Protein quality of normal barley cultivars and their proanthocyanidin-free mutants

by Margareth Overland

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
Proanthocyanidins have the potential to be antinutrients. Proanthocyanidin-free barleys have been cultivated and these, barleys may have an improved nutritional value over their parent barleys. Six pairs of normal and proanthocyanidin-free barley mutants were compared for their chemical composition, physical measurements and in biological trials with rats and chicks. Triumph and Galant (ANT-148) were compared in biological trials with pigs. Normal and proanthocyanidin-free cultivars included in this study were: Triumph, Galant; Moravian III, ANT-605; Andre, ANT-587; Robust, ANT-625; Advance, ANT-537; Karla and ANT-504, respectively. Clark was used as a control barley in chemical comparison and in the rat trials.

The chemical composition was similar for all barleys, except for protein and starch. Protein ranged from 11.2% to 14.7%, with a mean of 13.8%. Starch content ranged from 50.9% to 59.4%,with a mean of 56.2%. Physical measurements showed only slight differences between the normal and mutant barleys.

A chick growth trial, using 1-day-old broiler chicks, showed no differences in weight gains ($P>0.6$) , feed/gain ratios ($P>0.6$). or feed consumption ($P>0.1$) between the six barley pairs.

In a chick taste preference trial, using 1-day-old broiler chicks, there were no differences in preference for parent or mutant barleys as measured by the chick's feed consumption, except for the barley pair, Karla and ANT-504, where the consumption of diet prepared from Karla was higher than that of the mutant.

A rat nitrogen balance trial, using male weanling rats, showed differences between the barley cultivars for true digestible protein (TDP) ($P<.03$), biological value (BV) ($P<.003$) and net protein utilization (NPU) ($P<.007$). Comparison between the parent and mutant barleys showed that the average TDP was higher in the latter, although not significantly. BV and NPU were lower ($P<.01$) in the mutants; however, a significant interaction occurred ($P<.03$) between parent and ANT-barley for all measurements.

There were no significant differences i,n weight gains ($P>0.3$), feed/gain ratios ($P>0.9$) or feed consumption ($P>0.5$) of pigs fed diets prepared with either Triumph or Galant barley from 3-weeks of age to approximately 85 kg.

It is concluded that the proanthocyanidins have a negative influence on protein digestibility; however, the nutritional quality of these barleys was not affected by the presence of proanthocyanidins as measured by BV and NPU. Other constituents including beta-glucans, starch and total fiber will also have the potential of influencing animal and poultry performance.

PROTEIN QUALITY OF NORMAL BARLEY CULTIVARS AND THEIR PROANTHOCYANIDIN-FREE MUTANTS

by

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Date July 11, 1988

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ABSTRACT

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It is concluded that the proanthocyanidins have a negative influence on protein digestibility; however, the nutritional quality of these barleys was not affected by the presence of proanthocyanidins as measured by BV and NPU. Other constituents including beta-glucans, starch and total fiber will also have the potential of influencing animal and poultry performance.

INTRODUCTION

Cereal grains are important food staples of the world. Quantitatively, starch is by far the most important food reserve material in cereal grains. Cereals are also an important source of protein in the world, especially in developing countries. Discoveries of strains of maize, barley, and other crops with higher levels of essential amino acids than normal have demonstrated that differences in nutritional quality occur. Cereal protein has a relatively low biological value and efforts to increase the proportion of essential amino acids have not been totally adequate. Therefore, factors affecting the availability of amino acids could make an important contribution to solving the problem of protein malnutrition in many parts of the world.

Among barley cultivars currently in commercial production, nutritional value varies with respect to protein quality. Proteins play a crucial role in virtually all biological processes. They consist largely of chains of combinations of 20 individual amino acids, with each protein formed by a specific sequence of amino acids and having specific configuration (Stryer, 1981). The broad range of chemical functional groups that compose the amino acid side chains are responsible for the tendency of proteins to react with many components found in plants. For instance, protein will react with rancid fats, dietary fiber, reducing sugars and polyphenols. Any of these reactions

will decrease protein quality by reducing amino acid availability.

Phenolic compounds have been suggested as a factor that limit the protein value of barley. Most higher plants including barley contain a phenolic pigment called anthocyanidin. Proanthocyanidin is a collective term for flavonoids yielding anthocyanidins upon treatment with acid. (Weinges and Nader, 1982). The biological function of proanthocyanidins in the barley kernel is not well understood. It is suspected that many proanthocyanidins associate with proteins, forming insoluble complexes in the gastrointestional tracts of monogastric animals, thus reducing the availability of amino acids (Munck, 1964). Proanthocyanidin-free barley mutants have been developed through mutation breeding programs, and it is postulated that these barleys may have a higher nutritional value than their parent barleys.

The objectives of this study were: (1) to compare the chemical composition and physical characteristics of normal and proanthocyanidin-free barley, and (2) to determine if proanthocyanidin-free barleys have a higher nutritional value than normal barleys with respect to protein utilization in monogastric animals.

The general approach to achieve the objectives of the study is as follows:

1. Determine the chemical composition and physical properties of six pairs of parent (normal) and proanthocyanidin-free (mutant) barley.
2. Determine growth, feed consumption and feed efficiency of chicks and pigs fed these barleys.
3. Measure nitrogen metabolism in rats fed these barleys, including protein digestibility, biological value and net protein utilization.

LITERATURE REVIEW

This review includes literature concerning: (1) the physical and chemical characteristics of barley, (2) phenolic compounds in plants, (3) factors affecting protein utilization in cereals, and (4) animal models. A discussion of the phenolic compounds anthocyanidins, catechins, tannins and gossypol is presented. A second discussion describes proanthocyanidins in barley including their structure, biosynthetic pathway and genetic blockage of the biosynthetic pathway. In addition, a discussion of taste preference in chicks and protein evaluation using chicks, rats and pigs as models is reviewed.

