The nutritional value of six barleys and their proanthocyanidin-free mutants

by Kimberly Bolin Heintzman

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Home Economics

Montana State University

Abstract:

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Chick growth trials indicated a difference (P=0.00) for feed to gain ratios between the six barley cultivars. Comparison between the parent and mutant barleys showed the feed to gain means to be slightly higher (P=0.07) for the mutants (1.62) than the parents (1.58). Two pairs of parent and mutant barleys showed a significant difference for feed to gain; Robust was higher (P = 0.01) than its mutant and Advance was lower (P= 0.00) than its mutant. Feed to gain had a negative correlation (r =-0.85) with the starch content of the barleys.

Rat nitrogen balance trials showed a difference (P=0.00) between the six barley cultivars for true protein digestibility (TPD), biological value and net protein utilization (NPU). Comparison between the parent and mutant barleys showed the mutants to be higher (P <0.05) in TPD (86%) and NPU (66%) than the . parents (83% and 63%, respectively) .

Trained taste panelists could not distinguish the difference between parent and mutant barleys tested in a muffin. Consumers tended to favor a mild taste in a muffin and judged one Andre barley muffin to be ,similar to the wheat muffin. The majority of trained and consumer judges preferred the wheat muffin over the barley muffins.

It is concluded that all constituents of barley, including protein, starch, fiber, beta-glucans and proanthocyanidins, influence the nutritional value and must be considered when comparisons are made.

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APPROVAL

of a thesis submitted by

Kimberly Bolin Heintzman

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

December 5, 1986
Date

Rosemary K Newman
Chairperson, Graduate Committee

Approved for the Major Department

December 5, 1986
Date

Margaret Briggs
Head, Major Department

Approved for the College of Graduate Studies

December 8, 1986
Date

Henry L Parsons
Graduate Dean

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Date November 21, 1986

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ABSTRACT

The purpose of this study was to determine the effect of proanthocyanidins on the digestibility of protein in barley. Six normal barley cultivars (Triumph, Moravian III, Andre, Robust, Advance and Karla) and their proanthocyanidin-free mutants were analyzed for their chemical composition, physical measurements and animal trial performance. Two pairs were compared in a baked product.

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Rat nitrogen balance trials showed a difference (P=0.00) between the six barley cultivars for true protein digestibility (TPD), biological value and net protein utilization (NPU). Comparison between the parent and mutant barleys showed the mutants to be higher (P<0.05) in TPD (86%) and NPU (66%) than the parents (83% and 63%, respectively).

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It is concluded that all constituents of barley, including protein, starch, fiber, beta-glucans and proanthocyanidins, influence the nutritional value and must be considered when comparisons are made.

INTRODUCTION

The cultivation of barley (*Hordeum vulgare L.*) dates far back into human history; grains of 6-rowed barley have been discovered in Egypt dating from pre-dynastic and early dynastic periods (Kent, 1983; Profodcil Bulletin, 1981). Barley was used as a bread grain by the ancient Greeks and Romans. Greek coins dating from 413 B.C. to 50 B.C. incorporate ears or grains of barley into their design. Barley was the general food of the Roman gladiators, who were known as *hordearii*. Calcified remains of cakes made from barley and *Triticum monococcum*, dating from the Stone Age, have been found in Switzerland. Bread made from barley and rye flour formed the staple diet of the peasantry of England in the fifteenth century, while English nobles ate wheaten bread. As wheat and oats became more generally available, and with the cultivation of potatoes, barley ceased to be used for breadmaking. However, barley is still a staple food grain in the Near East (Kent, 1983).

Today, cereals are an important food staple of the world, meeting caloric and other nutritional needs of humans and animals. Barley composes about 12 percent of the world's total cereal production, ranking fourth in importance behind wheat, rice, and maize (MacKey, 1981).

The acreage of barley harvested in the United States is steadily increasing. Montana was second among the states in barley production in 1983 (77.7 million bushels) and then dropped to fifth in 1984 (59 million bushels) (Montana Agricultural Statistics, 1984). Preliminary statistics show production down to approximately 30 million bushels in 1985 (Montana Agricultural Statistics, 1985). The top five producing states in 1984, ranked from high to low, were North Dakota, Minnesota, Idaho, Washington, and Montana.

Barley, like many plants, contains various pigment compounds. Most brightly colored blue and red pigments in plants are anthocyanidins. Proanthocyanidins are defined as a group of flavonoids that yield anthocyanidin upon treatment with acid (Weinges and Nader, 1982). They are located between the aleurone layer and the seed coat (testa) of the barley kernel. Proanthocyanidins are suspected of reducing the nutritional value of barley by interfering with protein digestibility (Aastrup et al., 1984).

Much of the research on proanthocyanidin-free barleys has been generated by the brewing industry. Permanent haze and chill haze in beer are due to the precipitation of proteins by polyphenols such as proanthocyanidins known as anthocyanogens in the brewing industry. Beer brewed with proanthocyanidin-free barley has been found to have good haze stability without treatment with stabilizing agents

while maintaining all desirable beer characteristics (Wettstein et al., 1977).

These phenolic compounds may inhibit nutrient utilization of barleys by reducing the digestibility of protein in animals (Newman and McGuire, 1985). One study with rats and chicks (Newman et al., 1984) showed that the feed value of barley may be improved by using the proanthocyanidin-free mutant barleys.

The purpose of this study was to determine the effect of proanthocyanidins on the digestibility of protein in barley. The chemical characteristics of six varieties of barley and their proanthocyanidin-free mutants were compared for their effect on nutritional value. The normal barley cultivars were Triumph, Moravian III, Andre, Robust, Advance and Karla. These were chosen because of their potential as commercial cultivars. The general objectives of the study included:

1. Obtain gross chemical composition of six parent and six mutant barleys,
2. Determine the physical properties of the kernels,
3. Determine growth and feed efficiency rates for chicks fed the parent and mutant barleys,
4. Compare parent and mutant barleys for chick diet selection preference,
5. Measure nitrogen metabolism in rats for the parent and mutant barleys, and
6. Compare barley muffins to wheat muffins with chemical, objective and sensory evaluation.

LITERATURE REVIEW

Introduction

Barley is the world's fourth most important cereal crop, after wheat, maize, and rice. Barley was one of the earliest crops to be domesticated and has been cultivated since the beginnings of civilization. It is grown over a broader environmental range than any other cereal. Much of the world's barley is produced in regions with climates unfavorable for production of other major cereals. It has persisted as a major cereal crop through so many centuries because it has three unique characteristics: broad ecological adaptation, utility as a feed and food grain, and superiority of barley malt for use in brewing (Poehlman, 1985).

Kernel Structure and Composition

The chemical composition of different cereal grains varies widely, since it is influenced by genetic, soil, cultural and climatic factors. Amounts of proteins, lipids, carbohydrates, pigments, vitamins, minerals and total ash vary. Cereals are characterized by relatively low protein and high carbohydrate contents; the carbohydrates consist essentially of starch (90% or more), pentosans, and sugars (Pomeranz, 1982).

Breeding efforts to improve the nutritive value of cereal grains have concentrated on increasing protein content without decreasing protein quality (mainly retaining lysine concentration in the protein). The significance of protein distribution in the endosperm depends on the type of product that is likely to be consumed (Pomeranz, 1982).

Generally, the barley kernel is divided into the following botanical components: husk, pericarp, testa, germ, aleurone and endosperm (Figure 1).

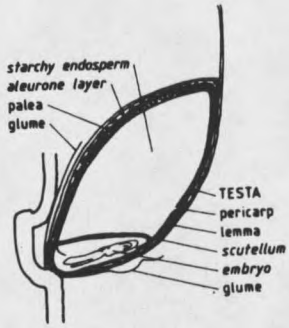


Figure 1. Structure of a barley kernel.

The husk is very low in protein, but rich in cellulose, hemicellulose and lignin. Nearly all the lignin of the kernel is assigned to the husk, making the cell wall very rigid and difficult to digest. The chemical composition of pericarp and testa resembles that of husk, but the fibers are not embedded in lignin. Polyphenols are present in the testa and between the testa layer and the aleurone. The aleurone cell walls are thick and mainly consist of arabinoxylan, carbohydrates and cellulose in that order. The aleurone consists of proteins and phytic acid

phosphorus. In barley, the aleurone layer is 2 to 3 cells thick and makes up approximately 7% of the dry matter and 13% of the protein. Starch is the major constituent of the endosperm (75-85%) together with the storage proteins hordeins and glutelins. The cell walls of these fractions are thinner than the cell walls from husk, pericarp, testa, and aleurone and are mainly composed of 1,3:1,4 beta-glucans. Hordeins are very rich in proline and glutamic acid, but low in essential amino acids such as lysine, methionine, and threonine (Bach Knudsen and Eggum, 1984; Aastrup and Outtrup, 1985).

Pigments and Phenolic Compounds

Anthocyanidins are a very large group of plant phenolics. These are the water soluble red, blue and purple pigments of flowers, fruit and vegetables. The name comes from a term proposed by Marquart in 1835 (cited by Berk, 1976), to denote the blue substance of *Centaurea cyanus*, cornflower (Berk, 1976).

All the names of anthocyanidin are derived from the names of plants. Pelargonidin is found in the strawberry; cyanidin in the purple fig, almond, mulberry, sweet cherry and elderberry; delphinidin in pomegranate and eggplant. The purple pigment of beetroot, betanin, was long thought to be an "unusual anthocyanin" as it contains nitrogen. The structure of betanin is now known to be quite different from that of flavonoid pigments (Berk, 1976).

Anthocyanidins may be degraded by oxidation, hydrolysis or polymerization. Influencing factors include temperature, pH, other cell constituents, enzymes and presence of metals (as in storage or cooking containers). The anthocyanin pigments change their color with a change in pH. Cyanine (the 3,5-diglucoside of cyanidin) for example, is red in acidic solution, purple at neutral pH and blue in alkaline medium. The change in color is assumed to be associated with a change in configuration (Berk, 1976).

The most serious problem which is posed by anthocyanin pigments in food technology is their pH dependence and lack of chemical stability. Reduction of anthocyanin destroys their color. Such reductive bleaching occurs in red berries, strawberries, etc., packed in unlacquered (plain) tin cans (Berk, 1976).

Anthocyanidins have -OH groups at C-3, 5 and 7. Glycosides usually occur in nature with attachment to the -OH at position 3. Substitutions on ring (B) result in formation of various anthocyanidins. Color varies with variation in molecular structure. Increased hydroxylation leads to increased blueness and increased methylation of -OH groups leads to increased redness. The combination and location of added groups affects the color of the pigment. Color is also influenced by the presence of other phenolic compounds (i.e. complexes with other flavonoids) (Outtrup, 1981). The general structure is shown in Figure 2.

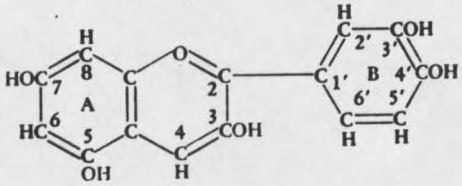


Figure 2. Cyanidin Structure

Proanthocyanidin is a collective term for all flavonoids that yield anthocyanidin upon treatment with acid (Weinges and Nader, 1982; Campbell et al., 1979).

(1) Leucoanthocyanidins (colorless) —H20→ Anthocyanidins; Dimeric Leucoanthocyanidins (substitution)

(2) Dimeric Proanthocyanidin —Acid→ Catechin; Epicatechin

(3) Proanthocyanidocatechins —Acid→ Anthocyanidins; Catechins

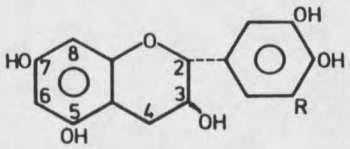
Fruits are particularly rich in proanthocyanidins. Seeds and unripe fruits contain more proanthocyanidins than ripe fruits. This may be evident in the puckery taste of underripe fruits (Berk, 1976; Weinges and Nader, 1982; Campbell et al., 1979).

Barley contains at least four trimeric proanthocyanidins, of which three are prodelphinidins (Outtrup and Schaumburg, 1981; Outtrup and Erdal, 1983;

Outtrup, 1981). The most abundant dimeric prodelphinidin in barley has a steric structure like procyanidin B3. Proanthocyanidins are built up of catechin units (Figures 3, 4, and 5). In Figure 3, the R group is substituted above or below the plane. In the proanthocyanidins such units are linked by either C4-C8 or C4-C6 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. From barley, only a few have been identified:

Procyanidin B3: 2 catechin units linked C4-C8, a dimeric prodelphinidin having either a procyanidin B1 structure or a procyanidin B3 structure,
Procyanidin B6: dimeric compound of 2 catechin units C4-C6 linked, and
Procyanidin C2: 3(+)-catechin units linked C4-C8.

A staining procedure (Aastrup, 1985) using vanillin-HCl has been used to locate the proanthocyanidins in mature barley grains. These flavonoids were found concentrated in the seed coat (testa) of the grains investigated (figure 1). Tannins reacting with vanillin-HCl have been demonstrated in the testa of sorghum. The proanthocyanidins are rendered visible by staining the sanded (or pearled) grains with a freshly prepared 1% vanillin-6M-HCl solution for 30 minutes. A distinct red color develops in the testa of the proanthocyanidin-containing grains, while the proanthocyanidin-free grains lack the red color. The color remains stable for at least 30 minutes.



