In Vivo and In Vitro Protein Digestibilities of Regular and Mutant Barleys and Their Isolated Protein Fractions[1]

R. S. BHATTY[2] and J. R. WHITAKER[3]

ABSTRACT

Cereal Chem. 64(3):144–149

Apparent protein digestibility (APD) of three pairs of parent and mutant barleys, Bomi and Risø 1508, Carlsberg II and Risø 86, Carlsberg II and Risø 56 grown under identical environments, mutant ANT-13-13, and Abee (a Canadian feed barley) was determined by in vivo (mouse feeding) and two in vitro procedures. The ANT-13-13 mutant had the highest in vivo APD. The in vivo APD of the mutants was lower, except in the Carlsberg II and Risø 56 pair, than those of the parents and seemed positively correlated with in vitro digestibility. Albumin plus globulin, hordein, and glutelin protein fractions isolated from one pair of parent and mutant barleys (Carlsberg II and Risø 86) and hydrolyzed in vitro with pepsin, trypsin, α-chymotrypsin, and peptidase showed the hordein fraction to be more susceptible to the mammalian proteases than the albumin plus globulin or the glutelin protein fractions. Wet heat improved digestibility of the protein fractions by pepsin, but both dry and wet heat reduced protein digestibility by trypsin. Treatment of the protein fractions with α-amylase before their hydrolysis improved their in vitro digestibility, particularly that of the glutelin fraction, both by pepsin and trypsin. The three protein fractions were poorly digested by the protozoan, *Tetrahymena thermophila*, compared to bovine serum albumin. Predigestion of the protein fractions with pronase for 3 hr at 55° C slightly improved their utilization. Under these conditions, the organism grew most on the hordein fraction, least on the glutelin fraction; the albumin plus globulin fraction was intermediate in promoting growth.

Barley is one of the major feed grains in Western countries. In Canada, approximately 85% of the annual barley production is used for animal feed, 10% for malt, and the rest for food and other purposes (Rossnagel et al 1981). Until recently, very little attention was paid to improving the nutritional quality of barley, but now hull-less barley cultivars having more digestible and metabolizable energy than hulled barley, particularly for swine and poultry, have been reported (Bhatty 1986). The discovery of Hiproly, a high-protein and high-lysine barley from the USDA World Barley Collection, by Munck et al (1970), and the development of high-lysine mutants of barley by Danish and other workers (Bansal 1970, Ingversen et al 1973, Doll 1973, Doll and Eggum 1974) has greatly improved the possibility of increasing the protein quantity and quality of feed barley.

Although the mutant barley protein has higher biological value because of its higher lysine content, a number of studies (Bansal et al 1977, Alexander et al 1979, Newman et al 1979) report lower digestibility of high-lysine barley mutant protein compared to its parent. The reasons for the lower digestibility of mutant protein are not known, although they may partly be caused by alterations in the proportions of endosperm proteins. However, comparative in vivo and in vitro digestibilities of isolated protein fractions from parent and mutant barleys have not been reported. As a consequence, the contribution of individual protein fractions to total protein digestibility in barley is not known. The objective of the present study was to determine the comparative in vivo and in vitro protein digestibilities of parent and mutant barleys grown under similar environments, and, in addition, of protein fractions isolated from one pair of parent and mutant barley.

MATERIALS AND METHODS

Barley Genotypes

Eight genotypes of barley were used in the study. The three genotype pairs, Bomi and Risø 1508, Carlsberg II and Risø 86, and Carlsberg II and Risø 56 are Danish malting barleys and their mutants, respectively. The mutants, Risø 1508, Risø 86, and Risø 56 were produced by Danish workers by treatment of the parent cultivars Bomi and Carlsberg II with chemical mutagens. The three genotype pairs were supplied by C. W. Newman of Montana State University, Bozeman, where they were grown under identical environmental conditions. In addition, two other genotypes were included in the study. Genotype ANT-13-13 is a proanthocyanidine-free mutant of barley. Cultivar Abee is a licensed, two-rowed Canadian feed barley.

The barley seed was ground in a Udy cyclone mill to pass 1.0-mm screen and the meals were stored at 5° C.