Chemical and Physical Characteristics of Barley Kernel

Physical Characteristics

The primary description of barley is dependant upon the arrangement of grains in the ear, either two-rowed and six-rowed. In two-rowed barley, the kernels are symmetrically arranged. In six-rowed barley, the median kernels are symmetrical on the spike, while the lateral grains are asymmetrical, with either a right or a left handed bias (Briggs, 1978). Two-rowed barleys generally produce larger kernels than six rowed barleys, although considerable variation exists in kernel shapes, from the long and slender to the short and plump in both row types (Briggs, 1978). Pomeranz et al., (1973) found that six-rowed barleys contained an average of 1 to 2% less protein than two-

rowed barleys and were lower in kernel weight.

The barley kernel consists of pericarp, testa, germ, aleurone and endosperm. Most barley cultivars are covered by a hull. In some cultivars the hull separates from the kernel during threshing leaving a nude kernel. These barleys are designated as hull-less types. The pericarp lies below the hull and acts as a protective covering of the entire barley kernel. Below the pericarp lies the aleurone which is the outermost layer of the endosperm (Briggs 1978). Proanthocyanidins are located in the aleurone layer and pericarp of the barley kernel (Aastrup et al.,1984). The starchy endosperm makes up a majority of the kernel and provides energy for the developing embryo during seed growth. The embryo is located at the base of the kernel and develops into the young plant as the seed germinates (Reid, 1985).

Kernel weight, plumpness, and the presence or absence of hull have been related to the nutritional quality of barley. The hull may comprise from 7 to 24% of the kernel, varying according to type, variety, and kernel size and the latitude of cultivation (Pomeranz, 1974). Normally, barley has a test weight of approximately 62.4 kg/hl, but this can vary from 46 to 72 kg/hl. These test weights vary with different growing conditions and as a result of changes in the endosperm relative to the hull. High test weight barleys tend to have full, plump kernels containing average protein levels and high starch levels, while low test weight barleys have relatively higher protein and fiber levels and low starch levels as a result of a shrunken kernel.

Chemical Composition

Carbohydrates. The major carbohydrates in barley are starch, cellulose, hemicellulose and water-soluble substances such as mixed linked 1,3-1,4 beta-glucans. Lignin is often associated with carbohydrates but makes up less than 1% of the kernel. Starch is the major chemical constituent of barley, making up 50 to 65% of the kernel by weight. The amount of starch varies inversely with protein percentage of the barley. Barley kernel starch is predominantly amylopectin (74-78%) in which chains of alpha-1,4-glucopyranose units are branched through alpha-1,6 linkages, and amylose (22-26%) containing straight chains of D-glucopyranose units linked alpha-1,4 (Briggs, 1978). Some barley starch contains amylopectin exclusively, and others contain amylose and amylopectin at a ratio of 1:1 (Newman and McGuire, 1985). Crude fiber content of covered barleys generally ranges from 4-8%, averaging about 6% (dry matter), while hulless barleys average 2% or less. The major crude fiber component of barley is cellulose which is derived from the hull covering the endosperm. However, mixed linked beta-glucans, which are chemically similar to cellulose, are present at high levels (up to 15%) in covered and hulless barleys (Newman and McGuire, 1985).

Beta-glucans are non-starch polysaccarides, containing mixed linear polymers of beta-D-glucopyranose. About 25 to 30% of the glucosidic linkages are in the 1,3 position, and the remaining linkages in the 1,4 position. The presence of beta-1,3 linkages in the beta-glucans causes an irregular configuration which renders the molecules partially soluble in water and susceptible to hydrolysis. Relative

viscosity of barley has a strong correlation with the total beta-glucan content (Aastrup, 1979); however, other carbohydrate components in barley such as pectin and xylan also contribute to the overall viscosity (Aastrup, 1979). Beta-glucans form the major component of the endosperm cell wall and a minor component of the aleurone cell walls in barley. They are linked to proteins in the cell wall, forming high molecular weight molecules. The beta-glucan component in barley varies from 1.5 to 15.0% of dry weight (Newman and McGuire, 1985). The relative percentage of beta-glucans in barley is thought to be influenced by both genetic and environmental factors (Bourne and Pierce, 1972). Beta-glucans are partially water-soluble, and are not retained as residue in the crude fiber analytical procedure. They are theoretically non-digestible in monogastrics and contribute little or no metabolizable energy.

Normal barleys also contain small quantities of simple sugars such as glucose and sucrose. The concentration ranges from 1 to 2% and has little influence on the overall nutritional value of the average barley (Newman and McGuire, 1985).

Protein. The amino acid composition and the total amount of protein is important to the nutritional quality of barley (Newman and McGuire, 1985). Large variation exists in the protein and amino acid content of barley, but on an average, it contains 11.6% protein. Lysine is the most limiting amino acid in barley protein, followed by methionine, threonine and tryptophan (Howe et al., 1965). Others have

reported threonine to be the second limiting amino acid in barley (Chung and Beams, 1974).

Proteins in barley were chemically classified by Osborne (1924) on the basis of solubility in either water (albumins), or solutions of salt (globulins), alkali (glutelins), alcohol (prolamins) or structural and metabolic proteins (glutelins, albumins, globulins). The albumin and globulin fractions are quite often extracted together and referred to as salt-soluble proteins. The storage protein prolamin, or hordein as it is commonly called, is located primarily in the endosperm, and is comparable to zein in corn. As with zein, it is of very poor nutritional quality, containing less than 1% lysine. The glutelins occur in the endosperm as well as in the germ, and are intermediate in nutritional quality, containing 4 to 5% lysine. Prolamin and glutelins are also poor in threonine but rich in glutamic acid and proline. The glutamic acid content is significantly higher in prolamins than in albumins and glutelins. The salt soluble proteins, which occur principally in the aleurone layers, and the kernel embryo account for 15 to 30% of total grain nitrogen. These proteins usually have a high lysine content of 6 to 8% and also exhibit a well balanced amino acid composition of superior nutritional value, similar to that of animal protein (Newman and McGuire, 1985). The total amount of essential amino acids of albumin and globulin proteins is over 40% of the total amino acids (Chung and Pomeranz, 1985).

Fats. Barley lipid content is low compared to that of maize and oats, ranging from 2 to 3% in most commercial cultivars (Newman and McGuire, 1985). Barley contains an average of 1.9% ether extract, of which the greatest portion is triglycerides. Linoleic acid is the major fatty acid present in these triglycerides. Smaller amounts of palmitic acid, and the unsaturated fatty acids oleic and linolenic acid are also present (Briggs, 1978).