R		compound
H	◢	(+)-catechin
	---	(-)-epicatechin
OH	◢	(+)-gallocatechin
	---	(-)-epigallocatechin

Figure 3. Catechin units of proanthocyanidins.

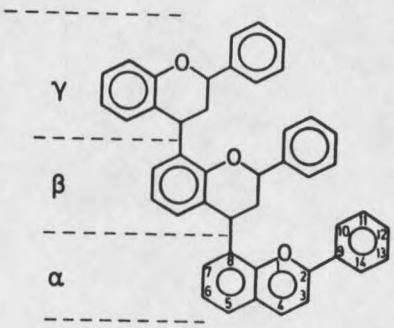


Figure 4. Numbering of units and ring atoms of trimeric proanthocyanidins.

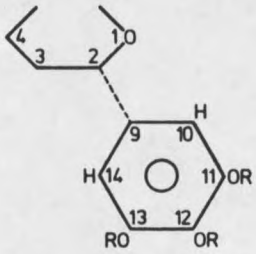


Figure 5. B-ring part of gallocatechin units.

The seed coat originates from at least three cell layers in the developing grain. Adjacent to the aleurone cells the nucleus cells with a thick epidermal wall and a cutica are found. Next are two cell layers of the testa bound by a cuticula bordering the cross cells of the pericarp. The technique presented by Aastrup et al. (1984) enables a study of the intercellular distribution of anthocyanins and proanthocyanidins in these three cell layers of the developing grain (Aastrup, 1985).

A study by Newman et al., (1984) compared proanthocyanidin-free barley to normal barley as a source of protein and energy in poultry and rat diets. The results suggest that the introduction of genetic blocks in the biosynthesis of flavonoids may enhance the feed value of barley.

Catechins take part in the enzymatic browning process of many foods. Together with leucoanthocyanidins they constitute the building blocks of the "condensed tannins." These make up the bulk of tannins in woods and barks. Tannins are responsible for the astringency of many foods, such as apples, pears, persimmons, dates, tea and cocoa. It is generally believed that astringency is associated with protein precipitation since other protein denaturants such as alcohols and heavy metal salts are also astringent (Berk, 1976).

Tannins are phenolic compounds which include catechins and leucoanthocyanidins and serve as substrates for enzymatic browning and contribute to astringency in many foods (Berk, 1976; Campbell et al., 1979). For example, in order for the browning in cut fruits and vegetables to occur, the following are needed:

SUBSTRATE (catechin or leucocyanidin)	+	ENZYME (called polyphenolase or polyphenol oxidase)	+	O_2

Eggum et al. (1983) performed two series of balance experiments with growing rats to test the effects of black tea, green tea, coffee and cocoa on protein and energy utilization. These products were added to the basal diet of barley and soybean meal. In both experiments, tea and coffee had significantly negative effects on true digestibility and biological value, while digestible energy was only slightly affected. Cocoa had no effect, its protein being completely indigestible. The strongest negative effect was recorded for black tea, which also had the highest tannin content. As tannin concentration in both teas and coffee increased the negative effects increased. These might in part be explained by the anti-nutritional effects of tannin. Proanthocyanidins may have similar anti-nutritional influence as tannins. Nonpigmented rice has been shown to have a higher nutritional value than pigmented rice (Eggum et al., 1982).

Several studies have demonstrated that reconstitution deactivates tannins and improves the nutritive value of high tannin sorghums for chickens and swine. In a study by Mitaru et al. (1985), two high and one low tannin sorghum type grains were used to study the effect of reconstitution (high moisture storage) and boiling treatments of the grain on protein and amino acid digestibilities. Reconstitution improved the protein and amino acid digestibilities in high, but not low, tannin sorghums. Boiling treatment had a detrimental effect on the protein and amino acid digestibilities in both high and low tannin sorghums.

Biosynthesis of Procyanidins

The site of blockage, as shown in Figure 6 may determine some of the biological and nutritional characteristics of mutant barleys (Kristiansen, 1984).

The most likely sites of action of ANT 13, 17, 18 and 19 in the biosynthetic pathway to catechin and procyanidins are indicated in Figure 6, together with the sites of the other ANT genes (ANT 1-12, 14-16) known to participate in anthocyanin but not proanthocyanidin biosynthesis. The leucocyanidin rather than dihydroquercetin apparently serves as the last common intermediate in the synthesis of anthocyanins and proanthocyanidins (Kristiansen, 1984).

The location of the gene blocks of the proanthocyanidin-free barleys included in the research for this thesis are as follows: ANT 13 (ANT 537 and ANT 605)

and ANT 17 (Galant, ANT 504, ANT 537, ANT 625) (B. Jende-Strid, personal communication, 1986).

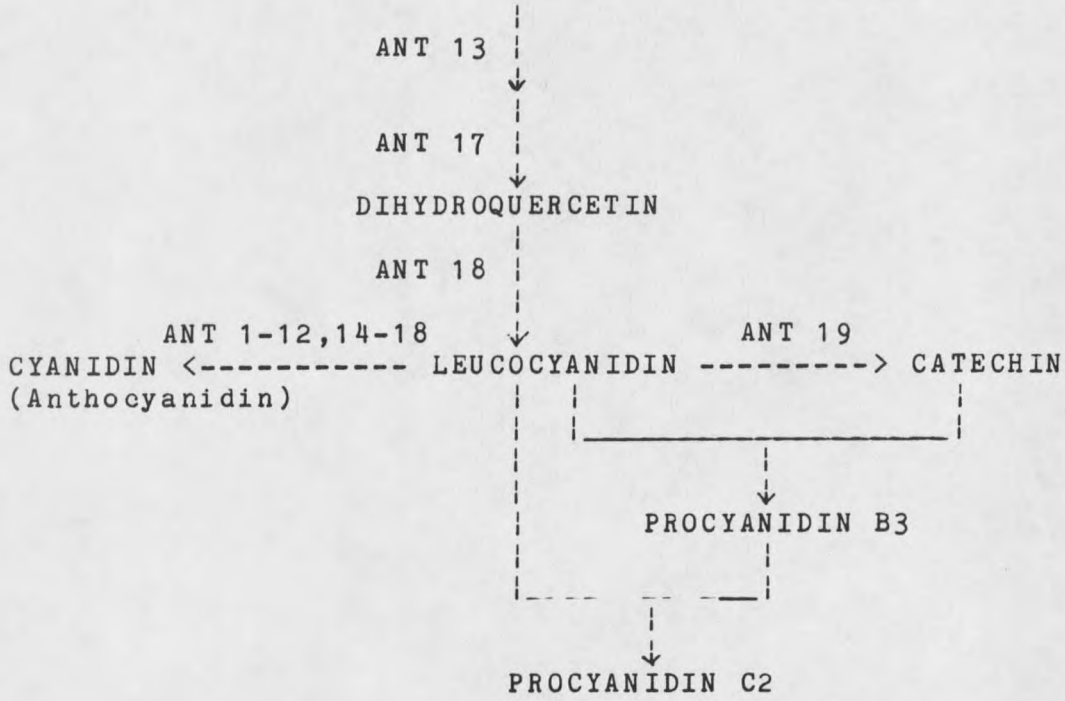


Figure 6. Proposed biosynthetic pathway leading from dihydroquercetin to catechin, procyanidins and cyanidin showing the likely sites of action of 19 ANT genes.

Jende-Strid (1978) studied the anthocyanin, anthocyanidin and proanthocyanidin content of 52 induced barley mutants with altered anthocyanin pigmentation in different organs of the plant. Comparing these mutants, no correlation between the amount of anthocyanin in the plant and the amount of proanthocyanidin in the dry grains was found.

Nutritional Value

Nutritional quality of barley refers to a relative merit in providing available food nutrients that are essential to humans and other animals. This encompasses the total nutrients present and the biological balance and availability of these nutrients. Certain components such as fiber and amino acids may be classified as important in determining the quality of barley for certain animal species being fed for a specific purpose (Newman and McGuire, 1985).

Cereals are not only the main crops for producing energy in food and feed, but they also supply most of the protein consumed by humans or used for animal production in certain areas of the world. The inferior nutritional value of cereal protein is primarily due to the high content of the storage protein prolamin. Storage proteins generally have a high content of proline and glutamine, and low content of lysine and other essential amino acids. The actual prolamin content depends on the nitrogen nutrition of the plants during seed development. If the nutritionally inferior storage protein could be converted into protein with a better nutritional value by plant breeding, it would have great impact on human nutrition in many areas as well as plant production (Doll, 1984; Newman and McGuire, 1985).

The potential for large variations of different barley varieties in protein synthesis is demonstrated by the differences in protein content, true digestibility,

biological value, net protein utilization and utilizable protein. Past research involved qualitative evaluation of cereal grains emphasizing physical properties rather than nutritional quality characteristics. However, recent years have brought increased awareness of the need for more plentiful as well as more nutritious foods (Eggum, 1984).

The grain yield and contents of the quantitatively predominant nutritional constituents of barley grain were determined by Torp et al. (1981) in nine adapted spring barley varieties each grown at seven European locations with three or four replications. The largest variation in nutritional composition was due to different environmental conditions, but genotypic effects were also present. The nutritional composition of the grain was influenced by the grain yield level. The percentage of dietary fiber and protein decreased with increasing grain yield, but some varietal differences which were independent of the grain yield level could be established. The protein quality depended upon the protein level, when the protein contained more low-lysine prolamin relative to non-prolamin protein at high than at low protein levels.

DeMan and Dondeyne (1985) studied the impact of nitrogen fertilization level on protein content and total fatty acid (TFA) content of barley grains. Increasing amounts of nitrogen fertilizer resulted in a higher protein content and a very slightly lowered TFA in the grains.

A study by Buckley and Devlin (1983) on the influence of processing on barley showed that various methods had little effect on digestibility, while cooking at high moisture levels tended to benefit digestibility (in vitro dry matter disappearance). The processing treatments compared were: dry ground, dry heated, ground reconstituted, reconstituted whole grain, steamed grain.

During the malting of barley, the vitamin B1 content changes little, but riboflavin and pyridoxine increase two fold. Pantothenic acid and biotin increase 30-40%, and a small increase in niacin from malting pearl barley contains, per gram approximately 1.2, 0.35, and 25ug of vitamin B1, riboflavin, and niacin, respectively (Kent, 1983).

Lysine Availability

Lysine is commonly the limiting amino acid in barley and other cereal grains. During the heating of proteins in food systems, chemical reactions can occur between the epsilon-amino group of lysine and a certain reactive carbohydrate or lipid grouping. The absorption and the retention of the lysine may both be reduced, and proteins so affected show lowered value when assayed by any bioassay technique. Thus, an amino acid can be present by chemical analysis but be effectively absent for an animal consuming it (Pellet, 1978).

With growth assay, the primary objective is to determine the slope of the response in growth to increasing concentration of some limiting nutrient. Gous and Morris (1985) conducted three experiments on male broiler chicks between one and three weeks of age to determine their response to dietary lysine concentrations. Intake of the most limiting amino acid (lysine) was the most important factor determining growth rate. Protein intake was of little or no importance.

The lysine content is highest in the aleurone layer and lowest in the endosperm. Since the endosperm possesses the highest digestibility and the lowest lysine content, lower true protein digestibility values for lysine will be obtained when estimated on total nitrogen (Eggum, 1973).

DeMuelenaere et al. (1967) examined lysine availability in cornand rice proteins, determined by growth and fecal analysis as well as the influence of the type of carbohydrate, amino acid pattern in the diet and caloric content of the diet. Values obtained by the growth method were influenced by changes in the composition of the diet and the method of calculating availability. They were most reproducible and least influenced by other factors when availability was calculated as a function of lysine consumption rather than lysine level in the diet. The growth method in which the standard curve of weight gain versus lysine consumed was subject to the least variation.

Differences in the ratio of available lysine to true digestibility show that lysine is not necessarily lost to the body proportionally to protein. Digestion of lysine (availability determined by fecal method) and digestion of the protein are therefore influenced by different factors.

A simple and rapid method involving the use of TNBS (2,4,6-trinitrobenzene-sulfonic acid) has been used to determine specifically the lysine content of proteins and the available lysine of protein foodstuffs. This method was used in research for this thesis. TNBS reacts specifically with primary amino groups (Kakade and Liener, 1969).

Rawson and Mahoney (1983) have used dye-binding techniques with Remazol Brilliant Blue R to measure reactive lysine in milk powder. Maillard-browning reactions in milk powder primarily involve the lysine residues. The authors modified techniques by doubling dye concentration and correcting for the dye which binds to the protein, independent of its free amino group content. The resulting dye-binding values reflect changes in reactive lysine, but tend to underestimate losses.