Enzyme, Reagents, and *Tetrahymena thermophila*

α-Amylase type X-A from *Aspergillus oryzae*; porcine stomach mucosa pepsin, 2X crystallized; bovine pancreas trypsin, 2X crystallized, type I; bovine pancreas α-chymotrypsin, 3X crystallized; porcine peptidase, grade III; pronase (protease) type V, from *Streptomyces griseus*; and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma Chemical Co., St. Louis, MO. TNBS was used without recrystallization; its calculated molar absorption at 340 nm was 1,000 $M^{-1}cm^{-1}$. All other chemicals and reagents were of analytical reagent grade.

For in vivo digestibility measurement of the protein fractions, the commonly used mouse or rat bioassay was not practical because of the limited sample. Instead, a protozoan, *Tetrahymena thermophila*, was used for the assay. This organism has amino acid requirements similar to rat and man (Wang et al 1979). *T. thermophila* (catalog no. 30008) was obtained from the American Type Culture Collection, Rockville, MD. The culture was maintained at room temperature (25° C) by weekly transfers under aseptic conditions into a series of test tubes each containing 5 ml of the AACC medium 357, 5.0 g proteose (peptone), 5.0 g tryptone, 0.2 g of K_2HPO_4 per liter, pH 7.2.

Chemical Analysis

Total nitrogen content of the samples was determined by micro-Kjeldahl (AOAC 1980), tannins by the method of Price et al (1978), and amino acids by hydrolyzing samples at 110° C with excess 5.7*M* HCl in evacuated, sealed Pyrex test tubes (16 × 125 mm) for 22 hr. A Durrum 500 amino acid analyzer with norleucine as an internal standard was used.

In Vitro Digestibility of Meal Protein

In vitro digestibilities of the eight barley meal proteins were determined by two procedures. Procedure I was the modified pepsin digestibility method of Mertz et al (1984), except that the meals were suspended in 0.035*M* HCl (pH 2.0), and the residue was dried overnight at 40° C. A portion of the dried residue was

[1]Part of this research was done during the first author's sabbatical leave at the Department of Food Science and Technology, University of California, Davis. Presented in part at the Fifth International Barley Genetics Symposium, Okayama, Japan, October 6–11, 1986.

[2]Crop Development Centre, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Canada S7N OWO.

[3]Department of Food Science and Technology, University of California, Davis 95616.

analyzed for total nitrogen. Blanks were run with each assay without the addition of the enzyme solution. Pepsin digestibility was calculated as follows:

(nitrogen in blank − nitrogen in assay)/(nitrogen in meal) × 100

Procedure 2 was the multienzyme technique of Hsu et al (1977). Protein digestibility was expressed as the pH of the assay mixture after 10 min of incubation with the enzyme solution.

In Vivo Digestibility of Meal Protein

In vivo digestibility of the meal protein was determined by feeding mice. The meals were incorporated into diets containing 94.3% meal, 0.2% choline bitartrate, 1.0 and 3.5% American Institute of Nutrition vitamin and mineral premix, respectively, 0.5% chromic oxide, and 0.5% corn oil. Each diet and water were fed ad libitum for five days to three individually housed four-month old female mice weighing about 45 g and randomized in two replicates (six animals per diet). Fecal samples were collected one and one-half days after the feeding started. The diets and the feces were analyzed for protein and chromic oxide (Bolin and Lockhart 1960) for the calculation of apparent protein digestibility.

Fractionation of Meal Proteins

The Carlsberg II and Risø 86 pair was used for sequential extraction (in duplicate) of the meal protein fractions using the following solvents: albumins plus globulins, 0.5*M* NaCl with 0.05*M* EDTA-Na_2; hordeins, 55% propan-2-ol containing 2% mercaptoethanol (Shewry et al 1978); glutelins, 0.0125*M* $Na_2B_4O_7$, 0.043*M* NaOH (pH 10), 1% sodium dodecyl sulfate, and 1% mercaptoethanol (Ingversen et al 1973). The pooled extracts of each protein fraction were dialyzed for 48 hr in spectropore dialysis tubing, molecular weight cut-off 3,500–5,000, against several changes of distilled, deionized water to remove the solvents. The nondiffusible fractions were concentrated on a rotary evaporator and freeze-dried.

The protein fractions were analyzed for total nitrogen, total carbohydrates (Dubois et al 1956), and starch content (Chiang and Johnson 1977).