The greatest portion of the lipids in the barley kernel is in the endosperm, with smaller percentages of the total occurring in the embryo and hull (Newman and McGuire, 1985).

Vitamins and Minerals. Barley is an excellent source of many of the B-complex vitamins compared to other grains, including thiamin (B_1), pyridoxine (B_6), riboflavin (B_2), and pantothenic acid. Concentrated niacin is reported in barley, but only about 10% of the total is thought to be biologically available. Lesser amounts of biotin and folacin occur in barley. Fat-soluble vitamins are limiting in barley, since the kernel contains no carotene or vitamins A, D, or K (NRC, 1988). A small concentration of vitamin E occurs in the barley germ (Newman and McGuire, 1985).

The ash content of barley ranges from 2.0 to 3.0% and is lowest in hulless types. The principal mineral constituents of barley ash are potassium and phosphorus, with smaller amounts of chlorine, magnesium, sulfur, sodium, and calcium (Owen et al., 1977). Smaller concentrations of iron, zinc, copper, manganese, and selenium also occur in barley kernel ash. Concentration of most minerals is often

influenced by season, soil type, and climatic conditions (Owen et al., 1977).

Phenolic Compounds

Plants contain a large number of phenolics and polyphenolic compounds. The phenolics can be divided into four major biochemical groups: benzoic acids, cinnamic acids, terpenoids, and flavonoids. A class of flavonoids, the anthocyanidins, are water soluble red, blue and purple pigments. These pigments are distributed throughout the plant kingdom and responsible for the colors of many flowers, fruits and vegetables. They are also pigments found in red wines. All the names of anthocyanidins are derived from the names of plants. Cyanidin is found in the purple fig, almond, mulberry, sweet cherry and elderberry; delphinidin is found in pomegranate and eggplant, and pelargonidin is found in strawberry. The anthocyanin pigments change their color with a change in pH. Cyanin for example, is red in acidic solution, purple at neutral pH and blue in alkaline medium. The changes in color are assumed to be associated with a change in molecular configuration (Berk, 1976).

Phenolic substrates in vegetables and fruits are involved in the well known spoilage phenomenon, enzymatic browning. On the other hand, certain phenolic compounds act as natural antioxidants, and therefore are important for the stability of many foods. The catechins, for instance, make up a class of flavonoids that takes part in the enzymatic browning process of many foods. They are also responsible for much of the taste in tea and wine. Catechins are characterized by

a single hydroxyl group on the C_3 portion at the 3-position (Berk, 1976).

Leucoanthocyanins is another class of flavonoids, and they are widely distributed in woody plants. Their name is due to the fact that they are colorless but produce anthocyanidins when treated with hydrocholoric acid (Berk, 1976). The polyphenolic compound tannins, a class of benzoic acids, are of relative high molecular weight. Tannins occur, sometimes in significant concentrations, in vegetable tissue of a wide variety of plants utilized for food or feed. Tannins are responsible for the astringency of many foods, such as apples, pears, dates, tea and cocoa. Most tannins found in food stuffs are condensed tannins. Condensed tannins constitute the bulk of tannins in wood and bark. Their structure is not completely understood, but they are formed by the polymerization of catechins or anthocyanidins (Berk, 1976). Tannins have the ability to aggregate and precipitate proteins under suitable conditions, and therefore can reduce the protein digestibility of feeds containing these compounds.

Several phenolics of vegetal and fungal origin are important toxic substances. Gossypol for instance is a terpenoid that is found in cottonseed. It has a specific polyphenolic structure, containing four benzene rings. Gossypol is mainly found in the cotelydons of cottonseed, concentrated in small bodies called pigment glands. It is intensely colored, dark brownish red. It is a toxic substance which may cause inflammation of tissues, hemorrhage and nervous disorders. Gossypol also complexes with the protein in the seed, thus reducing protein digestibility. The toxicity of gossypol in cottonseed meal may

be eliminated by heating the meal in the presence of moisture (Berk, 1976).

Phenolic Compounds in Barley

Barley cultivars contain anthocyanidins of the cyanidin type in their green tissue (Jende-Strid, 1981) and proanthocyanidins and catechins in the seed coat (testa) of the mature kernel (Aastrup et al., 1984). Proanthocyanidin is a collective term for all flavonoids that yield anthocyanidin upon treatment with acid (Weinges and Nader, 1982).

Research on proanthocyanidin-free barley started in the brewing industry at Calsberg in Copenhagen, Denmark. Proanthocyanidins in barley, known to brewers as anthocyanidins, have been subject to intensive studies, and their role in brewing as part of colloidal haze in beer is well documented. These phenolic compounds are known to associate with proteins forming insoluble complexes, which cause haze formation in beer (Jende-Strid, 1981; Munck, 1981) and could possibly reduce the digestibility of proteins in animals. Proanthocyanidin-free barley has recently been produced through mutation breeding (Jende-Strid, 1976; von Wettstein et al., 1977; Jende-Strid and Møller, 1981). These proanthocyanidin-free barleys produce beer with an excellent haze stability without influencing any other beer characteristics such as beer flavor. It is possible that such barley could have a higher nutritional value through more digestible proteins, as is the case with low-tannin grain sorghum (Cousins et al., 1981).

A staining procedure (Astrup, 1985) using vanillin-HCL has been

used to locate the proanthocyanidins in mature barley grain. The proanthocyanidins are believed to be located in the seed coat of the barley grain investigated, more specifically in the aleurone layer and pericarp of the kernel (Aastrup et al.,1984).

As the proanthocyanidins are secondary plant metabolites and thus not required for the normal growth and development of the barley plant, the breeding of proanthocyanidin-free malting barley should be feasible (von Wettstein et al., 1980). The proanthocyanidin-free mutant Galant, isolated from Triumph barley, has shown encouraging agronomic performance when tested at 13 different locations in Europe during 1982 and 1983. It can reach top yield levels (6 to 7 t/ha).