Nordheim and Coon (1984) compared four methods for determining available lysine for 23 animal protein meals. The correlation coefficients (r) for chick bioassay (CBA) versus 2,4,6-trinitrobenzene sulfonic acid (TNBS) lysine, 1-fluoro-2,4-dinitrobenzene (FDNB) lysine, digestible lysine

(DL) and total lysine (TL) were .90, .83, .97, and .93 respectively, for all animal proteins tested.

Starch and Fiber

Starch exists in two molecular forms, linear or branched, and is composed entirely of alpha-glucan or D-glucopyranose units. It can either be joined in straight chains linked alpha-1,4 called amylose or exist as amylopectin where the alpha-1,4 units are branched through alpha-1,6 linkages. Chains as long as 2000 glucose units make up amylose whereas amylopectin averages only 24 - 26 glucose units. Normal barley starch contains a 75:25 ratio of amylopectin to amylose, respectively (Briggs, 1978). Starch deposition parallels the increase in dry matter. About 75 - 80% of barley starch is found in the endosperm (Munck, 1981; Hofer, 1985).

Barley contains varying quantities of structural carbohydrates loosely defined as fiber; the total quantity is principally affected by the presence or absence of the hull or husk. Barley fiber has little or no energy value for nonruminant animals and certain of the water-soluble carbohydrate components of fiber may create digestive problems, especially in poultry (Newman and McGuire, 1985).

Schimberni et al. (1982) tested corn bran (CB), oat hull flour (OHF) and barley hull flour (BHF) as dietary fiber sources. Barley hull flour averaged 26.0% crude fiber. The dietary fiber content of 72.6% included 28.2%

cellulose, 33.9% hemicellulose, and 10.4% lignin. Water and oil absorption, density measurements and water holding capacity were comparable in all the samples investigated whereas CB exhibited the highest values of cation exchange capacity.

Increasing cereal fiber in human diets is known to decrease transit time and increase stool weight. In many studies the increase in cereal fiber intake has been provided by the addition of wheat bran to the diets or substitution of whole grain wheat for bread made from low extraction rate flour. Judd (1982) studied the acceptability of barley in human diets and to study its effects on the digestibility of nutrients in the subjects' diets. The barley used had a dietary fiber content of 15.3 g/100 g compared to a level of 9.6 g/100 g in whole wheat flour. Therefore substitution of barley for other cereals in the daily meals enabled a diet high in fiber to be provided without taking supplements. There were significant changes in number and type of bowel movements during consumption of the high fiber diet and fecal wet and dry weights were significantly reduced. Digestibilities of nitrogen, fat, dry matter and energy were all significanty lower on the high fiber diet.

Burger et al. (1984) investigated hepatic B-hydroxy-B-methyl-glutaryl CoA (HMG-CoA) reductase, cholesterol 7a-hydroxylase (7a-hyd) and fatty acid synthetase (FAS). The

activities of these enzymes, which are rate-limiting in the synthesis and degradation of cholesterol, and cholesterol levels were determined in chicks fed isonitrogenous corn and high-protein barley flour (HPBF) based diets. The HMG-CoA reductase (-27%), 7a-hyd (-30%), and serum cholesterol (-13%) were reduced, whereas FAS increased (28%) in comparison to a corn-based (control) diet. A petroleum ether-soluble fraction of HPBF produced increased body weight, decreased HMG-CoA reductase, FAS and serum TAG and cholesterol. The methanol-soluble fraction produced lower HMG-CoA reductase and serum cholesterol and increased FAS activity. These effects were duplicated in 7-week old broiler chickens which also showed a significant decrease in cholesterol-LDL (low density lipoproteins) levels by these fractions. This research points to a direct action of the plant material on cholesterol biosynthesis with a concomitant decrease of LDL cholesterol.

Chicks fed waxy barley with added dietary cholesterol had significantly lower serum cholesterol than chicks fed the same diet with supplemental beta-glucanase. This suggests the cholesterol-lowering effect was due to beta-glucans in the barley (Fadel et al., 1986).

Barley Uses

The principal uses for barley are as feed for domestic animals, for malting and brewing in the manufacture of beer, and for distilling in whiskey manufacture. Barley is now

the fourth most important cereal covering about 12% of the world's total cereal production (Munck, 1981; Profodcil Bulletin, 1981).

Relatively high consumers of barley live in the Far East, North Africa, and the Middle East Regions. In these regions, much of the barley is consumed as pearled grain for soups, as flour for flat-type bread, and as ground grain to be cooked and eaten as porridge. No country in the world has a diet based exclusively, or even mainly, on milled barley products (Kent, 1983; Profodcil Bulletin, 1981; Newman and McGuire, 1985).

Milling

Barley is milled to make blocked barley, pearl barley, barley groats, barley flakes, and barley flour for human consumption. Removal of the hull or husk of barley, which is largely indigestible, is an important part of the milling process. Good quality in barley for milling implies absence of sprouting, absence of discoloration due to weathering, freedom from fungal attack and insect infestation or damage, soundness of kernel and absence of undesirable aroma or flavor (Newman and McGuire, 1985; Kent, 1983; Munck, 1981).

The hardness of the barley grain is a characteristic dependent upon type and variety. Types with a blue-colored aleurone layer tend to be harder than types without it. For milling purposes the harder types are preferred, since the objective is generally not to produce flour but to remove

the hull and bran by superficial abrasion, yielding particles which retain the shape of the whole grain. Softer grains tend to fragment in the milling process, leading to a decrease in the yield of highest quality products. Barley for milling should have as low a hull content as possible. Thin kernels also decrease milling quality. With a higher hull content than normal, they make a small contribution to the yield of milled product (Kent 1983).

Barley is cleaned on machines similar to those used for wheat cleaning. The sizes of sieve apertures and indents are modified for the comparatively larger size of barley grains. Both blocking (de-hulling) and pearling (rounding) of barley are abrasive scouring processes, differing from each other merely in degree of removal of the superficial layers of the grain. Blocking removes only part of the husk. This procedure must be accomplished with minimal injury to the kernels. Pearling is carried out in two stages which remove the remainder of the husk and part of the endosperm. The average yield of pearl barley is ~67% of the whole barley. Pearl barley is used in soups and dressing and for the manufacture of puffed barley, a ready-to-eat breakfast cereal. Pearl barley is also a starting material for the manufacture of barley flour. Milled barley products are also used for extruded foods, snacks, as croutons for soup and salad dressings, and as crunches for nut substitutes (Kent, 1983; Newman and McGuire, 1985).

Flour

Conventional roller milling yields four major streams: flour, shorts, tailings flour and bran. Flour comes mainly from the endosperm; shorts and tailings flour represent a mixture of aleurone, pericarp, some germ, and starch endosperm; while bran is principally hulls and pericarp (Sorum, 1977; Robbins and Pomeranz, 1971).

Barley flour is milled from pearl barley, blocked barley or unpearled hulless barley. Average extraction rate of 82% of barley flour is obtained from pearl barley representing 67% of the grain, giving an overall extraction rate of 55% based on the original whole grain. The yield of flour from hulless barley milled in Korea was ~10% greater, at a constant milling rate, than that from hulled barley. Bread characteristics were better with the hulless barley flour than with the hulled barley flour when barley flour was blended with wheat flour in ratios of 10:90 and 30:70, but bread quality deteriorated as the proportion of barley flour increased (Kent, 1983).

Barley malt is ground or milled to make malted barley flour. Uses for malt flour include a high diastatic supplement for bread flours, a flavor supplement in malt loaves, kibbled malts, malt extract and cereal syrups (Kent, 1983; Newman and McGuire, 1985).

Beer

The second largest use of barley is for malt. In the USA, an estimated 3.27 million metric tons of barley were malted in 1979. Of this amount, approximately 3.06 million metric tons were utilized for brewer's malt, up from 1.97 million metric tons 20 years earlier (Poehlman, 1985).

Beer is made by yeast fermentation of a sugary solution called wort which also contains nitrogenous compounds, vitamins, and trace elements necessary for growth of yeast. The sugars are traditionally derived from cereals, i.e. barley in Europe, rye in the Soviet Union (for Kvass), maize in Central America, rice in Japan (sake'), sorghum in Africa (Kaffir beer).

Beer is manufactured from barley using the processes of malting and brewing. The condition of barley for malting has a considerable effect on the yield and quality of the products. Besides varietal and species purity and satisfactory grain color, malting barley should be clean to fit for storage. More specific characteristics of barley required for malting and brewing are: high germination capacity and energy, with adequate enzyme activity, absence of de-husked or broken grains, capacity of grain modification by malting to produce maximum of extract when mashed, low content of husk and low protein and high starch content (Kent, 1983). Malting is a controlled germination process which produces a complement of enzymes to convert

cereal starches to fermentable sugars, to secure an adequate supply of amino acids and other minor nutrients for yeasts, and to modify the quality of the macromolecules which have such important effects on the physical quality of beer.

High nitrogen barley is unsuitable for malting because yield of extract is lower and its quality impaired. The malt from high nitrogen barley contains more of the soluble protein or albuminoid material than that from low-nitrogen barley. This soluble protein will pass into the extract, forming "haze" and possibly impairing the keeping quality of the beer. Furthermore, development of bacteria is more likely to occur in liquids with a high albumin content (Kent, 1983). Permanent haze and chill haze in beer are caused by precipitation of proteins with polyphenols (proanthocyanidins) which are derived from barley and hops (Wettstein et al., 1977; Outtrup and Erdal, 1983; Erdal et al., 1983). The sequence of operations in malting are as follows: screening (cleaning the grain), storage, steeping, draining, spreading on the malting floor, turning or ploughing, germination, drying in malt kilns and screening.

Brewing is the procedure of converting the starch to an alcohol solution by means of yeast fermentation. About 75% of the original starch is converted to alcohol by brewing. The sequence of operations in brewing is as follows: grinding of malt, steeping, filtering, sparging, flavoring,

boiling, filtering, seeding with yeast, fermenting, removal of yeast and pasteurization.

Liquors are also distilled from cereals. Those in which barley is used include: Scotch whiskey, Irish whiskey, and Dutch gin (Kent, 1983).

Wettstein et al. (1980) investigated whether mutants which block the biosynthesis of catechins and proanthocyanidins in the barley grain can prevent the formation of beer haze. Some 50 proanthocyanidin-free mutants have so far been isolated in different barley varieties after mutagen treatments with ethyl methanesulfonate and sodium azide. The recessive mutant ANT 13-13 from the cultivar Foma has been propagated and malting and brewings performed in pilot as well as production scale. Malt, wort, and beer made from the mutant were free of catechins and proanthocyanidins. Without any stabilizing treatment, the bottled beer had an excellent haze stability. The specific elimination of catechins and proanthocyanidins had no detrimental effects upon beer quality including flavor.

Animal Feed

The largest use of barley is for animal feed. When used as feed, grain should be cracked, ground, or rolled. Primarily, grain supplies carbohydrates and protein in the ration. Protein content varies from ten to fifteen percent, depending on the cultivar, climate and soil conditions

under which the barley grows. A high protein content in feed barley is suggested by Poehlman (1985). However, Newman and McGuire (1985) state that approximately 12% protein is the most desirable maximum level of protein in barley in order to maintain a high energy content.

As barley contains some fiber which is relatively indigestible for monogastrics, the preferred type of barley for animal feeding is that with a low husk content. Bell et al. (1983) reported that as the percentage of hull increased, digestible energy and digestible crude protein decreased; this may be largely due to the higher crude fiber, lower crude protein, and the low digestibility of hulls. Hull content is a major factor affecting digestible energy content of barley (Kent, 1983; Profodcil Bulletin, 1981).

Bhatty et al. (1975) showed hulless barley to be superior to covered barley in rat and chick trials. Several trials at Montana State University in Bozeman have shown no benefit of hulless over covered barley, or that the improvement did not justify the reduction in yield that is observed in hulless cultivars (Newman and McGuire, 1985) .

Bach Knudsen (1983) reported on the feasibility of breeding barley for feed quality. The economical considerations point out the dominant role of available energy over that of lysine. Based on the economic considerations, it is recommended in future plant breeding

of feed barley to determine the priorities in the following order 1) improvement in yield, 2) energy content, and 3) protein/essential amino acid content.

Animal Diet Selection

Numerous investigators have shown that when animals are given the opportunity to select the components of their diets, they do so in a way which results in normal growth. It has been suggested, therefore, that self-selected diets may reflect the nutritional requirements of the organism.

Lilburn et al. (1984) reported that taste buds in the chick are rudimentary and few in number and palatability has not been considered a major factor in poultry nutrition when nutritionally balanced diets are fed. However, the chicken has been reported to have a sense of taste and this sense is said to be most acute when the flavoring agent is offered via the drinking water versus the feed. The broad taste classifications (sweet, sour) as perceived by man are not applicable to the fowl.

A study by Kaufman et al. (1978) showed that male broiler chicks, given access to high protein and high carbohydrate diet fractions, demonstrated an ability to select a protein-carbohydrate ratio sufficient to maintain growth at near control levels. The level of protein selected was below that present in either control diet and declined with age. It is hypothesized that a feedback loop involving learning controls the selection mechanism.