In Vitro Digestibility of the Protein Fractions

The extent of hydrolysis of the protein fractions was determined with pepsin, trypsin, α-chymotrypsin, and peptidase at enzyme-to-substrate ratios of 1:20 in a final volume of 2.0 ml, and an assay time of 24 hr in each case. For the assay of pepsin, the protein fractions were suspended in 1.0 ml of 10 m*M* HCl (pH 1.9), the enzyme (1.0 ml) was added in 1 m*M* HCl. For the assay of trypsin, α-chymotrypsin, and peptidase, the protein fractions were suspended in 1.0 ml of 5 m*M* phosphate buffer, pH 8.0; the enzymes were added in 1.0 ml of the same buffer except trypsin which was added in 10 m*M* HCl. In one experiment, the proteins were heated in an oven at 130° C for 15 min before the addition of the buffer (dry heat), or heated for 30 min in a boiling water bath after the addition of the buffer (wet heat). For α-amylase treatment, the proteins were hydrolyzed in 1.0 ml of 2 m*M* phosphate buffer, pH 6.9, at an enzyme-to-substrate ratio of 1:20 for 24 hr. The pH of the assay mixture was then adjusted to 2.0 by the addition of HCl for subsequent hydrolysis with pepsin, and to 8.0 by the addition of 5 m*M* NaOH for hydrolysis with trypsin. All assays were conducted in a shaking water bath at 37° C. Appropriate blanks containing substrate or enzyme only were run with each assay. At appropriate time intervals, 100 μl was withdrawn in triplicate from the assay mixture for the determination of peptide bonds hydrolyzed (Fields 1972). Glycine was used as a standard for the calculation of peptide bonds formed. Its calculated molar absorption (ε420) was 22,770 M^{-1} cm^{-1}. Enzyme activity was expressed as the degree of hydrolysis of the proteins, which was calculated by dividing the peptide bonds formed by the total number of bonds in the proteins (average molecular weight of amino acids taken as 113) × 100.

In Vivo Digestibility of the Protein Fractions

In vivo digestibility of the protein fractions and of bovine serum albumin (BSA), which was used for comparison, were determined using a *Tetrahymena thermophila* bioassay. The maintenance culture was centrifuged for 1 min at low speed in a table-top centrifuge, and the supernatant was removed with a Pasteur pipette. The sedimented cells were added to 20 ml of a complete defined medium (Rasmussen and Modeweg-Hansen 1973) in a 250-ml Erlenmeyer flask and allowed to grow without shaking at 30° C for 24 hr. In experiment 1, aliquots of the one-day-old culture were added to flasks containing 19.0 ml of the defined medium, 1.0 ml of sterile water (substituted for the amino acid solution in the complete defined medium), and appropriate weights of the proteins. In experiments 2–4, the sedimented cells, after centrifugation as described above, were suspended in 5 ml of sterile 50 m*M* phosphate buffer, pH 7.0 (Wang et al 1979), and then an aliquot of the suspension was added directly to each flask, which was then incubated at 30° C for 24 hr. The increase in cell concentration (turbidity) was measured periodically by reading absorbance at 600 nm. The absorbance of the media after the addition of the culture (0 time) was assigned a value of 100; the increase in absorbance was expressed relative to this value.

RESULTS AND DISCUSSION

Barley Composition

The eight genotypes of barley contained 9.2–14.2% protein and 0.00–0.16% tannins (data not given). The largest difference in protein contents (8.7%) was between the genotype pair Carlsberg II and Risø 56; the differences in protein content among the other two pairs were smaller (2.9–6.1%). The mutant ANT-13-13 contained 14.2% and the cultivar Abee 12.1% protein.

The differences in lysine content among the three genotype pairs were: Bomi and Risø 1508, 44.4%; Carlsberg II and Risø 86, 23.8%; and Carlsberg II and Risø 56, only 8.9% (Table I). The lysine content of ANT-13-13 and Abee was generally similar to that of the mutant parents Bomi and Carlsberg II. Eggum (1978) reported differences in protein and lysine contents (expressed as grams of lysine per 16 g of N) of Bomi, Carlsberg II, and their mutants. The lysine content of Risø 1508 was 38.7% and those of mutants Risø 86 and Risø 56 only 2.0–12.6% greater than their respective parents, Bomi and Carlsberg II. The largest difference in lysine found in the present study was between Bomi and Risø 1508. The increase in lysine content of the mutants is largely attributable to an increase in the proportion of the albumin plus globulin proteins and a corresponding decrease in the hordein proteins.