Structure of Proanthocyanidins

The composition of phenolic compounds is complex. These compounds are derivatives of phenolic amino acids, including tyrosine, phenylalanine and tryptophan. Proanthocyanidins are derivatives of the phenolic amino acid phenylalanine. Anthocyanidins are glucosides, and when hydrolyzed they yield a sugar and an aglycone, called anthocyanidine. The carbohydrate residues most frequently encountered are glucose, rhamnose, galactose and gentiobiose. The anthocyanidins have a common basic structure of a benzopyrylium nucleus and a phenol ring, the two together being called flavylium (Berk, 1976).

Barley grains contain two principal proanthocyanidins, one liberating cyanidin and the other delphinidin upon hydrolysis (von Wettstein et al., 1980). Proanthocyanidins in barley consist mainly of dimers and trimers of (+)-catechin and (+)-gallocatechin units, which

are linked by either C_4-C_8 or C_4-C_6 bondings. The minimum chain length is two units, but much larger molecules have been observed and many compounds have been isolated from various plant sources. Only a few proanthocyanidins have been isolated in barley: procyanidin B_3, consisting of two catechin units linked C_4-C_8, procyanin B_6 which is a dimer consisting of two catechin units C_4-C_6 linked, and procyanidin C_2 consisting of three (+)-catechin units linked C_4-C_8. In addition, two proanthocyanidins have been tentatively identified as trimeric compounds, one being a prodelphinidin. Ottrup and Schaumburg (1981) isolated four additional trimeric proanthocyanidins from barley, of which three are prodelphinidins, and Jende-Strid (1981) reported a number of unidentified proanthocyanidins. Both procyanidins and delphinidins occur in proanthocyanidins (Jende-Strid, 1981).

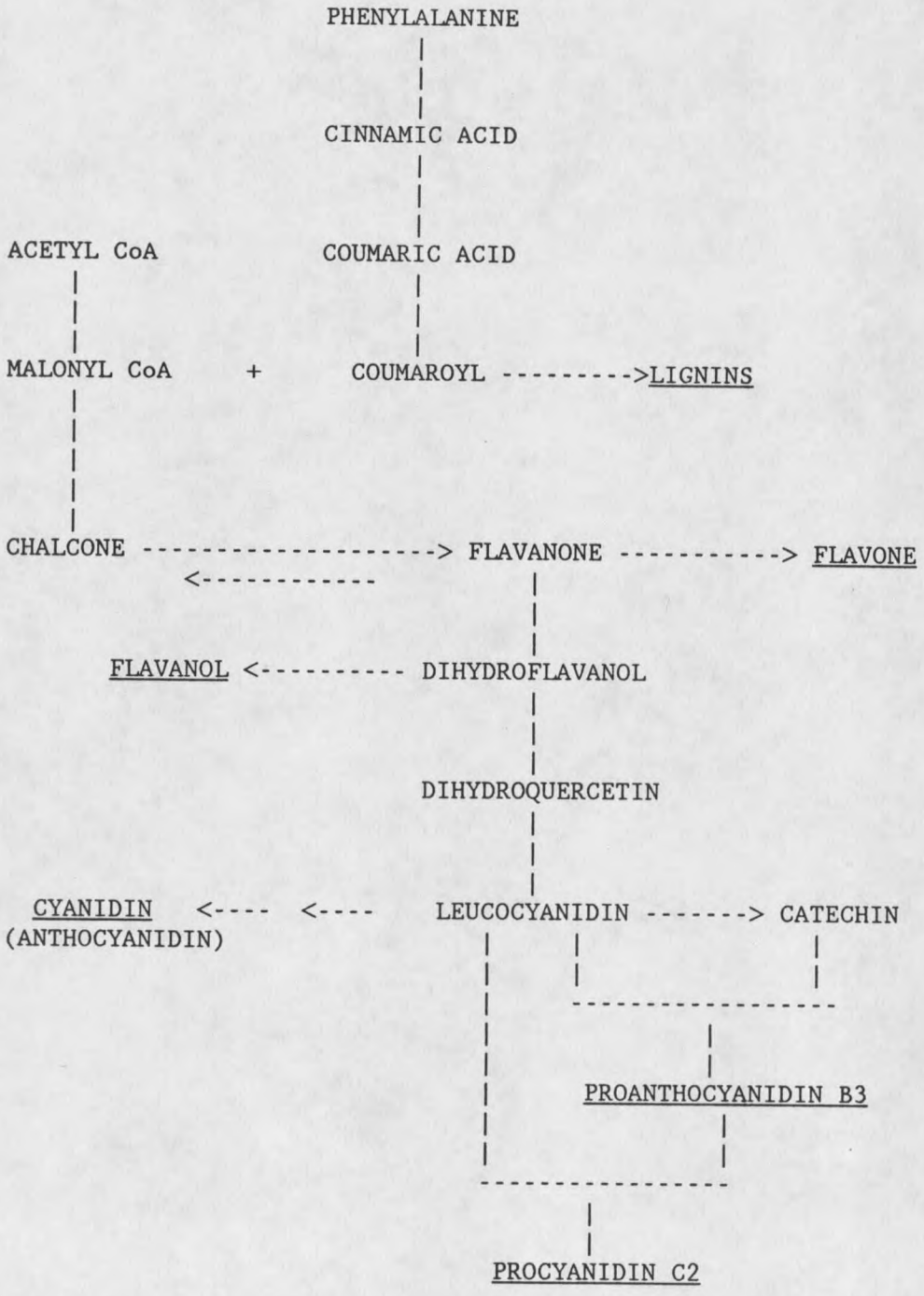
Biosynthesis of Proanthocyanidins

The proanthocyanidins are synthesized according to the general scheme for flavonoid formation. The enzymology of flavonoid biosynthesis is well established from the level of phenylalanine to dihydroquercetin. The biosynthetic pathway of proanthocyanidins is shown in Figure 1, and proceeds by the pathway chalcone --> flavanone--> dihydroflavonol --> dihydroquercitin. The leucocyanidin, rather than dihydroquercetin, serves as the last common intermediate in the synthesis of anthocyanins and proanthocyanidins (Kristiansen, 1984). Cinnamic acids from this central reaction in flavonoid biosynthesis are supplied by the phenolic amino acid phenylalanine. In the conversion of phenylalanine to cinnamic acid, it goes through a sequence of

reactions, the phenylpropanoid metabolism. Cinnamic acid is then converted, by a sequence of hydroxylations and methylation reactions, to a number of acids, including coumaroyl, which can be activated to the corresponding coenzyme A esters, malonyl-CoA. Malonyl-CoA is supplied from acetyl-CoA by the way of acetyl-CoA carboxylase reaction (Grisebach, 1982). These activated acids can enter different biosynthetic pathways leading to lignin, flavonoids, benzoic acids and other components.

