%, prolamins form 42%, and glutelin 23% (Balaravi et al., 1976; Koie and Nielson, 1977; Milflin and Shewry, 1978; and Kreis, 1979).

Materials and Methods

Sample Preparation

The five samples of barley grain from the Alberta Agriculture 1977 harvest were ground with a laboratory hammer mill (Christy and Norris Ltd., Chelmsford, England) using an 0.5 mm screen.

Reagents

Solutions were prepared by methods described by Koie and Nielson (1977). The salt solution consisted of 29.2 g NaCl and 0.02 g NaEDTA (sodium ethyldiaminetetra acetic acid) dissolved in water and made up to 1 litre

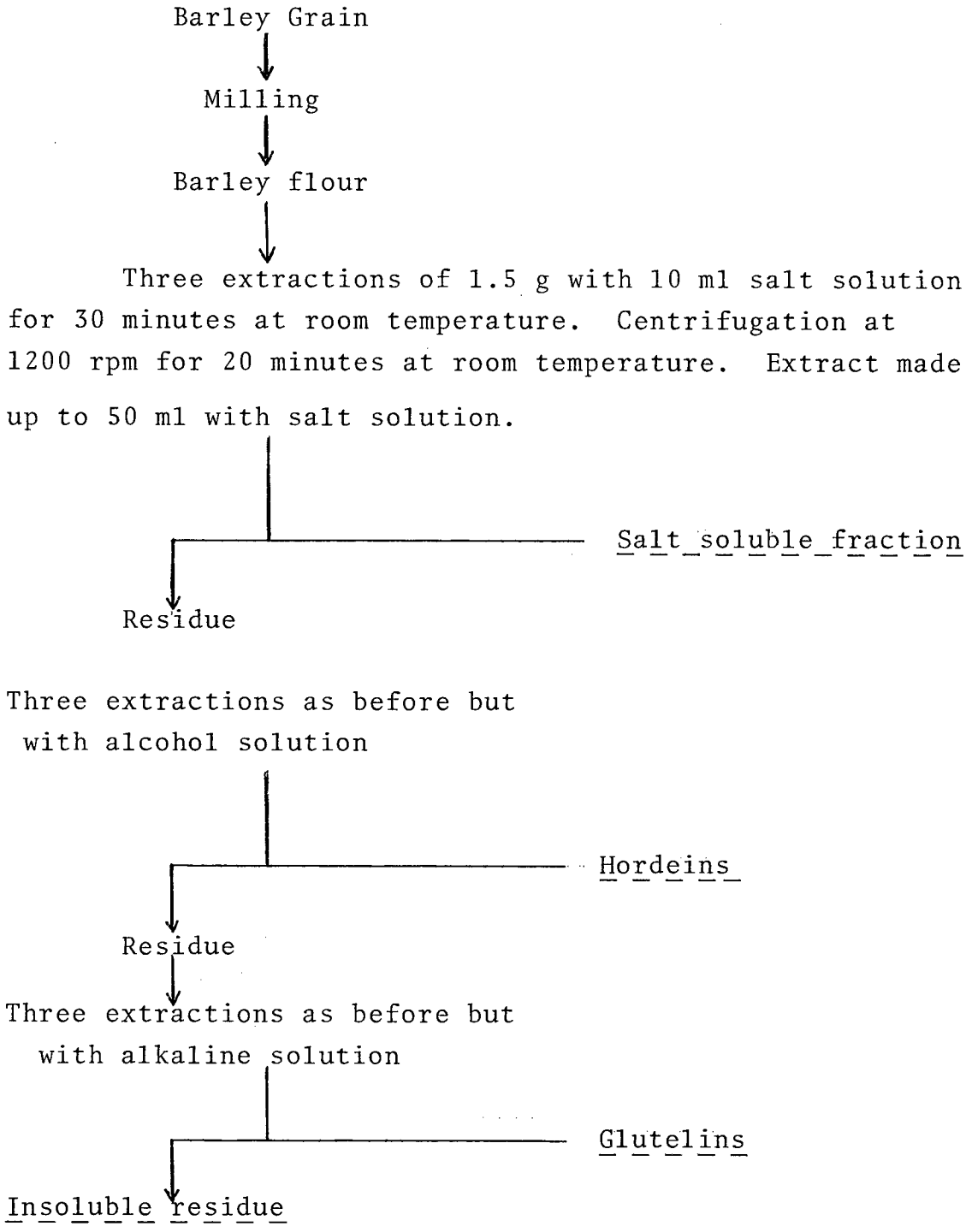
with water. The alcohol solution was made of 500 ml iso-propanol, 6 ml 2-mercaptoethanol and 500 ml water. The alkaline solution contained 4.8 g $Na_2B_4O_7 \cdot 10H_2O$ (sodium tetraborate), 1.7 g NaOH and 5.0 g SDS (sodium dodecyl sulphate) per litre.

Procedure

A ground sample of 1.5 g was weighed into a 60 ml plastic centrifuge tube and extracted three times with 10 ml of solvent. Extraction was done by mechanical shaking. The mechanical shaker was improvised from a water bath. Cut glass rods 3 cm x 0.5 cm diameter, rounded at both ends were placed in the centrifuge tubes and each tube fitted with a no. 4 rubber stopper. Each extraction was carried out for 30 minutes at room temperature which ranged from 23 to 25°C. Samples were centrifuged in a Sorvrall superspeed RC2-B refrigerated centrifuge at 12000 rpm for 20 minutes. The supernatants were pipetted into 50 ml volumetric flasks. After the third extraction all extracts were made up to 50 ml with more solvent. Samples were first extracted with salt solution for albumins and globulins as well as NPN, then with alcohol for hordeins (the prolamins of barley) and finally with the alkaline solution for the

glutelins. After all the extractions, the final residues were completely transferred into aluminium weighing dishes by washing with distilled water. Residues were dried in an oven at 85°C overnight. They were weighed after cooling in a dessicator. Ten millilitres of each extract was used for Kjeldahl nitrogen determination. The 10 ml of each alcohol extract was first evaporated on a water bath to about 3 ml before being digested. This ensured that there was no foaming which could lead to loss of samples during digestion. The nitrogen content of the final residues was also determined by the Kjeldahl method. The nitrogen content of the fractions were expressed as a percent of the total nitrogen which was determined in a 1.5 g sample. The percentages of nitrogen were summed up for each sample to determine the total percentage recovery of nitrogen.

Extraction Procedure for Soluble Protein Fractions



Results and Discussion

Table 2. Nitrogen content of barley extracts as a percentage of total nitrogen (a, b, duplicates).

Sample		Salt Extract	Alcohol Extract	Alkaline Extract	Residue	N-Recovery
Hiproly						
	a	20.38	38.11	30.49	8.04	97.02
	b	20.52	35.94	31.00	7.15	94.61
Galt						
	a	18.50	46.41	21.59	9.05	95.55
	b	19.27	47.10	21.88	7.85	96.07
Cultivar 1						
	a	22.57	41.23	22.53	8.85	95.18
	b	22.70	38.62	27.57	7.90	96.78
Cultivar 2						
	a	21.88	37.94	27.55	6.88	94.25
	b	21.87	39.40	28.86	6.32	96.46
Cultivar 3						
	a	19.20	44.78	20.64	8.67	93.29
	b	19.47	43.44	22.46	7.57	92.94

Cultivar 1 = H6901910900, Cultivar 2 = H69024038000,
Cultivar 3 = H690240004191

Table 2 shows the proportions in which the protein fractions occur in these five barleys. The salt soluble fraction which contains the albumins and globulins forms about 20% of the total protein, with a range of 18.5 to 22.7%. The alcohol fraction which contains the hordeins forms 37.0% in Hiproly protein, 46.5% Galt, 40% in cultivar 1, 38.5% in cultivar 2 and 44% in cultivar 3. In Hiproly, glutelins form 30.5% of the protein while in Galt it forms 21.7%, cultivar 1, 25.0%, cultivar 2, 28.0%, and cultivar 3, 21.5%. Ingversen and Koie (1973) found that the salt soluble fraction of normal Emir barley accounted for 24% of the total protein, and 44% of the total lysine. The difference between Ingversen and Koie's partitioning of the soluble fraction and that in this study could be due to incomplete extraction in the latter. It could also be characteristic of the type of barley being used. Balaravi, Bansal, Eggum and Bhaskaran (1976) in their study of the characteristics of induced high protein and high lysine

mutants in barley compared Hiproly, notch 1 and notch 2 and their normal parent for the proportions of the different fractions and for their nutritive value by animal experiments. Their results for soluble fractions are presented below.

	Salt Soluble	Alcohol Soluble	Alkaline Soluble	% Recovery
Normal Parent	20%	29%	45%	94
Notch 1	29%	22%	49%	99
Notch 2	28%	25%	41%	94
Hiproly	25%	22%	46%	92

If the salt soluble fraction contains the best quality proteins, the higher the proportion of this fraction the better. They observed that notch 1 and notch 2 had more albumins and globulins than Hiproly. In this present study cultivars 1 and 2 also appear to have more salt soluble fraction than Hiproly. Helm (1972) found the salt soluble fraction of Hiproly to be 21.12%, which is very close to the value obtained in this study (20.38%). Helm (1972) observed that the alcohol soluble fraction of Hiproly was only 19.26% of the

total protein and the alkaline fraction was 59.63%. The alcohol fraction seems rather low and the alkaline fraction too high when compared to the values obtained in the present study and those observed by Balaravi et al. (1976) for Hiproly, and values were obtained by Koie and Nielson (1977), Milflin and Shewry (1978) and Kreis (1979) for normal barleys. It appears that Helm's alcohol soluble fraction was not completely extracted and it got extracted into the alkaline soluble fraction. This may account for the low value and very high value obtained by Helm (1972) for his alcohol and alkaline soluble fractions.

According to Milflin and Shewry, the alcohol soluble fraction which contains the hordeins has low lysine and other basic amino acids but is high in glutamic acid and proline. They also suggest that cereal prolamins increase at a greater rate than other protein classes in response to added nitrogen. Thus, one can say that the percentage of hordein in barley is directly related to the nitrogen status of the soil on which it was grown. Folkes and Yemm (1956) separated barley proteins and determined the amino acid compositions of the fractions. They found that over 60% of the hordein nitrogen is made

up of glutamic acid, amides and proline. This is in agreement with that observed with normal Emir barley by Milflin and Shewry (1977). The amino acid composition of the barley protein fractions of Folkes and Yemm (1956) are quoted for comparison (Table 3).

From this table it is clear that albumins and globulins have a high basic amino acid content, especially arginine and lysine. Glutelin has a moderate level of lysine, an arginine and a histidine level which are similar to those of albumin and globulin. The glutelin fraction has a higher level of threonine, leucine, and isoleucine and other essential amino acids (EAA) than globulin. In the hordein fraction, lysine level is extremely low; histidine and arginine levels are also very low.

Generally the levels of EAA in the hordein fraction are much lower than the levels found in the other fractions. Animal performance tends to be affected to a greater extent by the total percentage lysine in the seed than by the lysine content of the separate Osborne fractions (Eggum, 1977a). Extraction experiments with normal and high lysine barleys have indicated that the solubility of the different proteins with the Osborne

Table 3. Amino acid nitrogen as a percentage of protein nitrogen (N x 6.25) (Folkes and Yemm, 1956).