In addition to lysine, the major differences in the amino acid composition of the three genotype pairs were in glutamic acid and proline, which were substantially reduced in the mutants (Table I). The largest reduction (30%) in these amino acids occurred within the Bomi and Risø 1508 pair, compared to a decrease of 5–10% within the other two pairs. Compared to their parents, the mutants, particularly Risø 1508, contained more histidine, arginine, aspartic acid, glycine, and alanine. An increase in lysine and a concomitant decrease in glutamic acid and proline are characteristics of most induced high-lysine mutants of barley.

All the genotypes except the mutant ANT-13-13 contained < 0.2% tannins. No tannins were detected in the mutant ANT-13-13.

Digestibility of Barley Meal Protein

Table II shows the in vivo and in vitro apparent protein digestibilities (APD) of the eight genotypes of barley. Mutant ANT-13-13 had the highest APD, followed by the cultivar Abee and the two genotype pairs. The highest APD of ANT-13-13 was most likely caused by the absence of tannins in this genotype. Tannins may bind to protein through hydrogen bonding or hydrophobic interactions (Oh et al 1980). In addition, condensed tannins may also inhibit some of the digestive enzymes (Tamir and Alumont 1969).

It is difficult to explain why cultivar Abee had a higher APD than the other genotypes (except Carlsberg II in one case), possibly

TABLE I
Amino Acid Composition (mol %) of Regular and Mutant Barley Meals[a]

Amino Acid	Bomi	Risø 1508	Carlsberg II	Risø 86	Carlsberg II	Risø 56	ANT-13-13	Abee
Alanine	6.70	8.17	6.16	6.72	7.10	8.90	6.34	6.49
Arginine	3.95	5.58	3.74	4.11	4.19	4.20	3.88	3.90
Aspartic acid	8.35	11.06	8.61	8.64	7.57	9.24	6.28	6.08
1/2 Cystine	1.38	1.25	1.47	1.45	1.52	1.28	1.25	1.31
Glutamic acid	18.84	13.23	19.56	17.75	18.16	16.84	19.82	19.81
Glycine	7.45	9.63	6.93	7.40	7.63	8.11	7.19	7.17
Histidine	2.03	2.60	1.95	2.22	2.04	2.06	2.09	2.00
Isoleucine	3.90	3.63	3.96	3.94	4.00	3.74	3.89	3.91
Leucine	7.60	7.17	7.49	7.40	7.63	7.27	7.64	7.67
Lysine	3.49	5.04	3.19	3.95	3.70	4.03	3.31	3.30
Methionine	0.72	1.05	0.69	1.17	1.25	0.97	1.40	1.40
Phenylalanine	4.53	3.93	4.97	5.15	4.43	4.44	4.92	4.71
Proline	13.17	9.18	14.17	12.85	11.89	11.30	13.84	14.10
Serine	5.39	5.83	5.53	5.51	5.81	5.84	5.74	5.71
Threonine	3.75	3.45	3.05	3.03	4.03	3.10	3.84	3.82
Tryosine	2.35	2.57	2.53	2.54	2.52	2.39	2.43	2.39
Valine	6.39	6.64	6.02	6.16	6.52	6.29	6.14	6.21

[a]Ammonia excluded for the calculation of molar ratios. Data are means of duplicate determination.

TABLE II
In Vivo and In Vitro Apparent Protein Digestibilities of Eight Genotypes of Barley

Genotype	In Vivo (%)	In Vitro Procedure 1[a] (%)	In Vitro Procedure 2[b] (pH)
Bomi	66.6 ± 0.1	50.0 ± 0.0	7.57 ± 0.03
Risø 1508	61.2 ± 1.2	38.8 ± 0.8	7.60 ± 0.00
Carlsberg II	74.7 ± 0.6	55.0 ± 0.0	7.60 ± 0.07
Risø 86	67.9 ± 0.7	45.3 ± 0.0	7.67 ± 0.11
Carlsberg II	64.1 ± 0.2	55.0 ± 0.0	7.67 ± 0.11
Risø 56	64.7 ± 0.2	45.2 ± 3.5	7.50 ± 0.00
ANT-13-13	79.2 ± 0.5	49.9 ± 0.9	7.60 ± 0.00
Abee	73.3 ± 2.1	54.7 ± 0.0	7.45 ± 0.21
LSD (0.05)	3.1	4.4	0.32

[a]Mertz et al (1984).
[b]Hsu et al (1977).