Tracer experiments and enzymatic studies proved chalcone is a common precursor for all flavonoids. Chalcone, the flavan skeleton, is synthesized in the plant from coumaroyl and malonyl-CoA by the action of the enzyme, chalcone synthase (Grisebach, 1982). Chalcone is then transformed to various flavonoids. Flavone and dihydroflavanol are formed from the isomeric flavonone. Dihydroflavanols are biosynthetic intermediates from anthocyanidins, flavonols, and catechins. Dihydroflavanol is converted to dihydroquercetin, which is a known precursor of anthocyanidin and is also thought to be a precursor of catechin and procyanidins. Little information is available concerning the biosynthetic step leading from the presumed dihydroquercetin precursor to catechin and procyanidins. The immediate precursor of anthocyanidins and proanthocyanidins is a leucocyanidin which is derived from a dihydroflavonol (Kristiansen, 1984). Leucocyanidin converts into catechin, condensation of leucocyanidin and catechin produces Procyanidin B_3, and condensation of leucocyanidin and Procyanidin B_3 produces Procyanidin C_2 (Kristiansen, 1984).

Figure 1. Biosynthetic Pathway of Proanthocyanidin Synthesis



Genetic Blockage of the Proanthocyanidin Synthesis

By chemical mutations proanthocyanidin-free mutants have been induced in 65 spring and 9 winter barley varieties (von Wettstein et al., 1980). These mutants have been induced by genetic blockage of the biosynthetic pathway of the proanthocyanidins. Proanthocyanidin synthesis and that of the anthocyanidins are controlled by the ant genes. A total of nineteen complementing genes, ant 1 to ant 19, have been identified in barley (Jende-Strid 1981). At least 6 ant genes which by mutation can block the synthesis of proanthocyanidins, including ant 13, ant 17 and ant 18. Mutants in ant 13, ant 17 and ant 18 are blocked in both proanthocyanidin and anthocyanin synthesis, mutants in ant 19 are blocked only in the proanthocyanidin synthesis, whereas mutants in ant 1 to ant 12 and ant 14 to ant 16 are blocked only in anthocyanin synthesis (Kristiansen, 1984). The genetic block caused by mutation in the gene ant 17 leads to an accumulation of naringenin, which is a related precursor to dihydroquersetin, while ant 18 mutants accumulate dihydroquercetin (von Wettstein et al., 1980). The location of genetic blockage in the barleys included in this study are ant 13 and ant 17. The genetic blockage in the gene ant 13 produces the barley mutants Ant 537 and Ant 605, whereas blockage in the gene ant 17 produces Galant, Ant 625, Ant 504 and Ant 587 (B. Jende-Strid, personal communication, 1986).

Factors Affecting Protein Utilization in Cereals

A number of factors in barley have been suggested as possible detrimental factors in protein utilization, including dietary fiber, protein composition, enzyme inhibitors and phenolic compounds. Barleys having high levels of albumins and globulins generally have less digestible proteins but have a better amino acid balance than barleys that contain high percentage hordein (Newman and El-Negoumy, 1980). Thus, most high protein barleys have a high relative protein digestibility due to increased concentrations of hordein, although observed improvements in protein digestibility may be influenced by changes in other constituents such as fiber. In the digestive tract, dietary fiber may interact with protein to affect nitrogen digestibility (Anderson and Køie, 1975). Eggum (1973) suggested that aleurone cell wall fiber is a major factor in protecting albumin and globulin proteins from digestive enzymes. Fiber from barley hulls has also been implicated as a factor in reduced absorption and utilization of protein and other nutrients (Bhatty et al., 1975). A negative curvilinear effect of barley hull fiber on protein digestibility was demonstrated in a study reported by Bell et al., (1983). Their data showed an increasingly depressive effect of barley hull fiber on digestibility as dietary protein level decreased. Thomke et al., (1980) concluded that beta-glucans, which are partially soluble fiber components, were responsible for decreasing the digestibility of protein, organic matter and energy in barley. Barley is inferior to corn when fed to broiler chicks for rapid weight gain (Hesselman,

1983). However, a considerable reduction in the frequency of sticky droppings and improvement of feed intake, growth rate and feed efficiency, and consequently in productive value, have been obtained when beta-glucanase has been incorporated into poultry diets (Hesselman et al., 1982). Supplementation of beta-glucanase in poultry diets showed an improved ileal digestibility of nitrogen as well as starch and beta-glucans (Hesselman and Åman, 1986). Consequently it was concluded that beta-glucans restricted protein and starch absorption thus reducing feed intake and diminished efficient utilization of nutrients. An improvement in nitrogen digestibility in the colon and caeca are also reported with beta-glucanase addition (Hesselman and Åman, 1986). Beta-glucans affect nutrient utilization less in swine than in poultry. When pigs were fed beta-glucanase supplemented barley diets they showed mixed response in growth improvement (Newman et al., 1985). Graham et al., (1986) reported an insignificant effect of beta-glucanase on overall performance of barley-fed pigs. Pigs fitted with duodenal and ileal cannulae to measure digestibility of nutrients in a barley-based diet showed that beta-glucanase supplementation did not affect the digestibility of nitrogen or starch.

Maillard reactions or browning reactions are also factors recognized in decreasing amino acid availability of cereals. These are nonenzymatic chemical reactions involving condensation of amino groups of proteins, peptides and amino acids to the carbonyl group of a sugar, resulting in the formation of intermediates which ultimately polymerize to form brown pigments (Erbersdobler, 1976).