	Albumin [Water soluble]	Globulin [Salt soluble]	Hordein [Alcohol soluble]	Glutelins [Alkaline soluble]
Amide	5.9	5.1	23.0	10.3
Aspartic Acid	8.0	5.6	1.2	4.3
Glutamic Acid	8.7	6.8	23.0	11.6
Proline	4.2	2.7	15.3	6.6
Glycine	6.7	10.7	1.7	5.2
Alanine	7.2	0.65	2.2	6.6
Valine	5.8	4.1	3.5	4.9
Leucine	5.7	4.5	4.6	5.8
Isoleucine	4.1	2.2	3.6	3.5
Phenylalanine	3.0	2.1	3.6	2.7
Tyrosine	2.7	1.5	1.6	1.9
Tryptophan	1.3	0.65	0.7	1.1
Serine	4.1	3.9	3.2	4.2
Threonine	3.4	2.4	1.9	3.1
Cystine+Cystein	1.5	2.6	1.5	0.9
Lysine	7.9	6.3	0.80	4.8
Methionine	1.4	0.9	0.75	1.1
Arginine	13.0	22.0	6.0	12.0
Histidine	4.3	3.1	0.22	4.3

technique is highly dependent on the ripeness of the seed and the character of the lys gene (Munk, 1977). It is believed that the lys gene which gives the high lysine content also changes the extractability of specific proteins (Munk, 1977). Eggum (1977a) has observed that fractionation of Bomi and mutant 1508 varied with locations of cultivation, so that the differences between the results of this work and that of other researchers could be due to differences in location.

Amino Acid Analysis

Introduction

Measurements of total amino acid content are very important in the study of the quality of any protein. It gives information on the total amino acids present, limiting amino acids and the proportions in which the amino acids occur. Amino acid balance is a good indicator of protein quality as in the measurement of amino acid score. The accuracy of amino acid measurements, however, depends on the efficiency of the hydrolysis procedure and the accuracy of the ion-exchange

chromatographic method. Hydrochloric acid is widely used for protein hydrolysis and it is known to give higher recoveries of most amino acids than any other reagent (Mason, Bech-Andersen and Rudemo, 1979). Due to the above fact, different reagents are used to hydrolyse proteins to analyse for different amino acids, thus we have acid hydrolysis or alkaline hydrolysis. To reduce losses of sulphur-containing amino acids in HCl hydrolysis, samples have to be oxidized with performic acid before being hydrolysed. Different oxidizing agents have been used and sometimes there may be reactions with some amino acids, thus reducing their recoveries. Certain constituents of feeds such as carbohydrates, lipids and nucleic acids are known to cause losses in certain amino acids during acid hydrolysis (Bech-Andersen, Rudemo and Mason, 1979). Thus the accuracy of the amino acid composition of a feed material depends on its constituents and method of hydrolysis.

Materials and Methods

Amino acid composition (except for cystine, methionine and tryptophan) of the five barley samples

was determined on hydrolysates prepared by the method of Kohler and Palter (1967). Cystine was determined as cysteic acid and methionine as methionine sulphone after oxidative hydrolysis with performic acid (Moore, 1963). The samples were analysed using an amino acid analyser (Durrum Model D500; Durrum, Palo Alto, California). Tryptophan was determined after alkaline hydrolysis (Hugli and Moore, 1972) using a Beckman-Spinco Analyser (Model 120B; Beckman Instruments Inc., Palo Alto, California).

Results

The amino acid composition as presented in Table 4 gives a general impression on the proteins in these five barleys. When expressed as g/100 g DM Hiproly had the highest level of most of the essential amino acids (EAA), except arginine and lysine where cultivar 2 had the highest levels and cystine where cultivar 3 had the highest level. For total lysine supply per unit of dry matter, cultivar 2 was the best. Cultivar 2 was mostly second to Hiproly in the levels of EAA contained on a DM basis, followed by cultivar 1

Table 4. Protein content and amino acid composition (total) of five barleys.

	HIPROLY		GALT		CULTIVAR 1		CULTIVAR 2		CULTIVAR 3	
	g/100g DM	g/16g N	g/100g DM	g/16g N	g/100g DM	g/16g N	g/100g DM	g/16g N	g/100g DM	g/16g N
Protein (N x 6.25)	18.57		15.16		17.45		18.10		16.81	
Alanine	0.67	3.61	0.50	3.30	0.68	3.90	0.65	3.59	0.56	3.30
Arginine	0.71	3.82	0.54	3.56	0.66	3.78	0.78	4.31	0.69	4.10
Aspartic Acid	1.04	5.60	0.74	4.88	1.01	5.79	0.92	5.08	0.81	4.82
Cystine	0.30	1.62	0.31	2.04	0.27	1.55	0.33	1.82	0.37	2.20
Glutamic Acid	3.88	20.89	3.26	21.50	3.67	21.03	3.67	20.28	3.80	22.61
Glycine	0.57	3.07	0.41	2.70	0.53	3.04	0.58	3.20	0.55	3.27
Histidine	0.37	1.48	0.30	1.98	0.34	1.95	0.34	1.88	0.32	1.90
Isoleucine	0.65	3.50	0.49	3.23	0.52	2.98	0.60	3.31	0.54	3.21
Leucine	1.26	6.79	1.00	6.60	1.20	6.88	1.17	6.46	1.09	6.48
Lysine	0.58	3.12	0.46	3.03	0.56	3.21	0.62	3.43	0.47	2.80
Methionine	0.30	1.62	0.20	1.32	0.27	1.55	0.27	1.49	0.22	1.31
Phenylalamine	0.99	5.33	0.73	6.53	0.91	5.21	0.89	4.92	0.77	4.58
Proline	1.79	9.64	1.45	9.56	1.62	9.28	1.62	8.95	1.71	10.17
Serine	0.65	3.50	0.50	3.30	0.61	3.50	0.69	3.81	0.60	3.57
Threonine	0.54	2.91	0.40	2.64	0.51	2.92	0.52	2.87	0.47	2.80
Tryptophan	0.50	2.69	0.40	2.64	0.45	2.58	0.45	2.49	0.45	2.68
Tyrosine	0.51	2.75	0.41	2.70	0.48	2.75	0.51	2.82	0.46	2.74
Valine	0.81	4.36	0.57	3.76	0.74	4.24	0.72	3.98	0.65	3.87
Total EAA	7.52	35.58	5.81	35.29	6.91	35.30	7.18	35.14	6.50	33.73

Cultivar 1 = H69019109000
Cultivar 2 = H69024038000
Cultivar 3 = H690240004191

Amino acid analysis was done by AAA Laboratories

which had the second lowest protein content of 16.81% on DM basis. Based on amino acid composition measured as g/100 g DM, the five barleys may be arranged in order of quality as: Hiproly, cultivar 2, cultivar 1, cultivar 3 and Galt in order of decreasing quality.

The high level of aspartic acid in Hiproly, cultivar 2 and cultivar 1 compared with Galt and cultivar 3 is worth noting. Pomeranz, Robbins, Wesenberg, Hockett and Gilbertson (1973) have shown that the high lysine content of Hiproly is associated with the high level of aspartic acid. It is known that aspartic acid is a key intermediate in the biosynthesis of lysine in bacteria, algae and higher plants (Pomeranz et al., 1973). The relationship between lysine and aspartic acid presumably holds for other high lysine varieties. The amino acid composition of notch-1 and notch-2 and their normal parent given by Balaravi et al. (1976) showed that the high lysine mutants have higher aspartic acid concentration than the normal parent.

In regular 2-row and 6-row barleys, the protein content determines the amino acid composition, i.e., the higher the level of protein, the higher the levels of the amino acids (Pomeranz et al., 1973). In Hiproly

the genes controlling protein content, lysine concentration in the protein and kernel development are not genetically linked, so that they segregate independently (Pomeranz et al., 1973; Munk, Karlsson, Hagberg and Eggum, 1970). If the lysine content (g/100 g DM) in Hiproly is given a value of 100 then:

Galt - 79.13
Cultivar 1 - 96.55
Cultivar 2 - 106.90
Cultivar 3 - 81.08

If the same is done for the other EAA, the values in Table 5 are obtained. From Table 5 with Hiproly as the standard, cultivar 2 is the next best source of EAA, followed by cultivar 1. It is clear from this table that cultivar 3 is not a very good source of EAA when compared with the other high lysine types. Galt being a normal commercial barley, is poor in the supply of EAA. For studies of protein quality, however, it is more meaningful to make comparisons in terms of g/16 g N. Table 6 shows such a comparison. In terms of g/100 g N, cultivar 2 was superior to Hiproly in the

Table 5. Amino acid content of four barleys (g/100 g DM) relative to amino acid composition of Hiproly.

Barley	Arg.	Lysine	Threo.	Hist.	Isoleu.	Leu.	Meth.	Tryp.	Tyro	Val
Hiproly	100	100	100	100	100	100	100	100	100	100
Galt	76.06	79.31	74.07	81.08	75.38	79.36	66.70	80.00	83.97	70.37
Cultivar 1	97.96	96.55	94.44	91.87	80.00	95.24	90.00	90.00	94.12	91.36
Cultivar 2	109.86	106.90	96.30	91.89	92.31	92.87	90.00	90.00	100.00	88.89
Cultivar 3	97.18	81.08	87.04	86.49	83.08	86.51	73.33	90.00	90.20	80.25

Table 6. Amino acid content of four barleys (g/16 g N) relative to amino acid composition of Hiproly.

Barley	Arg.	Lysine	Threo.	Hist.	Isoleu.	Leu.	Meth.	Tryp.	Typo.	Val.
Hiproly	100	100	100	100	100	100	100	100	100	100
Galt	93.19	97.12	90.72	133.78	92.29	97.20	81.48	98.14	98.18	86.24
Cultivar 1	98.95	102.88	100.34	131.76	85.14	101.33	95.68	95.91	100.00	97.25
Cultivar 2	112.83	109.94	98.63	127.03	94.57	95.14	91.98	92.56	102.55	91.28
Cultivar 3	107.33	89.74	96.22	128.38	91.71	95.43	80.86	99.63	99.64	88.76

supply of basic amino acids. Cultivar 1 was superior to Hiproly in the supply of lysine and histidine. Galt had the highest level of histidine among the five barleys. Cultivar 3 was superior to Hiproly but not cultivar 2 in the supply of arginine and histidine.

The essential amino acid levels of the barleys used in this study seem rather low. This is demonstrated by the fact that Hiproly had 3.12 g lysine/16 g N while Balaravi et al. (1976) obtained 4.08 g lysine/16 g N. Balaravi's amino acid composition of Hiproly is quoted here for comparison; all expressed as g/16 g N.

	Hiproly (Balaravi et al., 1976)	Hiproly (Imbeah, 1980)
Arginine	4.4	3.82
Histidine	2.1	1.48
Isoleucine	3.9	3.50
Leucine	7.0	6.79
Lysine	4.0	3.12
Methionine	2.0	1.62
Threonine	3.5	2.91
Tryptophan	1.2	2.69
Tyrosine	2.9	2.75
Valine	5.3	4.36

It appears that the lysine content and essential amino acid content of some normal barleys from the world collection (Pomeranz et al., 1976) are higher than that obtained for Hiproly in this study. Climate, soil and cultivation practices influence amino acid composition. Also the available nitrogen level in the soil is important. A high nitrogen level decreases lysine concentration (g/16 g N) as was observed with normal Bomi and mutant 1508 (Eggum, 1977a), by increasing the proportion of storage protein (hordein) in the total protein. Eggum (1977a) has reported variation in amino acid composition due to location. Balaravi's Hiproly and other high lysine barleys were grown in Denmark while the barleys for this study were grown in Alberta, Canada.

The same barley cultivars from the 1975 harvest of Alberta Agriculture program had the amino acid composition in Table 7. The amino acid composition of the same five barleys harvested in 1975 were higher than those obtained for the 1977 harvest. Since they were grown in the same geographical location, the difference in amino acid composition may be a result of climate. The year 1977 was wet compared with 1975. In a wet year, the rains bring about increased yield.

Table 7. Proximate analysis and total amino acid composition of five barleys from Alberta Agriculture program - 1975 harvest*.