because of its higher glutamic acid content (Table I). The APD of the mutants was lower than those of the parents except in the Carlsberg II and Risø 56 pair. These differences in the APD were not likely to be due to variable barley protein content, which has a curvilinear relationship with APD (Eggum 1985), but were more likely to be related to the higher lysine content of the mutants (Table I) as a result of increased level of albumin plus globulin proteins. Lysine, like tryptophan and threonine, is the least available of the essential amino acids of cereals (Misir and Sauer 1982, Tanksley and Knabe 1985). Furthermore, the albumin plus globulin protein fraction is distributed in the aleurone layer of barley. This layer has thick cell walls, and the proteins are tightly bound to the cellulosic matrix of the aleurone cells limiting accessibility to proteolytic enzymes of the gastrointestinal tract (Saunders and Kohler 1972). Aleurone in grains of wheat was largely unbroken during digestion by young chicks (Saunders et al 1969). It is most likely that the aleurone layer presents a similar problem in barley where it is tricellular compared to a monocellular layer in wheat.

The in vivo APD of the three pairs was positively related with in vitro APD values obtained by the pepsin digestibility procedure of Mertz et al (1984) but not with the multienzyme procedure of Hsu et al (1977). With the first procedure, the in vitro APD of the mutants was lower than the parents. However, unlike in vivo APD, the mutant ANT-13-13 showed reduced in vitro protein digestibility, suggesting that pepsin was more sensitive to tannins of barley in vitro than the proteases of the mouse gastrointestinal tract.

Figure 1 shows the rate of hydrolysis of meal proteins of eight genotypes of barley and, for comparison, of casein using the multienzyme technique of Hsu et al (1977). The largest drop in pH

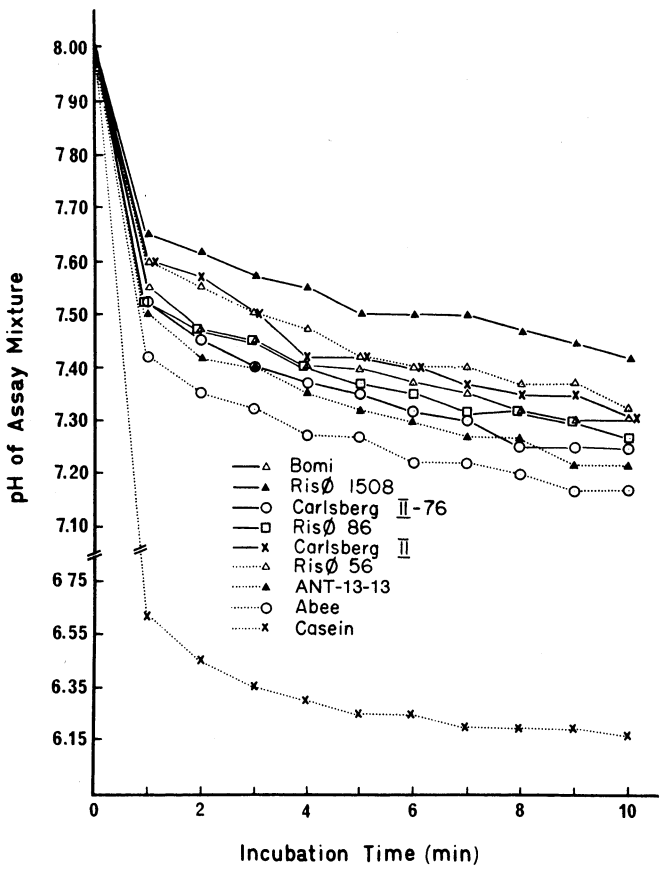

Fig. 1. The rate of hydrolysis of barley meal proteins and casein by multienzymes (Hsu et al 1977).

of the assay mixture due to enzymatic release of carboxyl groups from the meal proteins was in the first minute, after which it changed very little up to an assay time of 10 min. In one experiment, the incubation time was increased to 1 hr, but the change in pH was essentially same as that obtained with an assay time of 10 min. Thus, the multienzyme technique was unable to distinguish differences in APD among the barley genotypes. The poor sensitivity of this procedure may result from the strong buffering effect of barley meal. In contrast, hydrolysis of casein by the enzymes was rapid, although the major drop in pH was again in the first minute of the assay.