In addition, the high content of proline in cereal proteins is a

factor that might limit the digestibility of these proteins. Because of a large proline content, large numbers of lysylprolyl and/or arginylprolyl bonds occur, and these bonds are resistant to trypsin attack. It has been observed that because of its higher content of arginyl and lysyl residues, rice protein is cleaved by trypsin to smaller peptide fragments to a greater extent than any other cereal proteins (Bozzini and Silano, 1977). The total ratio of arginine and lysine to proline in rice is 4.0 compared to an average of 1.0 for other cereals, in line with the higher digestibility and biological value of rice protein (Bozzini and Silano, 1977). Another factor affecting the low digestibility of some cereal proteins depends on the protein conformation. Proteins with low solubility might have a higher resistance to proteases. The low solubility of proteins is related to their molecular conformation, and is an expression of highly organized, tightly folded, compact protein structures which prevent hydration of protein molecules and protect peptidase-susceptible bonds from enzyme attack. This may also be an important factor which is responsible for the lower digestibility of zein found in maize protein (Bozzini and Silano, 1977).

The presence of protease inhibitors in seeds and the negative effects on protein digestion in some legumes is well documented (Liener, 1975). Barley contains two main trypsin inhibitors and two main chymotrypsin inhibitors. The concentration of chymotrypsin inhibitors varies with cultivar, whereas the amount of trypsin inhibitors appears to be relatively constant in different genotypes. The two main chymotrypsin inhibitors are pepsin-labile and are not

considered to have any negative nutritional effect in animals. One of the trypsin inhibitors is also labile to pepsin; however, the other trypsin inhibitor is completely stable to pepsin. The activity of the pepsin-stable trypsin inhibitor represents about one-third of the total trypsin inhibitor activity in normal barley cultivars. This trypsin inhibitor is the only one found in the barley mutant Bomi Risø 1508, and its concentration is three to four times greater than in normal cultivars (Pedersen and Boisen, 1982). A lower protein digestibility of this mutant compared to its parent barley has been reported (Newman and El-Negoumy, 1980), but according to Pedersen and Boisen (1982) depressed protein digestibility was not caused by the relatively high concentration of pepsin-stable inhibitor. Barley contains low levels of trypsin inhibitors and it is therefore unlikely that they have a negative influence on the nutritional value of proteins (Warchalewski and Skupin, 1973).

The low nutritional quality of cereal protein is primarily due to the high content of storage protein, prolamin. It appears then that an improvement of cereal protein quality can be accomplished by developing new cultivars with a higher content of salt soluble proteins and a lower content of prolamins (Mertz et al., 1975). In fact, the major cause of the increase in lysine content in opaque-2 maize and in the Risø 1508 barley is a decreased amount of prolamines, as well as increased amount of soluble proteins and glutelins.

Other factors which reduce amino acid availability are inhibition of enzyme-binding sites and inaccessibility of the protein due to indigestible fibrous cell walls, bulky structure, or excessive cross-linkages in the molecule (Erbersdobler, 1976).

Protein-phenolic Compound Interactions

The largest group of plant constituents capable of interacting with proteins is the phenolic compounds, including tannins, gossypol and proanthocyanidins. These compounds are known to associate with proteins, forming insoluble complexes in the gastrointestinal tract of monogastric animals, thus limiting protein utilization. Proteins and phenolic compounds interact either reversibly through hydrogen or hydrophobic bonding or irreversibly via oxidation of the phenolic compound to a reactive quinone. Reversible bindings take place primarily with complex polyphenols, such as the condensed tannins, but have also been observed with monomeric phenols. This complex is easily disrupted by agents with hydrogen-bonding properties, the most important of which are polyvinyl pyrolidone and polyethylene glycol (Anderson and Køie, 1975). Covalent bondings of polyphenols with protein can occur after oxidation of the phenolic to a quinone. Because these compounds take on an intense coloration commonly observed when fruits become cut or bruised, this reaction has been termed "enzymatic browning" (Anderson andf Køie, 1975).

Gossypol, a toxic phenolic compound found in cottonseed can through its carbonyl groups react with the free amino acid group of proteins, such as the epsilon amino group of lysine, and convert to

"bound gossypol," which is not toxic. This reaction is analogous to the browning reaction with sugar. The nutritional value of cottonseed protein is severely reduced by this treatment, because the first limiting amino acid of cottonseed, lysine, is made unavailable. Alternative methods have been proposed for the removal of gossypol without damaging the protein, including extraction of gossypol with specific solvents and separation of the whole glands from the rest of the cotelydon. Also, through plant breeding "glandless cotton" has been developed, the seed of which contains no gossypol (Berk, 1976).

Tannins have been known to limit utilization of protein in animal and human diets. Numerous studies have shown that the protein degradability of high-tannin grain sorghum is much less than that of low-tannin cultivars (Cousins et al., 1981). Amino acid studies have shown declines in the digestibility of proline, histidine, and glycine both in rats (Ford and Hewitt, 1979) and pigs (Cousins et al., 1981) fed high-tannin sorghum. This agrees with work by Hagerman and Butler (1980), who found that the tannins from sorghum were associated with proteins with very high proline and glycine concentration. Hewett and Ford (1982) reported that addition of polyethylene glycol improves the digestibilty of high-tannin varieties of sorghum. They suggested that supplementing diets with polyethylene glycol may be a simple way to reverse the depression in the nutritional quality of sorghums. Although the interaction between tannin content and protein quality observed in sorghum is not found in any other cereal species, tannins are at least partially responsible for the relatively low protein digestibility of some barley cultivars. Eggum and Christensen (1975)

calculated a negative correlation between true protein digestibility and tannin content of 29 barley samples. Eckman (1981) reported a highly significant negative correlation (r=-0.74) between barley protein digestibility in vitro and polyvinyl pyrolidone-extracted tannins from 11 cultivars. It is generally believed that tannins interfere with the digestion of protein in growing animals either by complexing with proteins, thereby decreasing the availability of proteins, by interfering with the epithelial protective mucus of the digestive tract, or by inactivating various digestive enzymes, through similar processes of complex formation. In vitro studies have shown that digestive enzymes, including trypsin, alpha-amylase and lipase, were inhibited to a great extent by condensed tannins (Oh and Hoff, 1986). Thus, the cause of the nitrogen indigestibility in the presence of tannins is two-fold, as the tannins complex with both the dietary protein and the proteolytic enzymes. Because free tannins also have carcinogenic activity, it appears that protein-tannin complexes present in cereal and legume seeds may represent partially detoxified tannins. Proanthocyanidins may have similar antinutritional influences as tannins.