	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3
Dry matter	92.2	91.4	92.0	92.0	91.80
Components (DM basis)					
Protein (N x 6.25)	19.5	14.4	18.4	18.6	17.4
Ether extract	2.03	1.46	1.67	1.80	1.56
Acid-detergent fibre	2.50	6.92	4.80	4.78	5.35
Ash	2.22	2.63	2.26	2.35	2.21
g/100 g DM					
Alanine	0.90	0.59	0.87	0.82	0.67
Arginine	0.88	0.65	0.93	0.90	0.77
Aspartic acid	1.31	0.83	1.30	1.08	0.96
Cystine	0.27	0.27	0.34	0.33	0.33
Glutamic acid	4.60	3.91	5.07	4.57	5.08
Glycine	0.74	0.53	0.74	0.69	0.64
Histidine	0.43	0.32	0.43	0.41	0.38
Isoleucine	0.74	0.55	0.78	0.71	0.67
Leucine	1.35	1.04	1.42	1.28	1.25
Lysine	0.79	0.47	0.78	0.72	0.35
Methionine	0.36	0.22	0.37	0.32	0.27
Phenylalanine	1.10	0.80	1.20	1.04	1.08
Proline	2.15	1.81	2.33	2.13	2.36
Serine	0.86	0.69	0.89	0.84	0.80
Threonine	0.70	0.52	0.72	0.64	0.60
Tryptophan	0.38	0.24	0.43	0.30	0.24
Tyrosine	0.60	0.46	0.66	0.59	0.60
Valine	0.97	0.74	1.05	0.95	0.88

Cultivar 1 = H69019109000, Cultivar 2 = H69024038000, Cultivar 3 = H690240004191

* Beames, unpublished.

Increased yields are associated with higher proportions of non-protein nitrogen (NPN). Since NPN does not contribute any amino acids it causes low amino acid composition (g/16g N). This could be the reason for the difference between the amino acid compositions of the 1977 and 1975 harvest.

Determination of Available Lysine by Dye-Binding Procedure

Introduction

Methods for measuring reactive or available lysine in food proteins using FDNB or TNBS (trinitro-benzene sulphonic acid) followed by acid hydrolysis of the protein are relatively complicated (Hurrel and Carpenter, 1976). Measurements of DBC are simpler and faster. It has been found by Hurrel and Carpenter (1975) that for many food materials the DBC with acid orange 12 was equivalent to the sum of total histidine, arginine and reactive lysine. The difference in DBC before and after binding the reactive lysine has been used as a measure of available lysine specifically (Hurrel and Carpenter, 1976). Masking or binding of reactive lysine may be achieved by reacting with propionic

anhydride, ethylchloroformate or TNBS (Hurrel and Carpenter, 1976). Sandler and Warren (1974) used ethyl-chloroformate for binding the reactive lysine when they measured available lysine in fishmeals using the dye binding procedure. Hurrel, Lerman and Carpenter (1979) used the dye-binding method to measure available lysine in a number of feedstuffs including barley.

Materials and Methods

Five barleys from the 1977 harvest of Alberta Agriculture breeding program were used. Dye reagent was obtained from the Udy Analyzer Co., Boulder, Colorado. It contained 3.89 mmol/litre of acid orange 12 (MW 350.3), 200 g oxalic acid dihydrate, 34 g potassium dihydrogen phosphate and 600 ml glacial acetic acid with water added to make 10 litres. Propionic anhydride, sodium acetate, glass beads and Watman glassifbre filter paper (GF/A) were obtained from Fisher Chemical Company. Polyethylene bottles were provided by the Udy Company with the dye reagent.

For dye binding determination of available lysine there is a need to make two measurements, Dye

Binding Capacity (DBC) and Dye Binding After Propionylation (DBAP).

DBC

1.5 g barley, hammer-milled through a 0.5 mm screen, weighed in duplicates into polyethylene bottles and ten glass beads (3 mm) were added to each. Four millilitres of 2.2 M sodium acetate were added and mixed well (Hurrel et al., 1979 suggested that when the sample is more than 0.8 g 4 ml of 2.2 M sodium acetate should be used). Forty millilitres of dye reagent were added and the contents of the bottle were shaken on a mechanical shaker overnight at room temperature. The reaction mixture was filtered through Watman GF/A filters. An aliquot of the filtrate was diluted 50 fold (Carpenter, personal communication) and the absorbance measured at 475 nm. A reagent blank containing 40 ml dye reagent and 4 ml sodium acetate and no sample was diluted to bring it in the same absorbance range as the sample filtrate. This was used as the concentration of dye before reacting with the sample. Gilford Spectrophotometer Model Stasar II was used for all the measurements.

A standard curve was obtained by diluting the reagent dye to different concentrations and the absorbance measured. A regression of absorbance (Y) and concentration (X) [mmol/l] was obtained from the standard curve and this regression equation was used to predict the concentration of dye in the reaction filtrates from their absorbance. The equation being Y = 20.99 X; r = 0.99.

DBAP

Four millilitres of 2.2 sodium acetate solution and 0.4 ml propionic anhydride were added to 1.5 g ground samples and left to stand overnight. Forty millilitres of dye reagent were added and then the procedure was as for DBC. After measuring the dye concentrations, the total dye content left after the reaction was calculated by multiplying the corrected concentration by the volume of reaction mixture. DBC and DBAP were calculated as the difference between reagent blanks and sample filtrates. Available lysine was calculated as the dye binding lysine (DBL) which is the difference between DBC and DBAP in mmol/16 g N. These values were then expressed as percentages of total lysine in the various barleys.

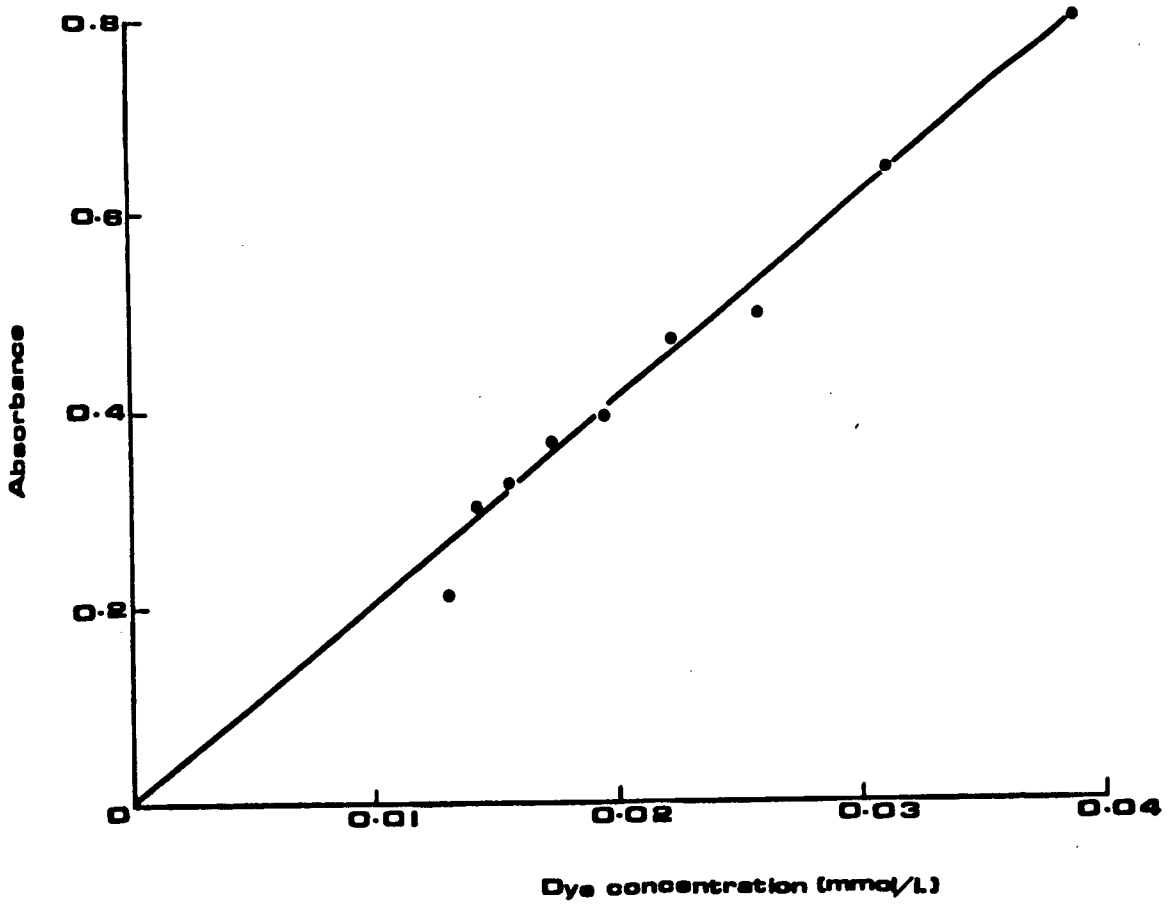


Fig. 1 Standard curve for dye concentration and absorbance

Results

Table 8. Standard curve for dye concentration.

Dilution	Concentration X	Absorbance Y
1/100	0.0389	0.799
1/125	0.0311	0.646
1/150	0.0259	0.496
1/175	0.0222	0.471
1/200	0.0195	0.396
1/225	0.0173	0.366
1/250	0.0153	0.323
1/275	0.0141	0.304
1/300	0.0130	0.210

Discussion

Dye binding measurements on the barleys are shown in Tables 9 and 10. It is believed that dye binding capacity is positively correlated with total basic amino acid content and with total lysine content

Table 9. Analysis of barley grain samples for dye binding determination of available lysine.

Barley	Hiproly	Galt	H69019109000	H69024038000	H690240004191
DBC (mmol/16 g N)	61.76	67.10	60.33	68.11	60.50
DBAP (mmol/16 g N)	43.89	50.95	47.10	50.97	47.57
DBD (DBC-DBAP)	17.87	16.15	13.28	17.14	12.93
available lysine ($\frac{\text{mmol}}{16\ \text{g N}}$)					
Total lysine from ion exchange ($\frac{\text{mmol}}{16\ \text{g N}}$)	21.33	20.71	21.94	23.44	19.14
% availability of lysine DBD/total lysine	83.78	77.98	60.53	73.12	67.55

Table 10. Protein content (g/100 DM), lysine and basic amino acid content (g/16 g N) and dye binding capacity (mmol/16 g N) of five barley samples.

Barley	Hiproly	Galt	H69019109000	H69024038000	H690240004191
Protein (N x 6.25)	18.57	15.16	17.45	18.10	16.81
Lysine (g/16 g N) from ion exchange	3.12	3.03	3.21	3.43	2.80
Total basic amino acids (g/16 g N) from ion exchange	8.42	8.57	8.94	8.64	8.80
DBC (mmol/16 g N)	61.71	67.10	60.38	68.11	60.50

(g/16 g N) (Munk, 1972; Tallberg, 1979; Walker, 1979). This fact is reported throughout the literature. In the present study, the dye binding capacities (DBC) were rather low and the correlation between DBC and total basic amino acids is too low (r = 0.502). Hurrel et al. (1979) have measured DBC in barleys and used this method to estimate lysine availabilities. DBC of 93.0, 88.5, 97.5 and 101.1 have been obtained for mutant 1508, barley hybrids 2188, 2187 and 2186 respectively (Hurrel et al., 1979). Lysine availabilities observed were 91.19%, 102.84%, 94.99% and 102.87% for mutant 1508, hybrids 2188, 2187 and 2186 respectively. With availabilities of more than 100%, one ought to be fairly cautious about the dye binding method for measuring available lysine. Biological measurements of available lysine elsewhere in this thesis have given values of 70.78%, 55.98%, 52.64%, 67.12% and 57.96% for Hiproly, Galt, cultivars 1, 2 and 3 respectively.