Newman and Sands (1983) studied broiler chicks that were provided choices of synthetic diets adequate or low in lysine, and adequate in or devoid of lysine. In each case, chicks consumed some of each diet offered, but preference was shown for the adequate lysine diet.

Active rats given a choice of protein and carbohydrate chose a higher proportion of carbohydrates than did non-active animals. Results were interpreted as showing that active rats differ in their nutritional requirements and that this difference reflects changes in intermediary metabolism (Collier et al., 1969). Yokogoshi et al. (1978) found that weanling male rats given simultaneous access to two foods, containing 18% casein and 15% or 70% carbohydrate (dextrin), tended to consume only 29-35% as much protein as carbohydrate (i.e., protein/carbohydrate ratios were 0.29-0.35). With maturation, about half continued this pattern of nutrient choice, but the others abruptly began to consume considerably larger proportions of protein, exhibiting protein/carbohydrate ratios as high as 0.80-1.00.

Leshner et al. (1971) studied animals housed in the cold that were given the opportunity to select the components of their diets from protein and carbohydrate fractions. The cold selection group accomplished the higher food intake, which was stimulated by cold stress, entirely by increasing their consumption of carbohydrate; their intake of protein remained the same as that for the

selecting group in the normal environment. Apparently, protein consumption is not determined by deviation from the growth curve, but rather, appears to be a function of age. Rats of a given age eat a determined amount of protein without regard to their size. On the other hand, the increased food consumption of the rats in the cold suggests that intake is sensitive to current caloric needs and is the best source satisfying these needs.

MATERIALS AND METHODS

Barleys

The six cultivars of barley and their proanthocyanidin free (ANT) mutants investigated include:

Parent Barley	ANT Mutant
Triumph	Galant
Moravian III	ANT 605
Andre	ANT 587
Robust	ANT 625
Advance	ANT 537
Karla	ANT 504

These barleys were grown in adjacent plots at the Montana State University Agricultural Experiment Station farm west of Bozeman in 1985.

Chemical Analyses

The following is a list of the analyses completed on the six parent and six mutant barleys researched.

1. Moisture
2. Kjeldahl nitrogen
3. Ether extract
4. Neutral detergent fiber
5. Acid detergent fiber
6. Starch
7. Ash
8. Calcium
9. Phosphorus
10. Amino acid profile
11. Lysine availability
12. Relative viscosity
13. Beta-glucan (total, soluble and insoluble)
14. Vanillin-HCl stain
15. Proanthocyanidin

Proximate analyses and acid detergent fiber (ADF)were determined according to Association of Official Analytical Chemists Methods (AOAC, 1980). Neutral detergent fiber (NDF) was measured according to the Robertson and Van Soest (1977) method as modified by Roth et al. (1982). Estimated dietary fiber (EDF) was calculated using the method described by Åman and Hesselman (1984). Calcium was determined with the Kramer-Tisdall method (Clark and Collip, 1925) and phosphorus content measured utilizing the method of Fiske and Subbarow (1925). Starch analysis was done by methods described by Åman and Hesselman (1984). Bendalow's fallingball technique as described by Coon et al. (1978) was utilized for measuring alkaline extract relative viscosity. Beta-glucans were analyzed according to methods developed at the Swedish University of Agriculture (P. Åman, personal communication). Amino acids were determined using ion exchange chromatography by the Animal Science Department at North Dakota State University, Fargo. Lysine availability was determined by the method described by Kakade and Liener (1969) at the Home Economics Research Laboratory at Montana State University.

Vanillin-HCl stain was conducted according to methods described by Aastrup et al. (1984) to indicate presence of proanthocyanidin pigments in the seed coat of barley grains. Proanthocyanidin content was analyzed by methods described

by Wettstein et al. (1977) at the Carlsberg Research Laboratory in Copenhagen, Denmark.

Physical Measurement

Percent plump was the weight of kernels on and above a 6/64" screen; percent thin was the weight of kernels passing through the 5.5/64" screen. Test weight was expressed as kg/hl. Kernel weight was determined as 30 g seed per number of kernels counted x 1000, which was expressed as thousand kernel weight in grams.

Color was measured on ground whole kernels (barley flour) with an Agtron (reflectance spectrophotometer, Model M-500-A), according to the instructions in the operating manual from Magnuson Engineers, Inc (San Jose, CA).[1] The Agtron measures the relative color of material in four monochromic spectral frequencies: red, green, blue and yellow. The above tests were all performed at the Cereal Quality Laboratory at Montana State University. Triplicate measures were performed on each sample.

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[1] Mention of a trademark, vendor, or proprietary product does not constitute or warranty use of the product by Montana State University and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Animal Studies

Chick Growth Trials

Chick trials were housed in Herrick Hall. Three barley pairs (parent and mutant) and one control (corn-soybean meal) were included in each of two trials. One day-old cockerel Hubbard broiler chicks from Fors Hatchery in Puyallup, Washington were housed in a battery-type cage with thermostatically controlled compartments with wire mesh floors. The room was temperature controlled with continuous lighting. Water and feed were given *ad libitum*. Chicks were number banded and allowed a 2-day adjustment period before data collection began. After the 2-day adjustment period, chicks were stratified by weight and randomly assigned to each diet.

Twenty-one chicks were assigned to each diet for 21 days, 7 chicks per cage, 3 replications per treatment. Beta-glucanase was added to the barley diets to reduce wet, sticky droppings (Newman and Newman, 1985). Diets were formulated at a 20.0% protein level and balanced for vitamins and minerals (Tables 1 and 2). Daily feed consumption was recorded and body weights were measured twice a week. Feed to gain ratio was calculated.

Table 1. Diet composition for chicks fed parent and mutant barleys, trial 1.

Ingredient[a]	MOR[b]	605	ROB	625	KAR	504	Corn
	%						
Cornmeal	-----	-----	-----	-----	-----	-----	55.41
Soybean meal	28.88	24.95	26.89	25.82	26.86	24.28	34.59
Barley	61.12	65.05	63.11	64.18	63.15	65.72	-----
Oil	5.59	5.59	5.59	5.59	5.59	5.59	5.59
Dical phos.	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Limestone	.60	.60	.60	.60	.60	.60	.60
Vit premix	.325	.325	.325	.325	.325	.325	.325
Salt	.50	.50	.50	.50	.50	.50	.50
DL-MET	.125	.125	.125	.125	.125	.125	.125
Biotin	.01	.01	.01	.01	.01	.01	.01
B-glucanase	.05	.05	.05	.05	.05	.05	-----
Cornstarch	-----	-----	-----	-----	-----	-----	.05

[a]Oil: 50% Mazola corn oil, 50% Crisco vegetable oil. Vitamin premix: furnishes the following (per kg diet) 7716 USPS vitamin A acetate, 2205 ICU vitamin D_3, 6.61 USPS vitamin E, 11 ug vitamin B_{12}, 6.61 mg riboflavin, 11 mg dl-calcium pantothenate, 496 mg choline chloride, 33 mg niacin, 3.3 mg pyridoxine hydrochloride, 1.1 mg menadione sodium bisulfite, 1.1 mg thiamine mononitrate, 0.66 mg folic acid, 55 ug d-biotin, 0.1 mg sodium selenite, 50 mg manganese sulfate, 50 mg zinc oxide, 50 mg iron carbonate, 5 mg copper oxide, 1.5 mg potassium iodide, 110.2 g oxytetracycline. Beta-glucanase: Enzeco (R) beta-glucanase, 200 units/g, Enzyme Development Corporation, Keyport, NJ.

[b]MOR = Moravian III, 605 = ANT 605, ROB = Robust, 625 = ANT 625, KAR = Karla, 504 = ANT 504, Corn = corn-soybean meal control, Dical phos. = dicalcium phosphate, DL-MET = DL-methionine.

Table 2. Diet composition for chicks fed parent and mutant barleys, trial 2.

Ingredients[a]	TRI[b]	GAL	AND	587	ADV	537	Corn
	----------	----------	----------	%	----------	----------	----------
Cornmeal	-----	-----	-----	-----	-----	-----	55.41
Soybean meal	27.92	27.30	28.52	26.47	25.82	23.82	34.59
Barley	62.08	62.70	61.48	63.53	64.18	66.18	-----
Oil	5.59	5.59	5.59	5.59	5.59	5.59	5.59
Dical phos.	2.80	2.80	2.80	2.80	2.80	2.80	2.80
Limestone	.60	.60	.60	.60	.60	.60	.60
Vit premix	.325	.325	.325	.325	.325	.325	.325
Salt	.50	.50	.50	.50	.50	.50	.50
DL-MET	.125	.125	.125	.125	.125	.125	.125
Biotin	.01	.01	.01	.01	.01	.01	.01
B-glucanase	.05	.05	.05	.05	.05	.05	-----
Cornstarch	-----	-----	-----	-----	-----	-----	.05

[a]See Table 1.

[b]TRI = Triumph, GAL = Galant, AND = Andre, 587 = ANT 587, ADV = Advance, 537 = ANT 537, Corn = corn-soybean meal control, Dical phos.= dicalcium phosphate, DL-MET = DL-methionine.

Chick Diet Preference Trial

A diet preference study was conducted under the same conditions and diets as in the chick growth trials. Ten broiler chicks were housed in each cage and were given the choice of diets prepared from parent and mutant barleys for 20 days. Position of the feed pans was alternated every 48 hours. Diet consumption was measured daily and body weight gain was recorded twice a week.

Rat Nitrogen Balance Trials

Rat nitrogen balance trials were conducted at the Department of Animal and Range Sciences Nutrition Center

using Holtzman male weanling rats. Two barley pairs (parent and mutant) and one control barley (Clark) were included in each of three trials. Rat diet composition is presented in Table 3.

Table 3. Composition of diets fed to rats comparing parent and mutant barleys, nitrogen balance trial.

Diet[a]	Barley	Corn-starch	Vit mix[b]	Min mix[c]	CaCO3	Anti-biotic[d]	Oil
	%						
TRI	70.86	22.09	1.00	2.00	.80	.25	3.00
GAL	69.23	23.72	1.00	2.00	.80	.25	3.00
MOR	64.75	28.20	1.00	2.00	.80	.25	3.00
605	63.83	29.12	1.00	2.00	.80	.25	3.00
AND	72.58	20.37	1.00	2.00	.80	.25	3.00
587	67.16	25.79	1.00	2.00	.80	.25	3.00
ROB	68.18	24.77	1.00	2.00	.80	.25	3.00
625	65.69	27.26	1.00	2.00	.80	.25	3.00
ADV	65.69	27.26	1.00	2.00	.80	.25	3.00
537	61.64	31.31	1.00	2.00	.80	.25	3.00
KAR	86.54	6.41	1.00	2.00	.80	.25	3.00
504	62.50	30.45	1.00	2.00	.80	.25	3.00
CLARK	72.58	20.37	1.00	2.00	.80	.25	3.00

[a]TRI = Triumph, GAL = Galant, MOR = Moravian III, 605 = ANT 605, AND = Andre, 587 = ANT 587, ROB = Robust, 625 = ANT 625, ADV = Advance, 537 = ANT 537, KAR = Karla, 504 = ANT 504, CLARK = Clark control barley.

[b]Vitamin Diet Fortification Mixture, ICN-NBC, Cleveland, OH.

[c]Bernhart and Tomarelli Salt Mixture (1966).

[d]Oxytetracycline, 110.2 g/kg.

Rats were housed in individual woven wire cages in an environmentally controlled room with twelve hours each of light and dark. Rats were allotted to diets and stratified by initial weight. Rats were individually fed 10 g of diet

per day in metabolism cages designed for separate collection of feces and urine. Water that had not been deionized was given ad libitum. Vitamin and mineral fortified diets were formulated at a level of 9.0% protein. Six rats were assigned to each diet for a 4- day adjustment period and a 4 day collection period. Feed, feces, and urine were analyzed for nitrogen to determine true protein digestibility (TPD), biological value (BV), and net protein utilization (NPU) (Eggum, 1973).

Muffin Evaluation

Two parent barleys and their proanthocyanidin-free mutants were compared in a standard muffin product. Muffins made from 100% whole barley flour, ground with a Magic Mill (Kitchenetics Corp., Campbell, CA) were compared to a wheat flour (a blend of 30% spring wheat and 70% winter wheat) muffin. Grated fresh apple was added to the formula which is shown in Table 4. Batter was weighed to 16 g per muffin and baked in a conventional oven at 210° C for 25 minutes. After cooling, muffins were placed in freezer bags and frozen at -10° C until needed for taste panel evaluation (2 days for consumer panels and 2 weeks for trained panels).

Proximate Analyses

Proximate analysis and lysine availability were determined using the methods described previously. Amino acids were determined by AAA Laboratories, Mercer Island,

Washington, using an automatic amino acid analyzer according to the procedure of Spackman et al. (1958).