Proanthocyanidins have been suggested as possible inhibitors of protein utilization of barleys. The function of proanthocyanidins is not well understood, although it is suspected that many proanthocyanidins associate with proteins, forming insoluble complexes in the gastrointestinal tracts of monogastric animals, thus reducing the availability of amino acids (Munck, 1964). As previously mentioned, proanthocyanidin-free barley mutants have been cultivated

through mutation breeding programs, and it is possible that these barleys may have a higher nutritional value through more digestible proteins, as in the case of low-tannin grain sorghum (Cousins et al., 1981). When proanthocyanidin-free barley mutants are fed to monogastric animals, an improved feed to gain ratio is frequently observed. Chicks fed proanthocyanidin-free barleys produced better gains and had an improved feed efficiency compared to chicks fed the corresponding control lines containing proanthocyanidins (Newman et al., 1984). Feeding trials with rats point in the same direction. Recent studies show that the proanthocyanidin-free mutant ANT-13-13 (Jende-Strid, 1978) had more digestible protein than normal barleys as measured with rats, and produced greater gains (Newman et al., 1984). These studies indicate that the feeding quality of proanthocyanidin-free barley is in most cases as good as or better than present day barley cultivars.

Animal Models

Taste Preference in Chicks

The ancestors of today's domestic fowl apparently were omnivorous, eating a diet consisting of seeds, plants, insects, etc. Thus, as with all omnivores, they were faced with the problem of a diet varying in nutritional quality and caloric density. Three possibilities exist for consuming a balanced diet in this situation. First, the pattern of variability of dietary items in the animal's habitat must be sufficient to provide a balanced diet. Second, the animal's preferences for the various feed stuffs must be such that the relative intake of available

items provides a balanced diet. Third, the animal must have a feedback loop which changes the relative intake of available items as deficiencies or surpluses develop in intake. The chicken presents an interesting model for the exploration of these possibilities since it differs from the usual test animal, the laboratory rat, in that it has few taste buds; its diet may include unhulled seeds, plants and intact insects; and that it stores ingested food in the crop prior to digesting it (Kaufman et al., 1978).

Taste Organs of the Chick. The function of taste in an animal is to encourage the ingestion of nutrients, to discriminate among foods that are available and possibly to avoid those that are toxic (Lindenmaier and Kare, 1959). It is suspected that the taste system in a particular species will serve to compliment the metabolic and dietary requirement of that species. (Lindenmaier and Kare, 1959).

Chickens have been reported to have a sense of taste, and this sense is said to be most acute when the flavoring agent is given via the drinking water versus the food (Lilburn et al., 1984). However, the classification of taste reception, as recognized by man, i.e. sweet, salty, sour and bitter, does not appear to be applicable to the fowl. For instance, a study done by Kaufman et al., (1978) demonstrated that the appeal of sucrose was totally lacking in chicks. Chicks were supplied with a sucrose solution in addition to their diet. The birds showed no preference for the sucrose, but increased intake of sucrose solution in response to decreased calories in the diet. Thus, only when caloric intake is restricted will a chicken select a sucrose

solution in order to make up for the deficiency.

Avian taste buds were first described by Botezat (1904). A study done by Lindenmaier and Kare (1959) showed that chicks have taste buds in reduced numbers and restricted distribution compared to other species. Taste buds in chicks are 30 microns wide and 70 microns long, and are found at the base of the tongue and the floor of the pharynx, caudal to the row of large horny papillae. Most of the taste buds are closely associated with the salivary glands. The highly cornified anterior part of the tongue is devoid of taste buds. Avian taste buds are intermediate in shape between those of fish and mammals, and resemble those of reptiles. (Lindenmaier and Kare, 1959). The number of taste buds in chickens increases with age; for instance day-old chicks have an average of 8 taste buds, which increases to 24 in 3-month old chicks (Lindenmaier and Kare, 1959).

Chicks and Feed Selection. Many studies have been conducted on the ability of animals to select a nutritionally optimum diet. It has been shown in several studies that when rats are given the opportunity to select the components of their diets, they do so in a way which results in a normal growth. These findings have led to the general hypothesis that selection patterns reflect the nutritional requirements of the organism. Collier et al., (1969) showed that active rats given a choice of protein and carbohydrates chose a higher proportion of carbohydrates than did non-active animals. It was concluded that active rats have a different nutritional requirement and this difference reflects changes in intermediary metabolism.

Many studies report that chicks were able to self-select nutrients in proportions adequate to sustain normal or near normal growth. Feeding trials have shown that when chicks are given the opportunity to select diets, growth can be maintained as they mature while levels of protein decrease. A study done by Kaufman et al. (1978) showed that chickens given the opportunity to select from two components varying widely in protein and carbohydrate content were able to construct a diet which resulted in a growth rate approximately equal to that of the control. This growth rate was achieved with a lower level of protein than that of the control diet.

It has been proposed that chicks are able to compensate for varying protein levels in the diet by altering intake. Graham (1934) demonstrated this in a study in which chicks were fed different levels of protein. Results showed a compensation for both increases and decreases in protein concentration. For instance, chicks increased their feed intake with low protein levels. Bray (1973), has reported decreases in pullets' feed intake from a preferred feeder in a free choice situation following increases as small as 1.0% in dl-methionine level of a standard diet.

A similar study done by Hill and Dansky (1954) showed contradicting results. Chicks' diets that were diluted with oat hulls from 20.1% to 18.1% or 16.1% protein showed no compensation in intake, which led to the conclusion that chicks were unable to distinguish differences in protein level. The dilutions used in this experiment might have been small enough that any compensation on the part of the chickens was masked by daily variability in intake, or that the protein

levels in all three diets were higher than that needed by chickens.