In this study, Hiproly gave the highest availability of 83.78% by dye binding difference. However, Hiproly gave only 70.78% by biological determination (i.e., faecal analysis). Galt which had 55.98% biological availability of lysine by faecal analysis

gave 77.98% availability by dye binding difference. Cultivar 1 had the lowest lysine availability by faecal analysis (52.64%) and also by dye binding difference (60.53). If one were to judge by lysine availability measured by dye binding difference in this study, Galt would be considered superior in protein quality to the three high lysine cultivars. With the low correlation coefficient obtained between dye binding capacity and total basic amino acids one ought to be critical about making conclusions about protein quality based on dye binding difference.

In the dye binding procedure described by Hurrel et al. (1979) and Walker (1979) they call for the use of polyethylene bottles for carrying out the reaction. This material absorbed the dye and could be a source of error in this procedure. However, Hurrel et al. (1979) and Walker (1979) made no reference to the possibility of the bottles absorbing the dye. Udy Analyszer Co. supplies these polyethylene bottles with the dye. I would recommend that corrections be made for the dye absorbed by the bottles, alternatively glass bottles should be used. In papers describing dye binding capacities (Hurrel et al. (1979) and

Walker (1979) had correction factors for machine drift. In the present study, no machine drift was observed.

BIOLOGICAL EVALUATION OF PROTEIN QUALITY

Rat Trial I

Rat Growth Assay and the Measurement of True Nitrogen Digestibility, True Biological Value and Net Protein Utilization

Introduction

The use of laboratory animals for nutritional studies is a well established practice. Change in body weight is a good estimate of changes in body protein in response to the test protein, under well controlled conditions such as restricted feeding, constant environmental conditions and young growing animals. Parameters that are commonly used in assessing protein quality include

True digestibility of nitrogen (TD)

Net protein utilization (NPU)

Biological value	(BV)
Protein efficiency ratio	(PER)
Relative protein value	(RPV)

(Munk, 1972; Eggum, 1973; P.A.G. Guidelines - FAO No. 16) Measurements of BV, PER and NPU are usually done with approximately 10% crude protein in the diet, fed to male rats. (Eggum, 1973; Chalupa and Fisher, 1963 and Hegsted and Chang, 1965). It is believed that sex affects the values of these parameters. BV and NPU are sometimes determined with restricted feeding.

Materials and Methods

Design of Experiment

A completely randomized design was used. There were six dietary treatments with 4 rats per treatment per period and two periods. Rats were housed individually in cages at a room temperature of 25°C.

Animals and Cages

Male rats (Woodlyn/Wistar Strain, Woodlyn Laboratories, Guelph, Ontario) 28 days of age at the beginning of the 14-day feeding period were used. The stainless steel cages had wire screen floors and were

fitted with trays for collecting faeces and urine. Trays were lined with paper towels which were changed daily. During the collection period, funnels with 0.5 mm wire mesh lining were fitted under the cages instead of the trays. The wire mesh separated the faeces from the urine.

Diets

Diets (Table 11) were based on barley grain ground through 0.5 mm screen on a laboratory hammer mill (Christy and Norris Ltd; Chelmsford, England). There were five test diets, one for each barley type, made to provide approximately 10% crude protein (DM basis). The sixth diet was a 4% egg protein purified diet. This was used to estimate metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) as suggested by Eggum (1973) and expressed as follows

EUN = mean N mg/rat/collection period

MFN = N mg/DM intake (g)

Table 11. Diet Composition (% DM basis).

Ingredients	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3	Egg Protein Concentrate
Barley	53.85	65.96	57.31	55.25	59.49	-
**Corn starch	39.15	27.04	35.69	37.75	33.51	88.63
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
*Mineral mix	4.00	4.00	4.00	4.00	4.00	4.00
*Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00
+Egg protein conc.	-	-	-	-	-	4.87
Dry matter	94.20	93.83	94.27	93.91	94.03	97.38
Crude protein	10.01	10.08	10.01	10.27	10.36	4.26
Cellulose	-	-	-	-	-	4.50
GE(cal/g)	4318	4342.32	4313.81	4321.57	4288.91	ND

+ Egg protein concentrate was obtained from United States Biochemical Corporation. It is fat free and 96% of it cholesterol has been removed.

* Compositions are shown in Tables 12 and 13.

** Corn starch was moistened and autoclaved for 3 hours, dried overnight in an oven at 95°C and ground through an 0.5 mm screen.

ND = not determined.

Table 12. Vitamin mix composition (g) (Eggum, 1973)

Vitamin A 325000 IU	0.3125
Vitamin D_3 200000 IU	0.0375
Thiamine HCl	0.0225
Nicotinamide	0.2000
Riboflavin	0.0500
Calcium pantothenate	0.1087
Pyridoxal HCl	0.0060
α-tocopherol phosphoric acid disodium salt	0.1285
Potato starch	to make up to 500 g.

Table 13. Mineral mix composition (g/kg) (Eggum, 1973)

Calcium carbonate ($CaCO_3$)	68.6
Calcium Citrate ($CaC_{12}H_{10}O_{14} \cdot 4H_2O$)	308.3
Calcium hydrogen phosphate ($CaHPO_4 \cdot 2H_2O$)	112.8
Dipotassium hydrogen phosphate (K_2HPO_4)	218.8
Potassium chloride (KCl)	124.7
Sodium chloride (NaCl)	77.1
Magnesium sulphate ($MgSO_4$)	38.3
Magnesium carbonate ($MgCO_3$)	35.3
Manganese sulphate ($MnSO_4 \cdot H_2O$)	0.201
Ammonium ferric citrate (Brown)	15.3
Copper sulphate ($CuSO_4 \cdot 5H_2O$)	0.078
Potassium Iodide (KI)	0.041
Sodium fluoride (NaF)	0.507
Aluminium ammonium sulphate ($AlNH_4(SO_4)_2 \cdot 12H_2O$)	0.090

Feeding Procedure

Food and water were provided ad libitum. Water was renewed every other day. Body weight and food consumption were recorded daily. Faeces and urine were separately collected in 5% H_2SO_4 during the last four days of the trial. Each day's collection was transferred into a 24 oz mason jar and stored at 0°C until ready for analysis. This procedure has been used by Eggum (1973).

Analytical Methods

Proximate analysis was done on each barley sample. After the diets were mixed, they were analyzed for crude protein by the Kjeldahl procedure (AOAC, 1970). Faeces and urine were thawed. Faeces samples were dissolved, each in concentrated sulphuric acid with 25 ml at a time. It was done four times; after each addition the mixture was stirred well and allowed to cool before further addition of acid. After the 4th time, the resultant solution was homogeneous. It was then transferred into a 500 ml volumetric flask and made up to volume with distilled water. 25 ml was used for each duplicate nitrogen determination.

The urine was transferred into 250 ml volumetric flasks. Collecting bottles and funnels were washed three times with distilled water into the flasks, and more distilled water was added to bring to volume. A volume of 25 ml was used for duplicate nitrogen determination for all samples. Energy content of the diets were determined using a Gallenkamp adiabatic bomb calorimeter (Model CB110). Faeces were also analyzed for energy for use in calculating digestible energy (DE).

Results and Discussion

Table 14 summarizes the feed intake and growth data. Those rats on the Hiproly diet gained significantly better than those on Galt and cultivar 3. Hiproly, cultivar 1 and cultivar 2 were not significantly different from each other in terms of weight gain of rats. However, neither were cultivars 1 and 2 significantly different from Galt and cultivar 3. Feed efficiencies also showed significant differences. Hiproly and cultivar 2 gave significantly higher feed efficiencies than Galt, cultivar 1 and cultivar 3, which were not significantly different from each other. Hiproly and cultivar 2 were not significantly different from each other.

Table 14. Average daily body weight gain, food consumption, growth rate and feed efficiency per rats.

Diets	1 Hiproly	2 Galt	3 Cultivar 1	4 Cultivar 2	5 Cultivar 3	SE	F-test	Newman-Keul's
Initial body weigh (g)	85.37	83.00	84.25	88.62	88.75	-	-	-
Food consumption (g/14 days)	211.00	193.00	189.00	210.20	199.50	9.90	NS	3,2,5,4,1
DM intake (g/14 days)	198.20	179.80	176.70	196.10	186.40	9.55	NS	3,2,5,4,1
Total gain (g)	37.81	25.56	31.13	33.63	27.06	4.96	*	2,5,3,4,1
Growth rate (g/day)	2.60	1.80	2.20	2.30	1.80	0.34	*	2,5,3,4,1
Feed efficiency (g gain/g DM)	0.20	0.14	0.16	0.19	0.15	0.026	*	2,5,3,4,1
DE Kcal/kg	3809	3628	3565	3684	3708	-	-	-

Cultivar 1 = H69019109000, Cultivar 2 = H69024038000, Cultivar 3 = H690240004191

In the nitrogen balance part of this trial, there were no significant differences between the diets for nitrogen intake, true nitrogen digestibility, biological value and net protein utilization (Table 15). Balaravi et al. (1976) observed true nitrogen digestibility (TD) of 78.9% for notch-1 and 83.6% for notch-2. Their normal parent had a TD of 86.6% which was higher than that of either notch-1 or notch-2. They obtained BV of 75.8%, 86.4% and 87.6% for the normal parent, notch-1 and notch-2 respectively. The same authors observed NPU values of 68.2 and 73.3 for notch-1 and notch-2. The findings of the present study agree fairly well with the results of Balaravi et al. (1976). Generally, normal barley has a biological value of 72.7% (Munk, 1972) and this is not changed significantly with amino acid supplements (it goes up to 73.2%). According to Munk (1972) true digestibility of nitrogen in barley grain increases as the crude protein content (g/100 g DM) increases. However, the lysine percentage and BV decrease simultaneously as the protein content of the grain increases. This is because there is an increased contribution by prolamins to the total crude protein and prolamins are poor quality proteins with low

Table 15. Nitrogen balance test (4 days collection period).

Barley	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3	S.E.	F-Test
N intake (g)	1.00	0.90	0.93	1.05	0.95	0.056	NS
True digestibility of nitrogen	0.88	0.85	0.86	0.86	0.86	0.010	NS
True BV	0.83	0.86	0.84	0.85	0.84	0.011	NS
NPU	73.09	73.00	72.09	74.19	72.62	0.77	NS

lysine content and a generally poor amino acid pattern. It is surprising then that Hiproly and cultivar 2 which are both high in lysine should tend towards reduced BV, (the differences were not significant). Munk (1972) observed that at the same level of protein intake, Hiproly had a higher TD and BV than normal high protein barley. In this present study, although the diets contained approximately equal levels of protein, total protein intake by rats were different because they were fed ad libitum and so feed intake and hence protein intake varied. The TD and BV values obtained were higher than those obtained by Eggum (1977a) who observed TD of 85.2% and BV of 76.0% for Hiproly, compared to values of 88% and 83% obtained in this study. However, Eggum (1977a) used restricted feeding, allowing 10 g DM and 150 mg N per rat per day and this could have made the difference.

NPU of about 70% has been observed for high lysine lines while normal barleys had about 60% NPU (Eggum, 1977a). It has been reported by Hegsted and Chang (1965) that BV values tend to overestimate the nutritive value of poor quality proteins. In this study the BV of Galt was the highest. One would

expect the high lysine cultivars to have higher biological values. However, the differences were not significant.

Rat Trial II

Relative Protein Value as a Measure of Protein Quality

Introduction

The use of Relative Protein Value (RPV) as a measure of protein quality assumes that the body weight response is linear over the range of test protein intakes studied (PAG, 1975; Eggum and Campbell, 1979). The accuracy, however, is dependent on the number of animals used, the variation around the regression lines and the range of protein intakes included in the test (PAG, 1975).