Objective Evaluation

Objective evaluation of the muffins included volume, pH and percent color reflectance. Product volume was measured 24 hours after removal from the oven using the rapeseed displacement method (Campbell et al., 1979). To measure pH, a slurry was made by blending ten grams of each muffin with 40 ml of distilled water in a Waring Commercial Blendor (Type SJT, New Hartford, CN) for 30 seconds. The pH of this slurry was determined using a pH meter (Model 815MP, Fisher Scientific Company, Pittsburgh, PA). The muffins were dried for 48 hours and blended for 30 seconds in an Osterizer (Galaxie Type, Oster Corporation, Milwaukee, WI) before color determinations were made. Color analysis was made using an Agtron with the methods described previously. Triplicate measurements were performed on each sample.

Table 4. Standard muffin formula.

Ingredient	Amount
Flour	204.0g
Baking powder	9.0g
Salt	5.5g
Sugar, granulated	24.5g
Oil	27.0g
Reconstituted NFDM[a]	250.0ml
Egg	50.0g
Grated apple	50.0g

[a]NFDM = nonfat dry milk.

Sensory Evaluation

Sensory evaluation was used to judge the parent and mutant barley muffins and the wheat muffin.

Trained taste panels

The wheat and barley muffins were judged by twelve trained panelists. Andre and ANT 587 and Robust and ANT 625 muffins were compared to wheat muffins in two separate trials. Panelists judged flavor by using a triangle test for difference and a nine-point hedonic test for like/dislike determination (Appendix A). Training was conducted according to the American Society for Testing and Materials recommendations (ASTM, 1968 and 1981). Judges rated coded samples in individual booths in a darkened room under 25 watt red colored lights. Distilled water at room temperature was provided for rinsing palates between samples. The judges consisted of 11 females and 1 male. Ages ranged from 21 to 57 with a mean age of 35 years, none of the judges used tobacco. Significance of triangle tests was determined using the appendix table E from Amerine et al. (1965).

Consumer taste panels

Two consumer taste panels were conducted in the Main Mall Shopping Center in Bozeman, Montana. Andre and ANT 587 muffins and Robust and ANT 625 muffins were compared to wheat muffins and presented in different panels. Untrained panelists recorded their age and sex and rated the barley

and wheat muffins using a nine point hedonic scale (Appendix A). The muffins were sliced and placed on coded trays. Water was provided to rinse palates between samples. Only properly completed hedonic forms were tabulated. Judges were required to judge all three muffins (two barley and one wheat muffin). The first panel, which judged wheat, Andre and ANT 587 muffins, consisted of 100 judges, of whom 66 were females and 34 were males. Ages ranged from 5 to 69 with a mean age of 36 years. The second panel, which judged wheat, Robust and ANT 625 muffins consisted of 100 judges of whom 71 were females and 29 were males. Ages ranged from 5 to 65 with a mean age of 33 years (Campbell et al., 1979).

Statistical Analysis

The chick and rat data were analyzed with an analysis of variance for barley cultivar and barley type (parent, mutant) (MSUSTAT, 1983). Control data were averaged and the mean values were used to eliminate trial effect. Data were analyzed by analysis of variance and paired parent and mutant barleys were compared orthognally (MSUSTAT, 1983). Pearson correlation coefficients were determined using SAS (1985).

Hedonic scores for the trained and consumer panels were analyzed for statistical significance using two-way analysis of variance technique (MSUSTAT, 1983). Significance of triangular tests was determined using the appendix Table E from Amerine et al. (1965).

RESULTS AND DISCUSSION

Barley Composition

Proximate Analyses

The chemical compositions of the barleys, as seen in Table 5, were quite similar, based on two analyses per sample.

Table 5. Protein, ether extract and fiber content of parent and mutant barleys, dry matter basis.

Barley	PROT[a]	EE	NDF	ADF	EDF
	%				
Triumph	13.9	2.5	13.0	5.6	16.2
Galant	14.1	2.5	14.6	5.2	22.6
Moravian III	15.1	2.4	13.0	5.6	19.8
ANT 605	15.4	2.4	14.0	5.7	19.1
Andre	13.6	2.5	12.9	5.2	19.9
ANT 587	14.5	2.6	12.7	5.1	20.7
Robust	14.3	2.0	14.6	4.8	20.7
ANT 625	14.7	2.4	14.0	5.0	19.8
Advance	14.6	2.3	16.3	6.3	21.4
ANT 537	15.5	2.9	16.1	6.8	27.0
Karla	11.3	2.3	16.1	6.0	23.1
ANT 504	15.5	2.9	15.0	6.1	25.2
Parent ($\bar{X}$)	13.8	2.3	14.3	5.6	20.2
Mutant ($\bar{X}$)	15.0	2.6	14.4	5.7	22.4

[a]PROT = protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, EDF = estimated dietary fiber.

The protein level of Karla was lowest, with ANT 537 and ANT 504 being the highest. The mutant barleys, on the average, were higher in protein (15.0%) than the parents (13.8%). Most of the protein difference was due to the difference between Karla and ANT 504. Karla was derived from Karl, a cultivar that is noted for being consistently low in protein (C.W. Newman, personal communication). Ether extract levels also averaged slightly higher in the mutant barleys as a group (2.6%), than the parent barleys (2.3%). Neutral detergent fiber and ADF mean levels were similar for the groups of parents and mutants. There was some difference by pair, however, with Advance and ANT 537 having the highest NDF and ADF, Andre and ANT 587 the lowest NDF and Robust and ANT 625 the lowest ADF. Mean estimated dietary fiber (EDF) values were higher for the group of mutants (22.4%) than the parents (20.2%).

Ash, calcium and phosphorus levels were similar for all barleys (Table 6). The mutants averaged lower in starch (53.5%) than the parents (54.9%). ANT 537 and ANT 504 had the lowest starch content, which inversely correlated with protein content ($r = -0.39$). Mutant barleys contained no proanthocyanidin, whereas the parents ranged between 195 and 259 mg/100 g.

Table 6. Ash, calcium, phosphorus, starch and proanthocyanidin content of parent and mutant barleys, dry matter basis.

Barley	ASH	Ca	P	STARCH	PROANT[a]
	----------%	----------			-mg/100g-
Triumph	2.5	.02	.38	57.8	226
Galant	2.7	.02	.43	52.1	0
Moravian III	2.4	.02	.40	54.2	200
ANT 605	2.6	.02	.42	53.7	0
Andre	2.5	.00	.38	55.2	214
ANT 587	2.4	.02	.37	53.9	0
Robust	2.3	.02	.36	54.4	199
ANT 625	2.4	.03	.38	55.3	0
Advance	2.6	.02	.40	54.2	195
ANT 537	2.4	.03	.34	47.7	0
Karla	2.8	.02	.40	53.6	259
ANT 504	2.7	.04	.41	48.2	0
Parent ($\bar{X}$)	2.5	.02	.39	54.9	216
Mutant (X)	2.6	.03	.39	53.5	0

[a]Ca = calcium, P = phosphorus, PROANT = proanthocyanidin.

Amino acid analyses (Table 7) showed a trend similar to protein, with total recovered amino acids being approximately one percentage point higher in the mutants (11.5%) than the parent barleys (10.4%).

Lysine was higher in all mutants than their parents, averaging 0.45% and 0.39%, respectively. The lysine content of Karla was the lowest (0.30%), with Galant , ANT 625 and ANT 504 being the highest (0.48%, 0.46% and 0.46%, respectively).

Table 7. Amino acid content of parent and mutant barleys, dry matter basis.

Amino acid[a]	Truimph	Galant	Moravian III	ANT 605	Andre	ANT 587
	%					
ALA	0.47	0.45	0.40	0.50	0.42	0.47
ARG	0.70	0.76	0.60	0.67	0.59	0.63
ASP	0.69	0.68	0.56	0.58	0.61	0.66
GLU	2.77	2.59	2.39	2.64	2.59	2.89
GLY	0.44	0.46	0.38	0.46	0.40	0.46
HIS	0.39	0.44	0.34	0.35	0.32	0.35
ILE	0.43	0.45	0.37	0.44	0.37	0.40
LEU	0.84	0.84	0.73	0.78	0.73	0.79
LYS	0.45	0.48	0.37	0.41	0.38	0.43
MET	0.08	0.09	0.06	0.10	0.16	0.10
PHE	0.81	0.75	0.68	0.77	0.66	0.75
PRO	1.73	1.55	1.57	1.74	1.22	1.21
SER	0.49	0.42	0.39	0.42	0.42	0.43
THR	0.39	0.33	0.30	0.37	0.33	0.34
TYR	0.35	0.30	0.28	0.38	0.35	0.34
VAL	0.71	0.64	0.59	0.63	0.54	0.60

[a]Cystine/2 and tryptophan were not determined. ALA = Alanine, ARG = Arginine, ASP = Aspartic acid, GLU = Glutamic acid, GLY = Glycine, HIS = Histidine, ILE = Isoleucine, LEU = Leucine, LYS = Lysine, MET = Methionine, PHE = Phenylalanine, PRO = Proline, SER = Serine, THR = Threonine, TYR = Tyrosine, VAL = Valine.

Table 7 (cont.). Amino acid content of parent and mutant barleys, dry matter basis.

Amino acid[a]	Robust	ANT 625	Advance	ANT 537	Karla	ANT 504	Ave par[b]	Ave mut
	%							
ALA	0.48	0.53	0.44	0.54	0.37	0.51	0.43	0.50
ARG	0.80	0.78	0.64	0.74	0.49	0.66	0.64	0.71
ASP	0.72	0.75	0.63	0.73	0.50	0.70	0.62	0.68
GLU	2.83	3.11	2.85	3.50	2.38	2.77	2.64	2.92
GLY	0.72	0.75	0.63	0.73	0.50	0.70	0.51	0.59
HIS	0.38	0.37	0.32	0.36	0.26	0.37	0.34	0.37
ILE	0.42	0.46	0.38	0.46	0.32	0.43	0.38	0.44
LEU	0.83	0.84	0.72	0.89	0.61	0.79	0.74	0.82
LYS	0.45	0.46	0.36	0.43	0.30	0.46	0.39	0.45
MET	0.19	0.07	0.10	0.10	0.07	0.10	0.11	0.09
PHE	0.76	0.78	0.66	0.80	0.57	0.70	0.69	0.76
PRO	1.22	1.29	1.23	1.57	1.19	1.31	1.36	1.45
SER	0.48	0.46	0.40	0.50	0.35	0.45	0.42	0.45
THR	0.37	0.38	0.33	0.40	0.27	0.39	0.33	0.37
TYR	0.39	0.34	0.30	0.36	0.27	0.38	0.32	0.35
VAL	0.62	0.66	0.55	0.66	0.46	0.64	0.58	0.64

[a]See previous footnote for Table 7.

[b]Ave par = average parent barley values, Ave mut = average mutant barley values.

Estimated available lysine and percentage lysine of recovered amino acids (Table 8) were also slightly higher in the mutants than the parent barleys.

Table 8. Estimated available lysine of parent and mutant barleys and lysine content, percent of recovered amino acids.

Barley	Estimated available lysine, as submitted	Lysine content of recovered amino acids
	----------%	----------
Triumph	0.29	3.83
Galant	0.14	4.27
Moravian III	0.21	3.70
ANT 605	0.24	3.65
Andre	0.20	3.77
ANT 587	0.38	3.96
Robust	0.30	3.94
ANT 625	0.30	3.90
Advance	0.31	3.50
ANT 537	0.36	3.43
Karla	0.27	3.42
ANT 504	0.34	4.13
Parent ($\bar{X}$)	0.26	3.69
Mutant (X)	0.29	3.89

The percentage of estimated available lysine varied considerably. This may, in part, have been due to the TNBS method, which is highly sensitive and subject to error. Therefore, total lysine will be considered a more important measure. Since proanthocyanidins are believed to bind lysine, this would theoretically lower available lysine. However, until there is a more precise method to measure available lysine, it is necessary to use bioassay and amino acid analyses. Rawson and Mahoney (1983) discussed the

problem with Maillard reactions in the analysis of samples, which contain a high level of carbohydrate.

Viscosity and beta-glucan results are presented in Table 9.

Table 9. Viscosity measurement and beta-glucan content of parent and mutant barleys.

Barley	Viscosity[a]	Beta-glucan[b]		
		Total	Soluble	Insoluble
	--cP--	----------%----------		
Triumph	1.75	3.5	1.8	1.7
Galant	2.04	4.1	2.2	1.9
Moravian III	2.20	4.2	2.2	2.0
ANT 605	1.85	3.7	2.0	1.7
Andre	2.23	4.3	2.1	2.2
ANT 587	2.28	4.3	2.2	2.1
Robust	1.97	3.7	1.5	2.2
ANT 625	1.84	3.7	1.5	2.2
Advance	2.65	4.5	2.5	2.0
ANT 537	2.09	4.4	2.1	2.3
Karla	1.86	3.7	1.3	2.4
ANT 504	2.11	4.2	2.3	1.9
Parent ($\bar{X}$)	2.11	4.0	1.9	2.1
Mutant (X)	2.04	4.1	2.1	2.0

[a]cP = centepoise units.

[b]Percentage dry matter.

Triumph showed the lowest viscosity (1.75 cP) and Advance resulted in the highest measurement (2.65 cP). The means for parents and mutants differed only slightly (2.11 vs. 2.04 cP, respectively).