In a study done by Leshner et al., (1971), chicks were housed in the cold (2°C) and given the opportunity to select the components of their diets from protein and carbohydrate fractions. Chicks housed in the cold had depressed growth, which was not overcome by allowing the birds the opportunity to select their diets. The cold selection group increased total caloric intake by increasing their consumption of carbohydrates, but protein intake remained similar to the control group. Thus, in the cold environment, birds allowed to select from carbohydrate or protein fractions were apparently able to partition their diet into that required for both growth and energy for maintenance. These data suggest that the level of protein selected is determined by age and growth factors, while the level of carbohydrates selected is determined by energy requirements.

Evaluation of Protein Quality

The quality of a protein is dependent on its amino acid composition and digestibility. Biological evaluation is the preferred method for determination of protein quality since it is the ability of a protein to support growth and maintenance that determines its ultimate value. Methods for evaluation of protein quality are therefore based on the retention of nitrogen in the body. Animal models used in this study were rats, chicks and pigs.

Biological Value (BV). A way of describing the nutritive value of protein is by its BV in nitrogen-balance with rats, which is the percentage of digested and absorbed nitrogen which is retained and not excreted in the urine (Pike and Brown, 1984). Standard conditions for measurements of biological value involve the use of low-protein diets (approximately 9-10% protein) and measurement of true digestibility and net nitrogen retention. The biological value indicates how closely the pattern of amino acids in a particular feed protein will satisfy the needs of the growing animal. Biological value technique to evaluate specific proteins is most commonly measured in rats, due to easy separation of urine and feces. Biological value represents the proportion of absorbed nitrogen retained.

True Digestible Protein (TDP). A method for evaluating a feed protein is measurement of its digestibility. True digestible protein is represented by the formula: N intake - Fecal N - Endogenous fecal N. Digestibility of a protein can be determined by measuring the intake of a feed nitrogen by an animal and its nitrogen output in feces (Scott et al., 1976).

Net Protein Utilization (NPU). Quantitatively, NPU is represented by the formula: N retained/N intake. The net protein utilization is equivalent to BV x TDP and it is a measure of both the digestibility and the BV of a protein. Net protein utilization represents the proportion of a food nitrogen retained (Pike and Brown, 1984).

Protein Efficiency Ratio (PER). One of the oldest methods of measuring protein quality is the protein efficiency ratio, which is the weight gain of an animal divided by protein intake. This test is based mainly on the relationship between growth of an animal and the level of essential amino acids in the diet (Pike and Brown, 1984).

$$\text{PER} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}}$$

MATERIALS AND METHODS

Barleys

Six pairs of malting barley cultivars and their proanthocyanidin-free mutant (ANT-) were grown in adjacent plots at the Montana Agricultural Experiment Station, Bozeman MT, in 1986. Parent and ANT- barleys included in this study are presented in Table 1. Clark was used as a control barley. The barleys were cleaned and a sample from each was obtained to use for chemical and physical analyses.

Table 1. The Six Normal Cultivars of Barley and Their Proanthocyanidin-free (Ant) Mutants

Pair no.	Normal cultivar	ANT- mutant
1	Triumph	Galant
2	Moravian III	ANT 605
3	Andre	ANT 587
4	Robust	ANT 625
5	Advance	ANT 537
6	Karla	ANT 504

Chemical Analysis

The following chemical analyses were conducted on samples: moisture, crude protein, ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, calcium, phosphorus, amino acid profile, starch, beta-glucans (total, soluble and insoluble), relative viscosity, proanthocyanidins and vanillin-HCL stain. The amino acid

determinations were conducted by Amino Acid Analyses Laboratory[1], and the proximate components were determined according to AOAC (1980). Calcium was determined according to the Kramer-Tisdal method (Clark and Collip, 1925) and phosphorus content measured by Fiske and Subbarow (1925). Determinations for NDF and ADF were done by methods described by Roth et al., (1982). Starch analyses were done according to Åman and Hesselman (1984) and relative viscosity was done according to Coon et al. (1978). Beta-glucans were analysed by the method of Åman and Graham (1987).

The kernels were stained with vanillin-HCL according to the method of Aastrup et al. (1984) to indicate the presence or absence of proanthocyanidins in the aleurone layer of the barley kernels. Proanthocyanidin content was determined according to von Wettstein et al. (1977) at the Carlsberg Research Laboratory in Copenhagen, Denmark.

Physical Measurements

The following physical traits were conducted: percentage plump and thin kernels, kernel weight and volume (test weight). Percentage plump was the percentage of kernels on and above a 6/64 inch screen and percentage thin kernels was the percentage of the kernels passing through a 5.5/64 inch screen. Test weight was expressed as kg/hl. Kernel weight was determined as 30g seed per number of kernels counted x 1000, and was expressed as thousand kernel weight in grams. Physical measurements were performed at the Cereal Quality Laboratory at Montana State University.

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Animal Studies

Chick Growth Trial

Three barleys and their proanthocyanidin-free mutants were compared in each of two growth trials with chicks. The barleys were ground through a 1.59mm hammer mill screen and incorporated into vitamin, mineral and antibiotic supplemented diets (Table 2,3). Diets were formulated to be isonitrogenous at a 21% crude protein level to meet or exceed NRC (1984) using soybean meal to equalize the nitrogen component. An isocaloric, isonitrogenous 21% diet prepared from Clark barley was used as a control in each trial. Beta-glucanase was added to the barley diet at .05% to eliminate the detrimental effects of beta-glucans on the performance of the birds.

Day-old cockerel Hubbard broiler chicks were obtained from the Fors Hatchery in Puyallup, Washington. This strain was selected for use in this study because of its rapid growth rate and availability. Chicks were housed in battery-type cages with wire meshed floors in a room with continuous lighting and constant temperature (26.7°C). Chicks were numbered, banded and stratified by weight and randomly assigned to each diet. A 3-day adjustment period was allowed prior to initiation of the experiment in which a standard 21% protein corn-soy diet was fed. Feed and water were provided ad libitum. Twenty-one chicks were assigned to each diet for 21 days in three replications with 7 chicks per cage per treatment group. Daily feed consumption was recorded and individual body weights were measured twice a week. Feed