Materials and Methods

Design of Experiment

A completely randomized design was used with 4 rats per treatment per period. There were six diets

with three levels of protein per diet, thus giving 18 different treatments. The experiment was done in two different periods of 14 days each.

Animals and Cages

Male rats (Woodlyn/Wistar strain, Woodlyn Laboratories, Guelph, Ontario) 23 days of age at the beginning of the experiment were used. Cages were the same as used for trial I. The room temperature was 25°C on the average during the trial period.

Diets

There were six different diets, each with three levels of protein. High nitrogen casein (Sheffield Chemical Co., Norwich, N.Y. 13815) was used as the reference protein. The other protein sources were the five barleys being evaluated for protein quality. Each protein source was fed at three levels, viz. 2, 5 and 8 g crude protein per 100 g DM. The composition of the diets is shown in Table 16.

Food and water were provided ad libitum. Water was renewed every other day and the bottom tray

Table 16. Composition of RPV diets - % DM basis

Ingredients	1 Hiproly	2 Galt	3 H69019109000	4 H69024038000	5 H690240004191	6 Casein
2% Crude Protein						
Barley	10.77	13.19	11.46	11.05	11.20	-
Corn starch	82.23	79.81	81.64	81.95	81.10	85.90
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0
Casein	-	-	-	-	-	2.10
Cellulose powder	-	-	-	-	-	5.00
Dry matter	95.68	96.41	97.18	96.55	95.64	96.32
Crude protein	2.18	2.14	2.08	2.06	2.16	2.07
5% Crude Protein						
Barley	26.93	32.98	28.65	27.62	29.74	-
Corn starch	66.07	60.02	64.35	65.38	63.26	82.80
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0
Casein	-	-	-	-	-	5.26
Cellulose powder	-	-	-	-	-	5.00
Dry matter	94.10	95.63	95.52	95.86	95.89	96.06
Crude protein	4.84	4.47	4.67	4.85	4.33	5.15
8% Crude Protein						
Barley	43.08	52.77	45.85	44.20	47.59	-
Corn starch	49.92	40.23	47.15	48.80	45.41	79.68
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0
Casein	-	-	-	-	-	8.40
Cellulose powder	-	-	-	-	-	5.00
Dry matter	94.50	95.00	94.88	95.43	95.53	96.25
Crude protein	7.58	7.32	7.98	7.97	6.69	8.05

was cleaned daily. The mineral and vitamin supplements used in this experiment had the same compositions as those used in trial 1. The body weights and food consumption were measured daily.

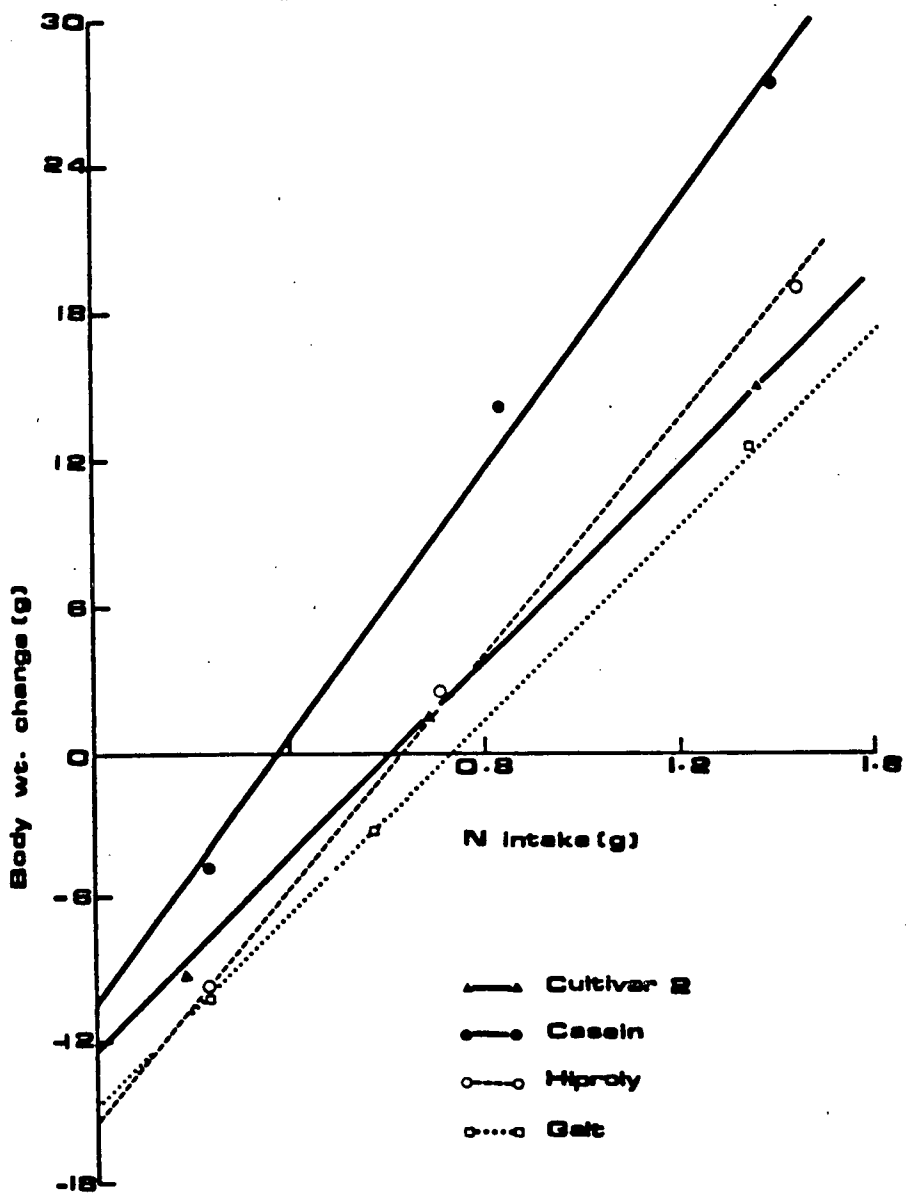


Fig. 2 Relative protein value determination

Table 17. Results of a 14-day RPV assay.

Dietary Regime	Protein Content g/100 g DM	Average Feed Intake (g)	Average DM Intake (g)	Average Nitrogen Intake (g)	Initial Body Weight (g)	Average Body Weight Change, g/14 days
Casein	2.07	72.50	69.83	0.23	47.75	-4.875
	5.15	104.50	100.38	0.83	46.00	14.127
	8.05	112.19	107.98	1.37	40.90	27.437
Hiproly	2.18	69.50	66.50	0.235	55.0	-9.75
	4.84	97.25	91.52	0.71	53.4	2.50
	7.58	125.50	118.66	1.44	51.0	19.003
Galt	2.14	68.75	66.23	0.23	53.25	-10.19
	4.47	83.00	79.37	0.57	52.94	-3.325
	7.32	119.875	113.88	1.335	48.63	14.50
Cutlivar 1	2.08	61.375	51.68	0.20	50.63	-9.57
	4.67	89.19	85.20	0.635	54.63	-1.415
	7.46	122.065	115.88	1.385	47.63	12.625
Cultivar 2	2.06	57.90	55.90	0.185	51.50	-9.312
	4.85	92.25	88.43	0.69	46.63	1.362
	7.97	116.07	110.76	1.36	47.50	14.94
Cultivar 3	2.16	58.56	56.01	0.19	51.44	-10.437
	4.33	83.44	80.01	0.555	46.00	1.937
	6.69	102.13	97.56	0.045	44.50	9.625

Discussion

From Table 18, Hiproly appears to have the highest RPV among the barleys, indicating a superiority in quality. Hiproly in this study had 87% of the quality of casein. Cultivar 3, according to this test, was next to Hiproly in quality followed by cultivar 1. This test showed cultivar 2 to be of the lowest quality.

There does not seem to be much literature on the use of RPV as a measure of protein quality. Most researchers doing biological evaluations use TD, BV and NPU as measures of protein quality. Eggum and Campbell (1979) have reported a number of points to be considered before accepting RPV as a standard method for protein evaluation. It is believed that the two low protein levels (2 and 5%) cause low and variable food intake, leading to small and varying weight gains, especially when the protein is of poor quality (Eggum and Campbell, 1979). This low and variable consumption was observed in this present study. It is believed that lysine deficient proteins may not yield a valid slope ratio (Eggum and Campbell, 1979).

Table 18. Estimates of RPV of five barleys

Protein Source	Intercept	Slope	RPV
Casein	-10.61	27.85	1.00
Hiproly	-15.53	24.24	0.87
Galt	-14.33	21.52	0.77
Cultivar 1	-14.96	23.19	0.83
Cultivar 2	-12.34	19.84	0.71
Cultivar 3	-13.73	23.36	0.84

Rat Trial III

Effect of Supplementing Barley with Soybean Meal (SBM) or Essential Amino Acids (EAA) on the Growth of Weanling Rats

Introduction

Cereals play an important role as a protein source in the diet of non-ruminant animals. It is known that cereal proteins are deficient in certain essential amino acids, especially lysine. In cereal-based diets such as barley-based diets for pigs, the cereal portion supplies about 60% of the amino acid requirements (Thomke, 1974). In order to meet all the amino acid requirements, such diets need to be supplemented with other sources of protein. Protein concentrates such as fish meal, meat meal and soybean meal can be used to improve protein status and the amino acid supply. Purified amino acids have been used as supplements for cereal based diets. Such supplements have been known to cause improved performance in animals. In the use of protein concentrates, it is believed that their amino acids complement those of the cereal protein.

Materials and Methods

Design of Experiment

The experiment was a 5 x 4 factorial with a completely randomized design. The two factors being studied were barley types and supplements. The barleys were Hiproly, Galt, cultivar 1, cultivar 2 and cultivar 3 and the supplements were none, soybean meal, lysine and lysine plus other essential amino acids. There were 20 different diets (treatments) with four rats per treatment per period and two periods. The rats were housed individually in cages at a room temperature of 25°C.

Animal and Cages

Male rats (Woodlyn/Wistar Strain, Woodlyn Laboratories, Guelph, Ontario) 28 days of age at the start of the experiment were used. The cages were the same as those used in trials I and II.

Diets

The diets were formulated to provide approximately 12% CP (DM basis), the NRC net protein requirement

for growing rats (NRC, 1972). This was achieved by diluting the barleys with starch. In the barley-SBM diet, SBM was added to supply 50% of the dietary protein and to maintain the protein level at approximately 12%. Lysine and the other essential amino acids were added to raise their levels in the diets to NRC (1972) recommended levels for growing rats, since the basal diets supplied these amino acids at levels lower than those required by growing rats. The compositions of the diets are shown in Table 19 and the calculated amino acid compositions of the basal diets are shown in Table 20.

Results and Discussion

The result of trial III can be seen in Table 22. The effect of supplementation can be seen clearly in this table. After starch dilution of each barley to produce a standard protein (N x 6.25) content of 12%, supplementation with lysine alone had no beneficial effect on rat performance. This indicates that lysine is not the only limiting amino acid in barley protein and thus could not correct the imbalance. The fact that addition of other EAA made a significant improvement

Table 19. Composition of basal and supplemented diets for trial III (DM basis to make 1 kg).