Total and soluble beta-glucans were similar for the mutants (4.1 and 2.1%, respectively) and the parents (4.0 and 1.9%, respectively). There was some difference in total beta-glucans by pair, with Robust and ANT 625 averaging the lowest (3.7%) and Advance and 537 the highest (4.5%); individual observation shows Triumph as having the lowest concentration (3.5%). Soluble beta-glucans showed similar differences with Robust and ANT 625 being the lowest (1.5%) and Advance and ANT 537 the highest (2.3%); individually Karla had the lowest (1.3%). Insoluble beta-glucans showed no apparent differences.

Physical Measurements

Kernel Measurement

Kernel measurements are presented in Table 10. Kernel weights averaged higher for the parent barleys (38.2 mg) than the mutants (37.0 mg). Triumph had the highest kernel weight (40.8 mg), with ANT 537 having the lowest (32.3 mg). This correlates with starch content (r = .70). Test weights were similar for both groups. Plump and thin kernel measurements indicated a wide difference between the parent and mutant barleys with percent plump averaging 81.7% and 72.4%, and percent thin 6.8% and 10.4%, respectively. This could be due to the ANT 537 and ANT 504 mutants which both had the highest percentage thin kernels. Plump and thin percentage also showed a significant correlation (r = .77) with starch content.

Table 10. Kernel measurements of parent and mutant barleys.

Barley	Kernel weight[a]	Test weights	Plump kernels	Thin kernels
	--mg--	-kg/hl-	------%-------	
Triumph	40.8	71.6	81.6	7.9
Galant	39.4	67.9	86.9	5.6
Moravian III	40.7	71.6	87.4	3.4
ANT 605	38.1	70.0	76.7	9.2
Andre	39.1	72.7	76.3	8.4
ANT 587	40.7	73.2	80.5	6.5
Robust	38.0	70.0	81.8	5.7
ANT 625	36.9	69.0	85.1	4.2
Advance	35.6	64.1	84.0	6.2
ANT 537	32.3	58.7	41.1	21.3
Karla	35.0	65.8	78.9	9.1
ANT 504	34.7	62.5	64.2	15.5
Parent ($\bar{X}$)	38.2	69.3	81.7	6.8
Mutant (X)	37.0	66.9	72.4	10.4

[a]The measurements were means of duplicate analyses.

Percent Color Reflectance

Differences occurred among all ground barley samples for the four colors measured (Table 11). The mutant barleys averaged a slightly darker or lower reflectance for all four colors than the parents. Karla and Galant had the highest reflectance color measurement (lightest) with Robust having the lowest (darkest). The wheat flour was measured for comparative purposes; it was the lightest sample, which is reasonable since the barley endosperm tends to be darker than wheat endosperm.

Table 11. Percent color reflectance of parent and mutant barleys.

Barley	Percent reflectance[a]			
	Blue	Green	Red	Yellow
	%			
Triumph	51	67	78	70
Galant	46	60	71	65
Moravian III	56	72	82	75
ANT 605	56	71	82	75
Andre	56	71	83	76
ANT 587	57	73	83	77
Robust	61	73	83	77
ANT 625	50	64	75	69
Advance	55	70	80	75
ANT 537	52	67	78	72
Karla	44	60	73	66
ANT 504	53	68	79	73
Parent ($\bar{X}$)	54	69	80	73
Mutant (X)	52	67	78	72
Wheat flour	72	83	91	87

[a]Percentages are the means of three samples.

Animal Experiments

Chick Growth Trials

Proximate analyses, calcium and phosphorus of chick diets fed in the growth trials are given in Table 12.

Table 12. Proximate components of chick diets prepared with parent and mutant barleys and corn, dry matter basis.

Diet	PROT[a]	EE	NDF	ADF	ASH	Ca	P
	%						
Trial 1							
Moravian III	21.4	8.5	14.0	6.5	7.8	1.02	0.87
ANT 605	21.8	7.7	13.8	6.5	7.0	0.80	1.07
Robust	21.6	8.0	14.2	5.9	7.0	0.80	0.98
ANT 625	21.6	8.5	14.1	6.0	7.0	0.79	0.94
Karla	21.8	8.4	15.0	3.8	7.2	0.83	1.01
ANT 504	21.5	8.1	15.3	6.7	7.0	0.81	0.98
Corn-soybean meal control	21.6	8.2	11.8	5.4	6.8	0.80	1.06
Trial 2							
Triumph	23.5	7.7	12.8	5.4	7.1	0.90	0.87
Galant	22.9	7.7	13.4	6.3	7.2	0.86	0.91
Andre	22.5	7.3	12.7	5.8	7.0	0.93	0.93
ANT 587	22.0	7.6	12.7	5.4	6.9	0.91	0.92
Advance	22.5	7.5	13.9	5.6	6.8	0.90	0.88
ANT 537	23.4	7.8	14.6	6.6	6.7	0.95	0.88
Corn-soybean meal control	23.8	6.5	11.9	5.5	6.9	0.98	0.96

[a]PROT = Protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, Ca = calcium, P = phosphorus.

Results of the chick growth trials comparing the parent and mutant barleys are shown in Table 13. Control data were averaged and the mean values were used to eliminate trial effect; original data are shown in Appendix B.

Table 13. Comparison of parent and mutant barleys for weight gain, feed consumption and feed/gain ratio of chicks, adjusted data.

Diets	n	Weight gain[a]	Feed consumed	Feed/Gain ratio
		-g- (P=)	-g- (P=)	(P=)
Triumph	21	648	962	1.49
Galant	19	630 (.52)	993 (.33)	1.58 (.07)
Moravian III	19	599	956	1.60
ANT 605	18	621 (.42)	987 (.32)	1.59 (.94)
Andre	20	603	968	1.61
ANT 587	20	649(.11)	1008 (.21)	1.56 (.29)
Robust	19	614	969	1.58
ANT 625	20	639 (.37)	922 (.14)	1.44 (.01)
Advance	21	587	956	1.63
ANT 537	20	520 (.02)	967 (.72)	1.87 (.00)
Karla	17	629	1001	1.59
ANT 504	17	583 (.11)	976 (.43)	1.68 (.09)
Parent ($\bar{X}$)		613	969	1.58
Mutant (X)		607 (.58)	976 (.59)	1.62 (.07)
S.E.[b]		7.75	6.42	0.06

[a]Probability values (P=) are listed in each column.

[b]S.E. = Standard error of the mean

Chick body weight gains showed a significant difference ($P<0.05$) with ANT 537 being the lowest (520 g). Parent barleys resulted in a slightly larger average gain (613 g) than the mutant barleys (607 g), although the difference was not significant ($P=0.58$). Weight gains for three of the mutants (ANT 605, ANT 587 and ANT 625) appeared to be higher than their parent barleys (Moravian III, Andre

and Robust, respectively); only the difference for Advance and ANT 537 was significant.

Feed consumed by the chicks showed no significant difference between barley cultivars or types. Feed consumed showed a negative correlation (r = -0.49) with protein content and a positive correlation (r = 0.60) with starch content.

Feed to gain ratio showed a significant difference between barley cultivars and types (Table 13). Average feed to gain ratio was slightly lower for the parent barleys (1.58), than the mutants (1.62) (P=0.07). Feed to gain ratio had a negative correlation (r = -0.85) with the starch content of the barley, which is presented in Figure 7. ANT 537 and ANT 504, which had the lowest starch contents (47.7% and 48.2%, respectively), had the highest F/G (1.87 and 1.68). Feed to gain ratios had a positive correlation with total beta-glucan (r = 0.54), which is shown in Figure 7.

Newman et al. (1984) found improved growth rate and feed efficiency with chicks for proanthocyanidin-free barley when compared to normal barley. Chick studies included in this research did not show consistent superior nutritional value for the mutant barleys when compared to their parent barleys.

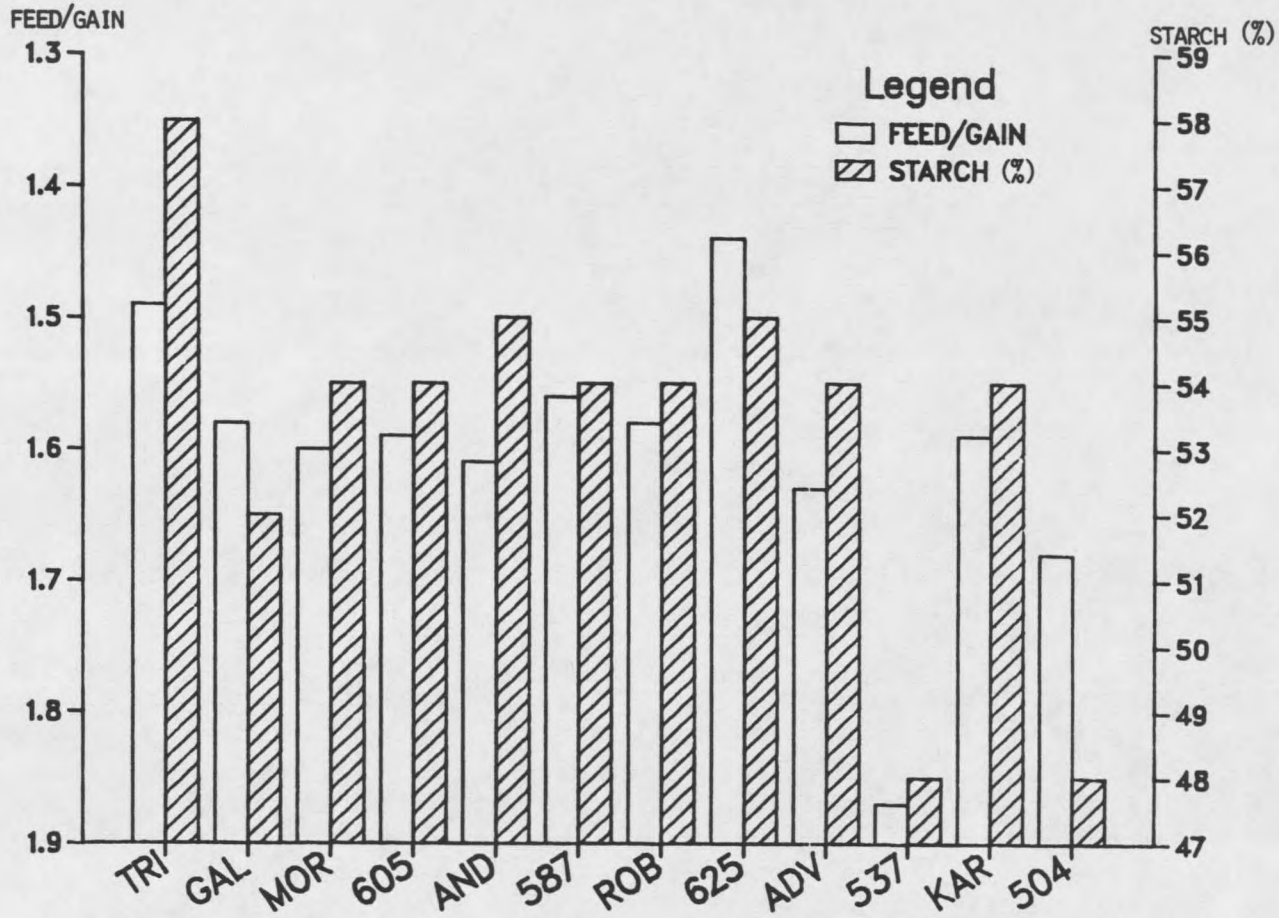

Figure 7. Feed to gain ratio of the chicks vs. starch content of the barleys (r=-0.85).