	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3
Basal Diet					
Barley	646.3	791.6	687.7	663.0	713.9
Corn starch	283.7	138.4	242.3	267.0	216.1
Corn oil	20.0	20.0	20.0	20.0	20.0
*Mineral mix	40.0	40.0	40.0	40.0	40.0
*Vitamin mix	10.0	10.0	10.0	10.0	10.0
Basal + Soybean meal					
Barley	323.15	395.8	343.85	331.5	356.95
Corn starch	486.85	414.2	466.15	478.5	453.05
Corn oil	20.0	20.0	20.0	20.0	20.0
*Mineral mix	40.0	40.0	40.0	40.0	40.0
*Vitamin mix	10.0	10.0	10.0	10.0	10.0
** Soybean meal	120.0	120.0	120.0	120.0	120.0
Basal + L-Lysine					
Barley	646.3	791.6	687.7	663.0	713.9
Corn starch	283.7	138.4	242.3	267.0	216.1
Corn oil	20.0	20.0	20.0	20.0	20.0
*Mineral mix	40.0	40.0	40.0	40.0	40.0
*Vitamin mix	10.0	10.0	10.0	10.0	10.0
L-Lysine	6.3	6.4	6.1	5.9	6.6
Basal + EAA					
Barley	646.3	791.6	687.7	663.0	713.9
Corn starch	283.7	138.4	242.3	267.0	216.1
Corn oil	20.0	20.0	20.0	20.0	20.0
*Mineral mix	40.0	40.0	40.0	40.0	40.0
*Vitamin mix	10.0	10.0	10.0	10.0	10.0
Arginine	1.8	2.4	2.2	1.5	1.8
Histidine	0.9	0.9	1.0	1.0	1.0
Isoleucine	1.9	2.2	2.5	2.1	2.2
Leucine	0.2	0.4	0.0	0.5	0.5
Methionine + cystine	2.8	2.7	3.0	2.7	2.5
Lysine	6.3	6.4	6.1	5.9	6.6
Threonine	2.1	2.4	2.5	2.6	2.4
Valine	1.5	2.2	1.6	1.9	2.1

* mineral and vitamin mix compositions are shown in Tables 7 and 8.
** soybean meal added to provide 50% of the protein.

Table 20. Barley content and calculated EAA content of basal diets (% DM basis).

Component	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3
Barley	64.63	79.16	68.77	66.30	71.39
Arginine	0.49	0.43	0.45	0.52	0.49
Histidine	0.24	0.24	0.23	0.23	0.23
Isoleucine	0.42	0.39	0.36	0.40	0.39
Leucine	0.81	0.79	0.83	0.78	0.78
Lysine	0.37	0.36	0.39	0.41	0.34
Methionine + Cystine	0.39	0.40	0.37	0.40	0.42
Pheylalanine + Tyrosine	0.97	0.90	0.96	0.93	0.89
Threonine	0.35	0.32	0.35	0.34	0.34
Tryptophan	0.32	0.32	0.31	0.30	0.32
Valine	0.52	0.45	0.51	0.48	0.46

Table 21. DM and crude protein analysis of diets

	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3
Basal					
Dry matter %	93.45	94.71	93.31	93.79	93.22
Protein (N x 6.25) (DM basis)	12.06	12.22	12.03	11.92	12.03
GE (Kcal/kg)	4328	4323	4314	4326	4298
Basal + SBM					
Dry matter %	94.05	94.92	93.87	93.98	93.61
Protein (N x 6.25) (DM basis)	12.56	12.73	12.55	12.43	12.63
Basal + Lysine					
Dry matter %	93.90	94.85	93.76	93.75	93.93
Protein (N x 6.25) (DM basis)	12.34	12.43	12.28	12.27	12.20
Basal + EAA					
Dry Matter %	93.98	94.80	93.56	94.03	93.80
Protein (N x 6.25) (DM basis)	12.75	12.80	12.29	12.46	12.91

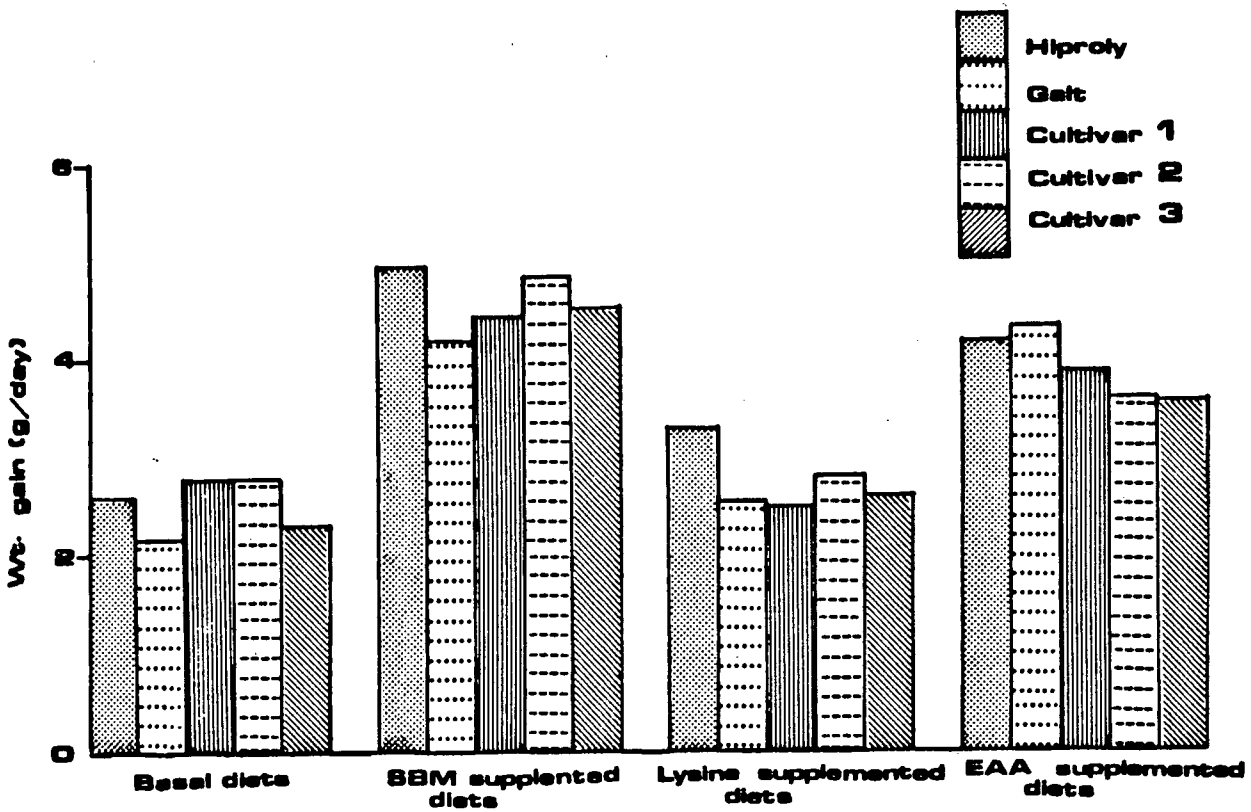


Fig. 3 Rat growth rates on basal and supplemented barley diets

Results

Table 22 Feed consumption and growth data, Trial III

Parameter	Supplement	Barley					
		Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3	Mean
Initial body weight (g)	None	80.50	84.38	81.13	82.00	83.13	82.23
	SBM	80.88	84.00	84.25	84.38	83.75	83.45
	Lysine	77.75	82.50	79.88	83.88	81.75	81.15
	Lysine+EAA	82.25	82.25	80.38	82.75	81.88	81.90
	Mean	80.35	83.28	81.41	83.25	82.63	
Food intake (g)	None	180.10	188.50	189.00	191.90	187.00	187.3^{b}
	SBM	213.50	214.60	224.40	225.50	210.90	217.8^{a}
	Lysine	200.14	186.13	178.63	200.63	183.13	189.5^{b}
	Lysine+EAA	202.00	220.38	197.00	195.13	201.25	203.1^{a}
	Mean	199.8^{a}	202.4^{a}	197.3^{a}	203.3^{a}	195.6^{a}	
DM intake (g)	None	168.50	178.50	176.80	180.00	174.30	175.6^{b}
	SBM	200.60	198.90	210.60	211.90	197.40	204.9^{a}
	Lysine	194.66	176.54	167.48	188.09	170.95	180.4^{b}
	Lysine+EAA	189.83	208.92	184.31	183.48	188.93	191.1^{a}
	Mean	189.4^{a}	191.9^{a}	184.8^{a}	190.9^{a}	182.9^{a}	
Total gain (g)	None	36.62	30.62	39.12	39.13	31.75	35.45^{b}
	SBM	69.37	58.62	62.12	68.00	63.25	64.27^{a}
	Lysine	46.43	35.50	35.38	39.38	36.25	38.38^{b}
	Lysine+EAA	58.38	60.75	54.13	57.38	56.75	57.47^{a}
	Mean	52.90^{a}	46.37^{b}	47.69^{b}	50.97^{ab}	47.00^{b}	
Growth rate (g/day)	None	2.62	2.18	2.80	2.80	2.33	2.544^{b}
	SBM	4.96	4.19	4.44	4.86	4.52	4.591^{a}
	Lysine	3.32	2.53	2.50	2.81	2.59	2.736^{b}
	Lysine+EAA	4.17	4.33	3.87	4.10	4.05	4.103^{a}
	Mean	3.779^{a}	3.309^{b}	3.400^{b}	3.640^{ab}	3.372^{b}	
Food Efficiency (g gain/g DM in take)	None	0.22	0.17	0.22	0.22	0.18	0.201^{b}
	SBM	0.35	0.29	0.30	0.32	0.32	0.313^{a}
	Lysine	0.25	0.20	0.21	0.21	0.21	0.214^{b}
	Lysine+EAA	0.31	0.29	0.28	0.31	0.30	0.298^{a}
	Mean	0.282^{a}	0.237^{c}	0.250^{bc}	0.264^{ab}	0.252^{bc}	

goes to support the fact that other amino acids could be limiting in these barley proteins. After diluting each barley to a standard protein level of 12%, SBM was just as good as EAA in improving rat performance.

On the whole, improvements with SBM were higher than with EAA but the differences between the two supplements were not significant. Hiproly was significantly superior in its response to supplementation, to the other barleys but not cultivar 2. All the other barleys including cultivar 2, were not significantly different in their response to supplementation in terms of total gain and growth rate. In terms of feed efficiency, Hiproly and cultivar 2 were not significantly different in their response to supplementation but Hiproly was significantly better than Galt, cultivars 1 and 3 which were not significantly different from each other in their response to supplementation.

Generally lysine supplementation of grain has been known to improve performance in animals and the addition of other essential amino acids especially threonine has proved beneficial (Pond et al., 1958).

Chung and Beames (1974) observed that supplementation of barley with lysine to provide 0.75 g/100 g DM improved the performance of growing pigs, and observed further improvement with the addition of threonine. This indicates that threonine could be the second limiting amino acid in barley grain. Chung and Beames (1974) have cited a number of papers reporting improvements in growth response with grain diet supplemented with lysine alone and also with other EAA. Bayley and Summers (1968) observed that supplementation of corn-SBM ration with 0.1% lysine and 0.05% methionine for growing pigs had no beneficial effect of growth rate on feed efficiency. However, when both were given together, there was a positive effect on gain. They also observed that response to lysine and methionine supplements depends on the addition of threonine, indicating that this amino acid is also deficient in low protein corn-SBM diets. In this trial, the responses to supplementation might have been greater without the starch dilution. In diluting the protein down to the recommended level of 12%, the amino acids are also diluted. For studies on protein quality this dilution is necessary. However, if barley had been used as a complete diet there would be no

dilution with starch. Beames (unpublished) fed the same types of barley (but from the 1975 harvest) to rats as a complete diet. He observed much greater responses with supplementation.

Determination of Apparent Availability of Lysine in Barley Using the Faecal Analysis Method

Introduction

Availability of amino acids may be defined as the proportion of the particular amino acids that are accessible to the organism. This assumes that all amino acids that are absorbed from the gastro-intestinal tract are available to the animal. Throughout the literature one encounters a term like digestible amino acids which I consider as a misnomer. This is because amino acids are not digested; they are the smallest units of the protein molecule. They are absorbed as such and used as such. Amino acids may be deaminated but after that they are of no use to the animal for protein synthesis. I believe that when the protein is digested, the amino acids do not change form before absorption. Therefore amino acids are absorbed after protein digestion without any further break down. Availability is therefore a better word to use with amino acids than digestibility.