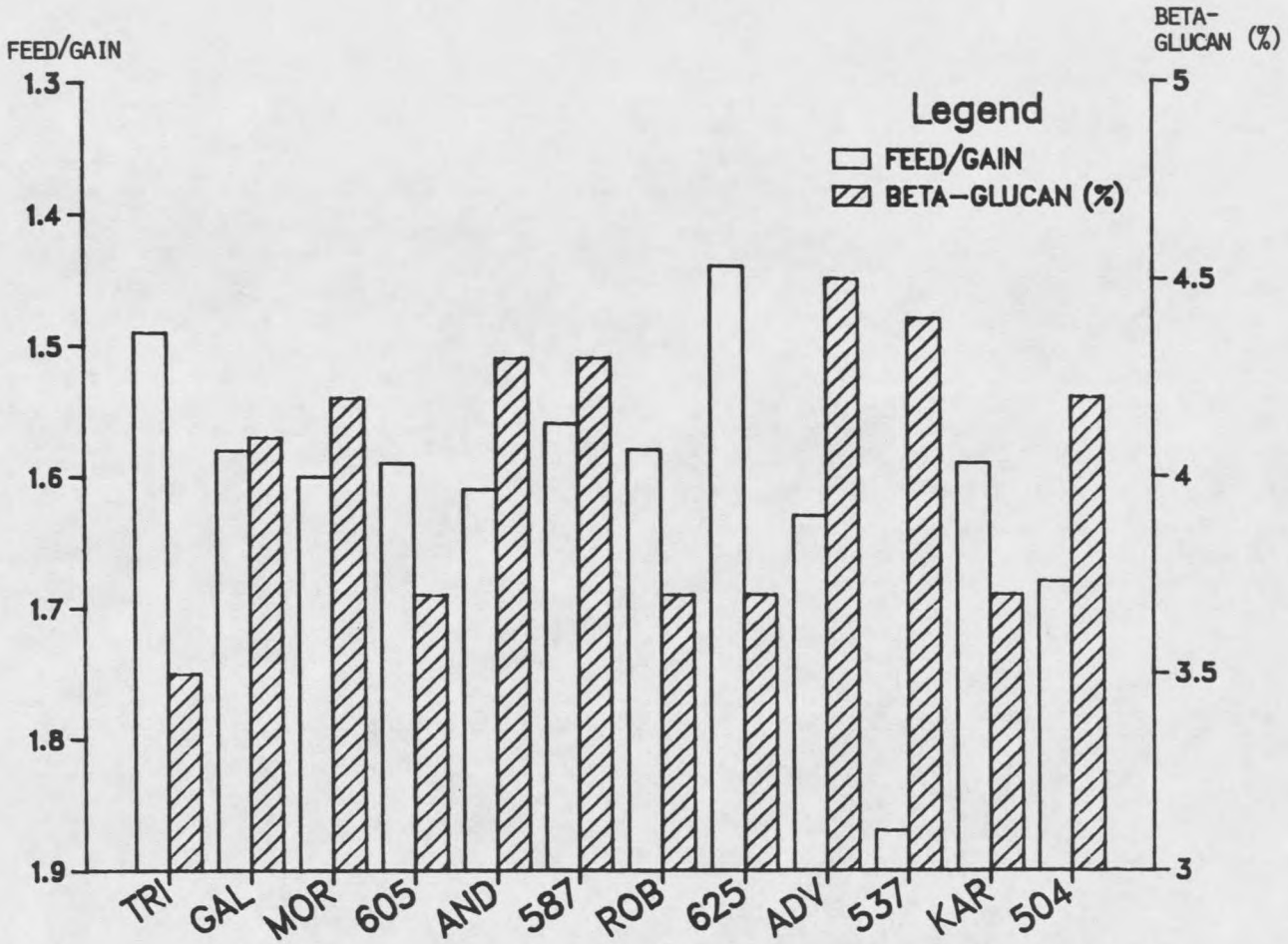

Figure 8. Feed to gain ratios of the chicks vs. total beta-glucan content of the barleys (r=0.54).

Chick Diet Preference Trial

Results from the chick diet preference trial are presented in Table 14. Chicks' feed consumption versus starch content of the barleys is presented in Figure 9.

Table 14. Chick preference for diets composed of parent and mutant barleys.

Barley	Feed consumed		Total
	--g--	--%--	--g--
Triumph	380	54	
Galant	321	46	701
Moravian III	191	28	
ANT 605	495	72	686
Andre	384	57	
ANT 587	293	43	677
Robust	305	44	
ANT 625	393	56	698
Advance	618	92	
ANT 537	53	8	671
Karla	556	77	
ANT 504	169	23	725
Parent ($\bar{X}$)	406	59	
Mutant (X)	287	41	

Chicks preferred ANT 537 grain the least as evidenced by consumption of 53 grams by 10 chicks in 20 days. They consumed 618 g of Advance as the alternate choice to ANT 537. Chicks preferred Karla over ANT 504 by 556 g to 169 g. These two mutant barleys, ANT 537 and ANT 504, have the lowest starch and were among the highest beta-glucan content, which may account for the low preference by the chicks.

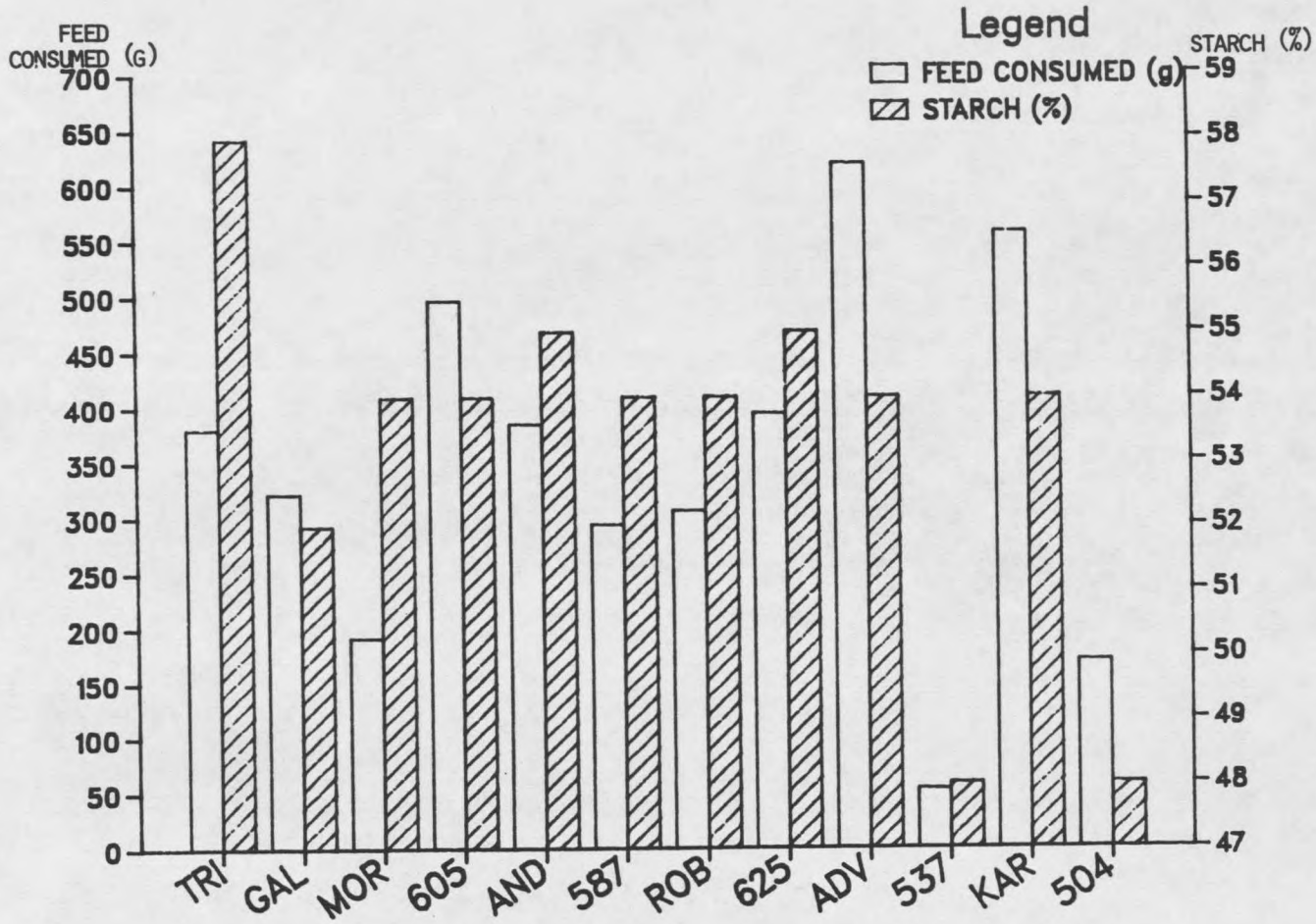

Figure 9. Chick diet preference trial feed consumption vs. starch content of the barleys.

There also may be an unknown factor present which affects the chick feed selection. Kaufman et al. (1978) reported that chicks have demonstrated the ability to select diets with sufficient protein-carbohydrate ratio to maintain normal growth.

Rat Nitrogen Balance Trial

Proximate components of rat diets fed in the nitrogen balance studies are shown in Table 15.

Table 15. Proximate composition of rat diets prepared with parent and mutant barleys, dry matter basis.

Diet	PROT[a]	EE	NDF	ADF	ASH	Ca	P
	%						
Triumph	10.1	5.0	9.8	3.9	4.7	0.91	0.67
Galant	10.1	6.0	10.0	3.9	4.8	0.95	0.70
Moravian III	9.9	6.1	8.4	3.2	4.6	0.87	0.65
ANT 605	10.3	5.7	9.1	3.4	4.5	0.83	0.68
Andre	10.6	4.9	10.0	3.4	4.7	0.91	0.66
ANT 587	9.4	5.4	7.7	2.9	4.4	0.86	0.59
Robust	9.5	5.4	8.2	2.5	4.8	1.08	0.65
ANT 625	9.6	5.2	8.4	3.4	4.4	0.85	0.59
Advance	9.9	5.3	9.7	3.8	4.5	0.86	0.65
ANT 537	10.0	4.9	9.7	3.7	4.3	0.85	0.60
Karla	9.8	5.1	11.9	4.6	5.2	0.91	0.73
ANT 504	9.8	5.4	9.8	3.9	4.6	0.86	0.66
Control (Clark)	9.8	5.3	9.6	3.9	4.8	0.90	0.66

[a]PROT = protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, Ca = calcium, P = phosphorus.

Results of the rat nitrogen balance trials, comparing the parent and mutant barleys, are presented in Table 16. Control data were averaged and the mean values were used to eliminate trial effect; original data are shown in Appendix B.

Table 16. Comparison of parent and mutant barleys for true protein digestibility, biological value and net protein utilization, rat trials, adjusted data.

Diets	TPD[a]		BV		NPU	
	-%-	(P=)	-%-	(P=)	-%-	(P=)
Triumph	82.6		71.6		59.3	
Galant	82.2	(.76)	86.5	(.00)	71.5	(.18)
Moravian III	85.1		80.6		68.7	
ANT 605	84.8	(.82)	75.5	(.20)	64.2	(.18)
Andre	81.0		66.2		53.4	
ANT 587	83.9	(.07)	74.6	(.01)	62.7	(.01)
Robust	82.4		84.8		70.3	
ANT 625	85.5	(.06)	76.9	(.04)	66.0	(.21)
Advance	86.6		70.6		61.2	
ANT 537	90.4	(.02)	75.2	(.23)	67.7	(.06)
Karla	81.8		79.9		65.0	
ANT 504	87.7	(.00)	74.4	(.16)	65.0	(.99)
Parent ($\bar{X}$)	83.3		75.6		63.0	
Mutant (X)	85.8	(.00)	77.2	(.32)	66.2	(.00)
S.E.[b]	0.35		0.98		0.85	

[a]TPD = true protein digestibility, BV = biological value, NPU = net protein utilization. S.E. = Standard error of the mean difference. Probability values are shown in each column.

[b]SE = standard error of the mean.

True protein digestibility was slightly higher for the mutants (85.8%) than the parents (83.3%). True protein digestibility was positively correlated with protein content ($r = 0.79$, $P<0.01$).

The biological value of the protein that was digested was slightly higher for the mutants (77.2%) than the parents (75.6%). Biological value showed a trend for being negatively correlated with total beta-glucan content ($r = -0.41$) and soluble beta-glucan ($r = -0.45$) but neither were statistically significant. Figure 10 represents the relationship between BV and total beta-glucan content.

Net protein utilization was different ($P=0.00$) for the mutants (66.2%) and the parents (63.0%). There was, however, no correlation between NPU and the barley composition that was analyzed.

Newman et al. (1984) found the protein more digestible for proanthocyanidin-free barleys with rats when compared to normal barleys. Rat studies included in this research did not show consistent superior nutritional value for the mutant barleys when compared to their parent barleys.

Muffin Evaluation

Andre/ANT 587 and Robust/ANT 625 were selected for baked product evaluation.