Availability of amino acids may be determined chemically, biologically, enzymatically or microbiologically. In biological determination, laboratory

animals such as rats and mice have been used or sometimes farm animals such as pigs are used (Batterham et al., 1978). In biological evaluation one can measure true or apparent availability of amino acids using ileal or faecal analysis methods. Sauer, Stothers and Parker (1977) determined apparent availabilities of amino acids using ileal and faecal analysis for wheats and milling by-products for growing pigs. They showed that the use of a correction for metabolic faecal amino acid levels (i.e., amino acid excretion on zero nitrogen intake) for calculating true availability was not accurate. They observed that metabolic amino acid levels in the faeces were greater for some amino acids and less for others than the levels found at the terminal ileum. This they explained as being due to net effect of microbial synthesis and degradation between the terminal ileum and the faeces. For example, arginine at the terminal ileum was 0.49 g/kg DM intake but was 0.22 g/kg DM intake in faeces; histidine was 0.21 g/kg DM intake at the terminal ileum but 0.27 g/kg DM intake in the faeces; lysine was 0.27 g/kg DM intake at the terminal ileum but 0.37 g/kg DM intake in the faeces. In all the diets, the calculated true faecal availabilities

of some amino acids were markedly in excess of 100% and the results were not included in the publication (Sauer et al., 1977). Faecal analysis method may be far from accurate for the determination of true availability of amino acids (Sauer et al., 1977). This is because these authors have found differences of 9.1% and 17.3% between ileal and faecal recoveries of lysine and threonine respectively. One would expect ileal and faecal recoveries to be the same if there is neither absorption nor synthesis in the lower gut. However, the microbial population in the colon and caecum must affect the final level of amino acids in the excreta.

True amino acid availability determined by the faecal analysis method can be found throughout the literature on biological availability of amino acids (Eggum, 1973, de Muelenare and Feldman, 1960). In the literature authors of such publications do not tell us anything about the possible interference by microbial action. Due to the modifying action of microflora in the large intestines, it should be more appropriate to measure just apparent availabilities by faecal analysis and to use ileal analysis if true availability is to be measured. Metabolic faecal amino

acids determined from faeces of animals on N-free diets or 4% egg protein diets is not accurate since microbial effects are variable. For a true measure of true availability of amino acids, chemical or enzymatical methods may be better than faecal or even ileal analysis. This experiment was done to compare results with values obtained in the chemical determination of available lysine using a dye binding method.

Procedure

Rats on the unsupplemented diets of trial III were used for this determination. Rats were fed ad libitum for 14 days. Faeces were collected from day 11 to day 14 for lysine determination. Lysine intake was calculated from DM intake and lysine concentration in the diets. Faecal lysine was measured by the ninhydrin method described by Beckwith, Paulis and Wall (1975).

Principles of the Ninhydrin Method for Lysine Determination

The coloured derivative produced by the reaction of ninhydrin with alpha and epsilon amino groups

on amino acids, peptides and proteins is the basis for the rapid spectrophotometric estimation of lysine in corn meal (Beckwith et al., 1975). If the meal is first extracted with Trichloroacetic acid (TCA) to remove free amino acids, the amount of coloured derivative produced in a dimethyl sulfoxide solvent system can be used for a quantitative determination of lysine content of grain to within the accuracy found with amino acid analyses for lysine (Beckwith et al., 1975). The amount of free lysine in the grain is relatively small compared to the total lysine, hence its contribution of amino groups is very small. When free lysine is removed together with other free amino acids by precipitation with TCA, the loss of amino groups from free lysine is negligible. In measuring lysine in faeces by the ninhydrin method the removal of free amino acids (of endogenous origin) with TCA leaves the undigested protein, thus lysine may be measured fairly accurately in faeces.

Several chemical methods have been used for lysine determination but they all lack simplicity or accuracy (Beckwith et al., 1975). The reaction of alpha and epsilon amino groups is facilitated by the use of a special solvent system. In developing the ninhydrin reagent for use in amino acid analyses, it was

found that the reduced form, hydrindantin, is necessary for colour formation but it is insoluble in water. Dimethyl sulfoxide (DMSO) was used as the solvent in this method because it has better solvent action than other organic solvents (Beckwith et al., 1975).

Determination of Lysine in Faeces

Twenty milligrams (20 mg) ground faeces were placed in pyrex screw cap tubes and 2 ml of 10% (W/V) TCA was added. This was shaken vigorously on an electric shaker (Canlab) for one minute to remove free amino acid. Samples were centrifuged at 1000 g for 10 minutes and the supernatant was decanted. Sodium metabisulfite (0.2 ml of 0.125 M) was added to the residue followed by 2.3 ml of DMSO-Water (4:1 V/V) and 1 ml of ninhydrin reagent. The bisulfite was added to break disulfide bonds in glutelin to yield smaller protein particles, and to remove colours due to DMSO-soluble pigments in the sample. The reaction mixture was heated for 30 minutes on a boiling water bath. Five millilitres of DMSO-water was added and the mixture was centrifuged at 1000 g

for 10 minutes. The supernatant was decanted into a clean tube, diluted with 5 ml DMSO and the absorbance measured at 580 nm with the necessary dilutions to make reading possible.

Known weights of Hiproly were used to obtain a standard curve. Beckwith et al. (1975) used maize with known lysine content to obtain their standard curve. A regression of absorbance on lysine content was done. The regression equation obtained was used to predict the lysine content in the faeces. The regression equation was $Y = 0.83 X$, $r = 0.9968$ where y = absorbance and x = lysine content (mg).

Results and Discussion

The results on apparent availability of lysine indicate that Hiproly has the highest availability of lysine, this value being 70.31%. It is an indication of superior quality to the other barleys. Cultivar 2 has 93.57% of the lysine availability of Hiproly; cultivar 3 has 83%, cultivar 1 has 75.68% and Galt has 78.3% of the quality of Hiproly based on apparent availabilityof lysine determined by faecal analysis.

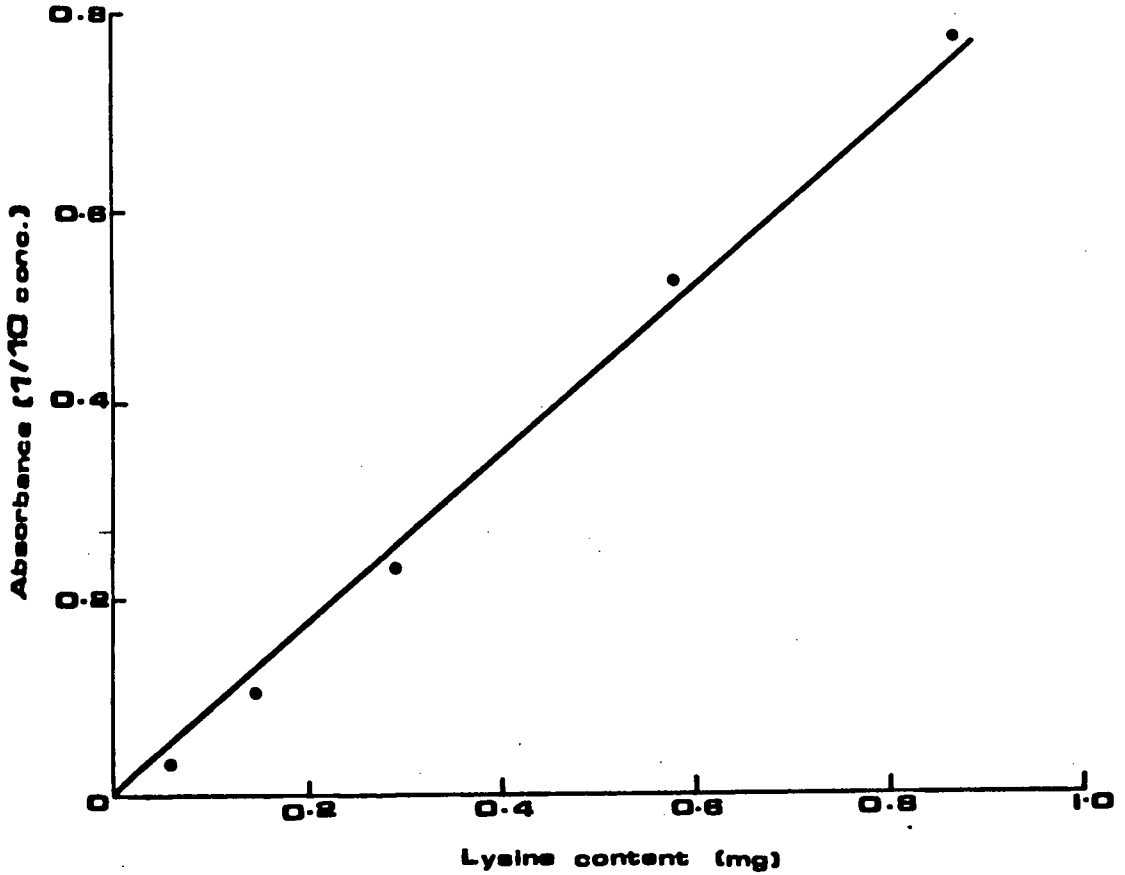


Fig. 4 Standard curve for lysine determination

Table 23. Standard curve for lysine determination by ninhydrin reaction.

Weight of Hiproly (g)	Total Lysine (g)	Absorbance (10% concentration)
0.0108	0.58	0.034
0.0270	0.145	0.105
0.0540	0.290	0.234
0.1080	0.580	0.524
0.1620	0.870	0.768
0.2700	1.45	1.163

Table 24. Faecal measurements for lysine determination.

	Hiproly	Galt	Cultivar 1 (H69019109000)	Cultivar 2 (H69024038000)	Cultivar 3 (H690240004191)
Dry weight of faeces used (g)	0.0133	0.01325	0.0121	0.0123	0.0120
Absorbance (1/10 conc)	0.085	0.095	0.093	0.085	0.090
Estimated lysine content in faeces used (mg)	0.1243	0.1359	0.136	0.1243	0.1301
Estimated lysine conc in faeces (mg/g Dry faeces)	9.346	10.2566	11.0413	10.1057	10.8417

Table 25. Apparent availability of lysine by faecal analysis.

	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3
DM intake (g)	55.50	49.30	54.94	56.82	51.16
*Lysine intake (mg)	205.40	177.50	214.30	233.00	173.94
Dry weight of faeces (g)	26.10	31.05	36.30	31.55	26.70
Lysine content of dry faeces (mg)	60.98	79.74	100.20	79.71	72.37
Apparent % lysine availability	70.31	55.08	53.21	65.79	58.39

* Calculated from amino acid composition of barley by ion exchange chromatography, and dry matter intake.

Sauer et al. (1977) observed apparent lysine availability of 77.5% for normal barley (Herta) using barrows as experimental animals. They observed values of 83.0% and 80.7% for commercial corn and hard wheat.

The values obtained in this study are markedly lower than those obtained by Sauer et al. (1977). Eggum (1973) observed true digestibility of lysine (i.e., true availability) in normal barley to be 76% using rats and 72.3% using pigs. The results of the apparent availability test seem to follow the same trend as the other measures of protein quality except for the relative protein value results. The chemical availability of lysine measured by dye binding difference did not follow the same trend as the biological availability figures. However, because the correlation coefficient for dye binding capacity and total basic amino acids in this experiment was low (0.502), the interpretation of the results must be done with caution.

GENERAL DISCUSSIONS AND CONCLUSIONS

This project was designed to evaluate the protein quality of five barley cultivars from the 1977 harvest of the Alberta Agriculture breeding program. This was done by:

proximate analysis
measurement of protein fractions
amino acid composition
availability of lysine
nitrogen balance studies
relative protein value
growth performance and feed utilization of rats
the effect of supplementation on growth performance and feed conversion.

A summary of all the experimental results can be seen in Table 26.

Based on the proximate analysis values, Hiproly and the other high-lysine cultivars supply more protein (N x 6.25) than Galt, the commercial variety. On this criterion alone, these high lysine cultivars would appear to be a better source of protein for pigs

Table 26. Summary of experimental results.

Experiment	Parameter	Hiproly	Galt	Cultivar 1	Cultivar 2	Cultivar 3
Proximate analysis	Dry matter % (DM)	92.62	92.69	91.92	92.55	92.64
	Crude protein (% DM basis) (N x 6.25)	18.57	15.94	17.45	18.10	16.81
Solubility test	Albumin + globulin (% of total protein)	20.45	18.89	22.63	21.88	19.34
	Hordein (% of total protein)	37.02	46.75	39.93	38.67	44.11
Amino acid composition	Total EAA (g/100 g DM)	7.52	5.81	6.91	7.18	6.50
	Total EAA (g/16 g N)	35.58	35.29	35.30	35.14	33.73
	Total basic amino acids (g/16 g N)	8.43	8.59	8.94	8.64	8.80
	Lysine (g/16 g N)	3.12	3.03	3.21	3.43	2.80
	Lysine (g/100 g DM)	0.58	0.46	0.56	0.62	0.47
Availability of lysine	*Chemical % availability	83.78	77.98	60.53	73.12	67.55
	**Biological % availability	70.31	55.08	53.21	65.79	58.39
Trial I	Growth rates (g/gain/day)	2.60	1.80	2.20	2.30	1.80
	Feed efficiency (g gain/g DM intake)	0.20	0.14	0.16	0.19	0.15
	True N digestibility	0.88	0.85	0.86	0.86	0.86
	Net protein utilization	73.09	73.00	72.09	74.19	72.62
Trial II	Relative protein value	0.87	0.77	0.83	0.71	0.84
Trial III	Growth rates (g gain/day)					
	Basal	2.62	2.18	2.80	2.80	2.33
	Basal + SBM	4.96	4.19	4.44	4.86	4.50
	Basal + lysine	3.32	2.53	2.50	2.81	2.59
	Basal + EAA	4.17	4.33	3.89	4.10	4.05
	Feed efficiencey (g gain/g DM intake)					
	Basal	0.22	0.17	0.22	0.22	0.18
	Basal + SBM	0.35	0.29	0.30	0.32	0.32
	Basal + lysine	0.25	0.20	0.21	0.21	0.21
	Basal + EAA	0.31	0.29	0.28	0.31	0.30

* measured by dye binding difference
** measured by faecal analysis

and would require less protein supplement than Galt. Cultivar 3 does not have as high a protein level as the other high lysine cultivars (Table 26). However, protein level alone is not enough for judging the protein contribution of these barleys; the quality of the protein is more important than the quantity.

According to Ingrersen and Koie (1973), the quality of a protein is related to the proportions of albumins, globulins, glutelins and prolamins it contains. Albumins and globulins are high quality proteins so the higher the proportions of these in a protein the better the quality of that protein. On this basis and on the results of the protein separation experiment, it can be said that cultivar 1, cultivar 2 and Hiproly are superior to cultivar 3 and Galt in quality. The amino acid composition of the different protein fractions of barley (Folkes and Yemm, 1956; see Table 3) support the fact that prolamins are poor quality proteins with low levels of essential amino acids, especially lysine. Cultivar 3 and Galt which have low levels of albumin and globulin have high levels of prolamin and this would contribute to their poor quality. This is not reflected in the amino acid composition (g/16 g N)

of the barleys. One would expect that Hiproly and cultivars 1 and 2 would have higher total essential amino acids, especially lysine because they had higher levels of albumins and globulins in their protein, but this was not observed. The total essential amino acid (EAA) levels in the proteins were approximately the same for Galt and the high lysine cultivars except in cultivar 3 which had a slightly lower total EAA than the rest.

The amino acid composition (g/16 g N) (Table 4) indicate very little or no differences between the barleys. If amino acid composition were to be used as the sole criterion for judging protein quality one could say that the five barleys have the same protein quality. However, if amino acid composition is expressed as g/100 g DM (Table 4) Hiproly and the other high lysine cultivars are superior to the normal variety in their ability to supply EAA. For protein quality studies amino acid composition expressed as g/16 g N is more meaningful. High lysine barleys are bred for the lysine content expressed as g/100 gm DM. For practical feeding purposes this is a better expression than g/16 g N, since the amino acid composition of the diet of pigs and other farm animals is expressed on a DM basis. Also an animal's requirement of amino acids is expressed as a percentage of the DM of the diet.

Lysine availability measured by dye binding differences gave values that do not follow any particular trend though Hiproly was still portrayed as being superior to the other barleys as other tests in this study have shown. Based on the results of the dye binding test in this study it is difficult to make conclusions about the protein quality. On the other hand, measurements of apparent availability of lysine done by faecal analysis show that Hiproly and cultivar 2 are superior in quality to the other barleys because of their relatively high lysine availability (70.31% and 65.79% respectively). Cultivar 3 which has shown low values for quality parameters thus far has a better availability of lysine than cultivar 1. This does not seem very likely, considering the prolamin contents of the two. There appears to be no obvious explanation for this inconsistency.

The nitrogen balance tests showed no significant differences between the five barleys. The tests used (TD, BV, NPU) called for 10% CP and this was achieved by diluting the barleys with starch as suggested by Eggum (1973). The results obtained for the nitrogen balance tests in this study (Table 4) are comparable to results obtained by Eggum

(1977b) who worked on some high lysine barleys. For example, he obtained a TD of 85%, true BV of 83% and NPU of 71% for Hily 71/669; Mutant 1508 had TD of 78%, true BV of 90% and NPU of 70; Hiproly had TD of 85.2%, true BV of 76% and NPU of 64%. For nitrogen balance tests, restricted feeding is recommended (Eggum, 1973). Animals are fed 10 g DM per day with 150 mg protein per day. The rats used in this study (i.e., trial I) even though feeding was ad libitum, consumed on the average 11 g DM per day, which is close to the recommended 10 g DM per day.

Relative protein value (RPV) as a measure of protein quality gave a different picture altogether (trial II). Hiproly still maintained its superiority (87%) but cultivar 3 became second to Hiproly (84%) while cultivar 2 was the poorest by this test. This does not seem to fit in very well with the quality trend set by the other methods. There was so much variation in food consumption and body weight changes (Table 17) that it is not certain how much weight should be attached to the RPV results. Eggum and Campbell (1979) have suggested that lysine deficient proteins may not give valid slope ratios. For high lysine barleys

one would imagine that the RPV would be a suitable test for protein quality. However, with the amino acid composition expressed as g/16 g N, these high lysine barleys are not different from the normal varieties and their protein can still be described as lysine deficient. Eggum and Campbell (1979) believe that the 2% and 5% CP levels are too low and are responsible for the variation in food intake and body weight changes.

Rat growth assays (trials I and II) indicate that Hiproly has a significantly better ($P \ll 0.05$) ability to support growth than the other barleys being studied except cultivar 2. This is demonstrated in the growth rates and feed efficiencies of rats fed these barleys as the sole source of protein. In terms of the ability to support growth cultivar 3 was as poor as Galt which is an indication of the quality of its protein. Work done on high lysine barleys has been centred more or less on nitrogen balance tests and amino acid analyses. There appears to be no information on growth studies with high lysine barleys; however, Beames (unpublished) has worked on the same varieties of barley as the ones being discussed in this report, but from a 1975 harvest. He fed the barley undiluted with starch and obtained growth

rates of 6.3, 4.6, 4.8, 5.9 and 4.6 g/day for Hiproly, Galt, cultivar 1, cultivar 2 and cultivar 3 respectively, compared with respective values of 2.6, 1.8, 2.2, 2.3 and 1.8 g/day in trial I and 2.62, 2.18, 2.8, 2.8 and 2.33 g/day in Trial III. In the present study the protein level in the barleys were brought down to 12% crude protein (DM basis) but NRC (1972) recommends 12% ideal protein (DM basis) for growing rats. It is possible that if the 1977 barleys were undiluted, the rats would have done just as well as those on the 1975 harvest. With the dilution of barley, the amino acid concentration was reduced and this led to the low growth rates and feed efficiencies when compared with the results of Beames (unpublished). The NRC recommended 12% net protein apparently applied to good quality proteins and that with very high quality proteins 9% CP in the diet is adequate to support rat growth. In this study a level of 12% CP in the diet was selected because the aim of the investigation was to evaluate the quality of the barley proteins. Without knowing the quality of the proteins in advance allowance could not have been made for poor quality by feeding above the recommended level.

The fact that supplementation of these barleys with lysine alone made no significant improvement in rat performance and that addition of other essential amino acids made a significant improvement ($P < 0.05$) in the performance of all rats is an indication that there may be some other essential amino acids limiting in these barleys. It seems that because other amino acids are limiting, lysine supplementation could not correct the imbalance. Also that soybean meal made a significant ($P < 0.05$) improvement in rat performance shows that SBM protein has an amino acid pattern that complements that existing in these barleys.

To conclude I would say that cultivar 2 is as good as Hiproly in quality since there were no significant differences between them for almost all the tests except in one case where there was an inconsistency in the results. Cultivar 1 appears to have intermediate quality while cultivar 3 has a quality as low as that of Galt, the commercial variety.

Also that relative protein value may not be a good test for protein quality in barley. Eggum and Campbell (1979) have suggested that lysine deficient proteins may not yield valid slope ratios. High

lysine barleys, although they contain higher levels of lysine in the DM than normal barley, the lysine concentration (g/16 g N) in their protein is not much different from those of normal barleys. In this case, I would still consider high lysine barleys as a source of lysine deficient protein. Possibly this will explain the type of results obtained with the RPV test. However, since animal diets are formulated to provide amino acids on a DM basis, the high lysine barleys on this basis had a better nutritional quality or nutritional value than the normal variety Galt used in this study.

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APPENDIX I

Essential ámino acid requirements of growing (0-6 wks) chicks (layer replacements) (NRC, 1971)

Arginine	1.20
Glycine	1.00
Histidine	0.40
Isoleucine	0.75
Leucine	1.40
Lysine	1.10
Methionine + cystine	0.75
Phenylalanine + tyrosine	1.30
Threonine	0.70
Tryptophan	0.20
Valine	0.85

APPENDIX II

Essential amino acid requirements (% DM diet) of the growing rat.

	NRC (1972)	Rama Rao et al. (1979)
Arginine	0.67	-
Histidine	0.33	0.21
Isoleucine	0.61	0.55
Leucine	0.83	0.69
Lysine	1.00	0.90
Methionine ± cystine	0.67	0.49
Phenylalanine + tyrosine	0.89	0.72
Threonine	0.56	0.51
Tryptophan	0.17	0.11
Valine	0.67	0.56

APPENDIX III

Essential amino acid requirements (% DM diet) of the growing pig (NRC, 1973)

	Live weight (kg)				
Amino acid	5-10	10-20	20-35	35-60	60-100
Arginine	0.28	0.23	0.20	0.18	0.16
Histidine	0.25	0.20	0.18	0.16	0.15
Isoleucine	0.69	0.56	0.50	0.44	0.41
Leucine	0.83	0.68	0.60	0.52	0.48
Lysine	0.96	0.79	0.70	0.61	0.57
Methionine + cystine	0.69	0.56	0.50	0.44	0.41
Phenylalanine + tyrosine	0.69	0.56	0.50	0.44	0.41
Threonine	0.62	0.51	0.45	0.39	0.37
Tryptophan	0.18	0.15	0.13	0.11	0.11
Valine	0.69	0.56	0.50	0.44	0.41