Judd (1982) studied the acceptability of barley into human diets by substituting barley for other cereals in

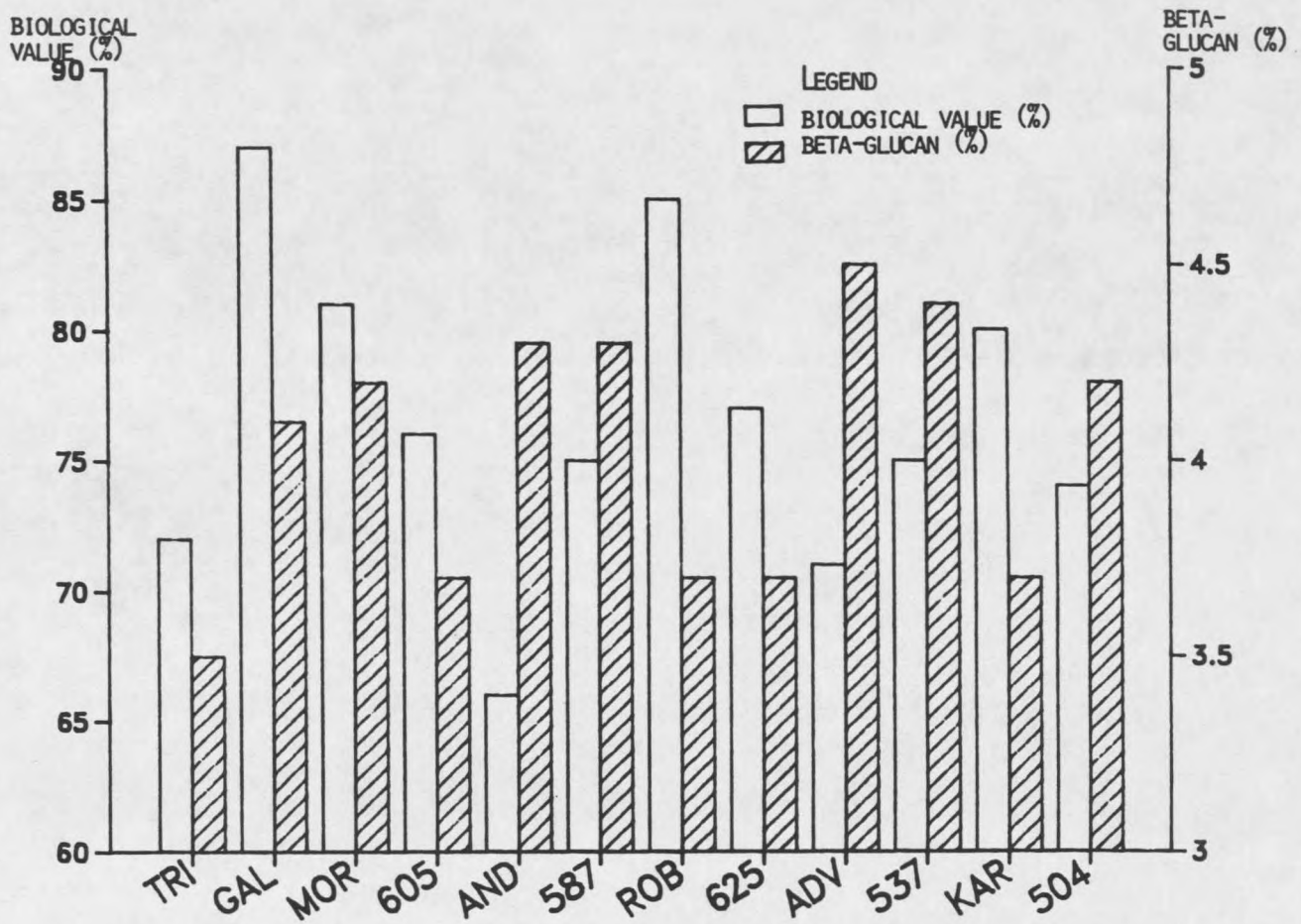

Figure 10. Rat nitrogen balance trial biological values vs. beta-glucan content of the barleys (r=-0.41).

daily meals; fiber content of the diets were increased without taking supplements.

Proximate Analysis

Proximate components of the muffins are presented in Table 17. As presented in Table 17, the four barley muffins averaged higher estimated dietary fiber content (32.0%) than the wheat muffin (19.8%).

Table 17. Protein, ether extract, fiber analyses, ash, starch and energy of barley and wheat muffins, dry matter basis.

Quality[a] parameter	Wheat flour	Andre	ANT 587	Robust	ANT 625
	%				
PROTEIN	14.3	14.5	14.9	15.3	14.7
EE	10.8	11.6	11.9	11.8	11.5
NDF	2.6	10.3	9.9	11.2	11.5
ADF	1.8	4.7	5.4	4.8	5.3
EDF	19.8	31.8	32.0	31.3	32.7
ASH	4.3	5.3	5.4	5.2	5.4
STARCH	45.7	32.1	31.1	31.8	31.2
Kcal/g	4.4	4.4	4.4	4.3	4.4

[a]EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, EDF = estimated dietary fiber.

All barley muffins were higher in protein and estimated dietary fiber than the wheat flour muffin. Starch content was higher in the wheat muffin and caloric content was similar in all five muffins. The protein content of the wheat flour was 11%. Amino acid analysis is shown in

Table 18. As with protein, lysine was slightly higher in the barley muffins than in the wheat muffin.

Table 18. Amino acid content and estimated available lysine of barley and wheat muffins.

Amino acid[a]	Wheat	Andre	ANT 587	Robust	ANT 625
	----------	----------	%	----------	----------
Alanine	0.339	0.393	0.420	0.418	0.405
Arginine	0.375	0.442	0.453	0.512	0.480
Aspartic acid	0.608	0.719	0.768	0.793	0.752
Glutamic acid	2.920	2.260	2.420	2.520	2.400
Glycine	0.288	0.300	0.346	0.330	0.321
Histidine	0.233	0.229	0.244	0.257	0.247
Isoleucine	0.495	0.474	0.494	0.506	0.495
Leucine	0.882	0.860	0.915	0.923	0.889
Lysine	0.372	0.447	0.468	0.481	0.455
Methionine	0.148	0.159	0.151	0.161	0.148
Phenylalanine	0.631	0.576	0.623	0.724	0.698
Proline	0.761	0.742	0.802	0.813	0.796
Serine	0.544	0.502	0.520	0.544	0.519
Threonine	0.337	0.370	0.394	0.407	0.389
Tyrosine	0.341	0.353	0.361	0.401	0.369
Valine	0.549	0.590	0.625	0.630	0.586
TOTAL	9.820	9.420	10.000	10.400	9.950
Estimated available lysine	0.370	0.300	0.400	0.410	0.410

[a]Cystine/2 and tryptophan were not determined.

Objective Evaluation

Percent color reflectance of the muffins is presented in Table 19. The wheat flour muffin had higher (lighter)

readings for all four colors. Andre was the lightest of the barley muffins and ANT 625 the darkest. This color difference could affect consumer preference, as some people prefer a lighter muffin, while others may prefer a darker, whole grain-type product. The proanthocyanidin-free mutant barleys were slightly darker in the red reflectance than their parent barleys.

Table 19. Percent color reflectance of barley and wheat muffins.

	Percent reflectance[a]			
	Blue	Green	Red	Yellow
	%			
Wheat flour	27	50	64	57
Andre	18	38	55	46
ANT 587	16	35	49	41
Robust	17	35	50	42
ANT 625	15	33	47	39

[a] Percentages are the means of three batches.

Volume measurements and pH results are presented in Table 20. Volume measurements were slightly higher for the barley muffins when compared to the wheat muffins; pH values were similar for all muffins.

Sensory Evaluation

Muffins were evaluated by trained and consumer taste panelists to determine differences and acceptability.

Table 20. Volume and pH of barley and wheat muffins.

Muffin	Volume[a]	Specific volume	pH
	-cc-	-cc/g-	
Wheat flour	38.7	2.98	6.57
Andre	43.7	3.36	6.40
ANT 587	42.0	3.23	6.22
Robust	45.3	3.48	6.49
ANT 625	47.0	3.62	6.42

[a]Volume and pH values are the means of three measurements.

Trained taste panels

Trained taste panel results are presented in Tables 21 and 22. The trained panelists were able to tell the difference between the wheat and barley muffins. They could not tell the difference ($P<0.01$) between the muffins baked with the parent and mutant barleys. The different proanthocyanidin content of the barleys did not affect the flavor of the muffins enough for the trained panel to differentiate between parent and mutant barley muffins.

Hedonic scores for the trained taste panel are presented in Table 23. There was no significant difference for the scores of wheat, Andre and ANT 587 muffins judged by the trained panelists. When compared to the wheat muffin, Robust and ANT 625 had significantly lower scores than the wheat muffin ($P<0.01$).

Table 21. Taste panel response using triangle tests to compare barley and wheat muffins.

Triangle	Correct	Incorrect
Panel 1		
Wheat vs. Andre	33	3***
Wheat vs. ANT 587	35	1***
Andre vs. ANT 587	13	23
Panel 2		
Wheat vs. Robust	30	1***
Wheat vs. ANT 625	28	3***
Robust vs. ANT 625	7	24

*** Indicates a significant correct identification ($P<0.001$).

Consumer taste panels

Hedonic scores for the consumer panels are presented in Table 23. Although the wheat muffin obtained a higher score when compared to the Andre muffin, consumers judged them to be similar ($P<0.05$). There was a significant difference ($P<0.05$) between the wheat and ANT 587 muffins, with the ANT 587 muffin scoring the lowest. There was no difference between the scores for the Andre and ANT 587 muffins. The Andre muffin was reported as having the mildest flavor of the barley muffins.

Hedonic scores for the Robust and ANT 625 muffins were significantly lower than those of the wheat muffin ($P<0.01$). These barley muffins were reported as having a very strong, almost rye-like flavor.

Table 22. Mean hedonic scores of barley and wheat muffins, consumer and trained taste panels.

Muffin type	Consumer panel n	Consumer panel Score	Trained panel n	Trained panel Score
Wheat	100	7.0±1.4 b*	36	6.5±1.3
Andre		6.8±1.6 ab		6.1±1.5
ANT 587		6.6±1.6 a		6.3±1.6
Wheat	100	7.1±1.3 b**	31	6.6±1.0 b**
Robust		6.4±1.5 a		5.3±1.8 a
ANT 625		6.6±1.4 a		5.5±1.6 a

* Values in a group that do not share the same letter (a,b) differ significantly ($P<0.05$).
** $P<0.01$.

Results from the taste panels show that products made from Andre and ANT 587 barleys have promise of being accepted by consumers. Robust and ANT 625 barleys, having stronger flavors, could be incorporated into products containing rye or in other strong flavored products.

CONCLUSIONS

Proanthocyanidins are chemical constituents of barley that have been previously shown to have a negative influence on protein digestibility and nutritional quality (Newman et al, 1984). Rat and chick studies included in this research did not show consistent superior nutritional value for the proanthocyanidin-free barleys when compared to the normal barleys. Other constituents of barley including protein structure, total fiber, beta-glucans, and starch will influence the results of animal and poultry evaluations. These factors must be "equalized" as much as possible when comparisons are made to prevent incorrect conclusions.

Consumer and trained taste panels were conducted to compare muffins made from wheat and two parent mutant barleys. Trained taste panelists could not distinguish the difference between parent and mutant barleys tested. Consumers tended to favor a mild taste in a muffin and judged one normal barley muffin to be similar to the wheat muffin. The majority of trained and consumer judges preferred the wheat muffin over the barley muffins.

It is concluded that all constituents of barley, including protein, starch, fiber, beta-glucans and proanthocyanidins, influence the nutritional value and must be considered when comparisons are made.

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APPENDICES

APPENDIX A

Taste Panel Recording Sheets

BOOTH NUMBER_____

FOOD EVALUATION

Triangle Test

Product:_____

Two of these samples are identical and the other different, please circle the odd sample and score for preference.

Circle the odd sample(s):

¤ &

Check your preference:

[ ] Pair

[ ] Single

[ ] No Preference

Name_____

Date_____

Comments:

Booth number_____

Food Evaluation Test

Product_____

Check the appropriate block:

	SAMPLE NUMBER		
	[ ]	[ ]	[ ]
Like Extremely	[ ]	[ ]	[ ]
Like Very Much	[ ]	[ ]	[ ]
Like Moderately	[ ]	[ ]	[ ]
Like Slightly	[ ]	[ ]	[ ]
Neither Like or Dislike	[ ]	[ ]	[ ]
Dislike Slightly	[ ]	[ ]	[ ]
Dislike Moderately	[ ]	[ ]	[ ]
Dislike Very Much	[ ]	[ ]	[ ]
Dislike Extremely	[ ]	[ ]	[ ]

Comments:

Name_____
Date_____

APPENDIX B

Animal Data

Table 23. Comparison of parent and mutant barleys for final body weight, weight gain, feed cosumption, and feed/gain ratio of chicks, original data.

Diets	n	Final weight	Weight gain	Feed consumed	Feed/gain ratio
		g			
Trial 1					
Moravian III	19	671	607	981	1.62
ANT 605	18	694	630	1013	1.61
Robust	19	687	623	994	1.60
ANT 625	20	712	648	948	1.46
Karla	17	702	638	1026	1.61
ANT 504	17	656	592	1001	1.70
Corn-soybean meal control	20	766	702	1003	1.43
Trial 2					
Triumph	21	699	640	937	1.47
Galant	19	681	622	967	1.56
Andre	20	654	595	943	1.59
ANT 587	20	700	641	983	1.54
Advance	21	638	579	931	1.61
ANT 537	20	570	511	942	1.85
Corn-soybean meal control	19	744	685	952	1.39
Parents		675	614	969	1.58
Mutants		669	607	976	1.62

Table 24. Comparison of nitrogen balance, rat trials, original data.

Diets	TPD	BV	NPU
	----------	%	----------
Triumph	85.0	70.6	60.1
Galant	84.6	69.4	72.3
Moravian III	87.5	79.5	69.6
ANT 605	87.2	74.4	64.9
Andre	83.0	71.4	59.2
ANT 587	85.9	79.8	69.5
Robust	84.4	89.9	76.0
ANT 625	87.5	82.0	71.8
Advance	82.2	66.5	54.6
ANT 537	85.9	71.1	61.1
Karla	77.4	75.8	58.4
ANT 504	83.3	70.3	58.5
Parents	83.2	75.2	62.6
Mutants	85.7	77.2	66.2
S.E.	0.35	0.98	0.85

[a]TPD = true protein digestibility, BV = biological value, NPU = net protein utilization.