In Vitro Digestibility of the Isolated Barley Protein Fractions

Carlsberg II (parent) contained 13.9, 38.1, and 24.2% albumin plus globulin, hordein, and glutelin proteins, respectively,

expressed as percent of the meal protein. The corresponding values for the mutant were 12.6, 27.8, and 19.7%. The mutant contained almost three times more (17.3%) nonprotein nitrogen (lost on dialysis) than the parent barley.

All the three protein fractions were contaminated with carbohydrates. The albumin plus globulin fraction contained 30.8%, the hordein and glutelin fractions 3.6–4.5% total carbohydrates. While no starch was detected in the hordein fraction, the albumin plus globulin and the glutelin fractions contained 13.8 and 7.6% starch, respectively (data not given).

Table III shows the extent of in vitro hydrolysis of the albumin plus globulin, hordein, and glutelin protein fractions by mammalian proteases. The extent of hydrolysis of the same protein fraction isolated either from the parent or mutant barley was almost identical. Therefore, only one set of data (Risø 86) are given in Table III. A generally similar extent of hydrolysis obtained for the same protein fractions isolated either from the parent or mutant barleys was not surprising in view of the almost identical amino acid composition of the protein fractions (Table IV). Each protein fraction, therefore, isolated either from the parent or the mutant, seemed to have a similar primary structure and offered a nearly equal number of susceptible bonds to the proteolytic enzymes; however, the susceptibility of different protein fractions to the enzymes was quite variable. The albumin plus globulin fraction had lower digestibility than the hordein fraction, and the glutelin fraction was least susceptible to the enzymes. The simplest explanation for these differences may be the relationship between amino acid composition and protein digestibility. Although discrepancies on the different digestibility of amino acids from the same protein source have been reported (Silano 1977), it seems the higher digestibility of the hordein fraction, particularly compared to the albumin plus globulin fraction, was attributable to its lower lysine and threonine and higher glutamic acid contents (Table IV). Eggum (1985) reported that glutamic acid is highly digestible and lysine poorly digested, with other amino acids having intermediate digestibility between these amino acids. The lower digestibility of lysine may be caused by its ϵ-NH_2 group, which can react with sugars (Maillard products), phenols, and oxidized lipids to form lysine complexes that resist hydrolysis of the protein (Hurrell and Finot 1985). However, amino acid composition of the protein fractions alone was not sufficient to explain differences in their susceptibility to the enzymes. Albumin plus globulin and glutelin protein fractions had almost identical amino acid composition, particularly their lysine and glutamic acid contents (Table IV), yet the glutelin fraction was highly resistant to the proteases. Undoubtedly, other factors were responsible for the observed differences in hydrolysis of the protein fractions. These factors may be specificity of the enzymes, amino acid composition and sequence of the protein, molecular size, inter- and intra-chain disulfide bonds, and tertiary structure as well as presence in the protein of starch and sugars (Kakade 1974).

Data in Table V show that denaturation of the protein fractions by wet heat improved their digestibility by pepsin. Dry heat had no beneficial effect, and even slightly lowered the digestibility of the albumin plus globulin fraction. Both forms of heat lowered hydrolysis of the proteins by trypsin, most likely caused by destruction of lysine, as this enzyme preferentially hydrolyzes lysyl and arginyl peptide bonds. Treatment of the protein fractions with α-amylase (in the absence of Cl^- ions) greatly improved their hydrolysis both by pepsin and trypsin, suggesting that carbohydrates inhibited activity of these enzymes. Although no starch was detected in the hordein fraction, α-amylase treatment improved its hydrolysis by trypsin almost threefold. These data suggest that the lower digestibility of the glutelin fraction by mammalian proteases (Table III) largely resulted from starch or sugars present as contaminants or covalently bound to the protein fractions, although other factors may also be responsible as its digestibility was still lower than the albumin plus globulin or hordein fractions.

In Vivo Digestibility of the Protein Fractions

Table VI shows the in vivo digestibility by *T. thermophila* of barley protein fractions and BSA under different conditions. The protozoan grew extremely poorly and in many cases not at all when barley proteins were added to the media. The least growth occurred when the culture was applied to BSA or the protein fractions after first growing it in the complete defined medium for 24 hr (experiment 1). Figure 2 shows the growth of the organism in the complete defined medium for 24 hr. The organism grew linearly for about 10 hr (inset Fig. 2), and apparently was in the lag phase at 24 hr of growth. In all subsequent experiments, therefore, the culture was applied directly to the medium, which allowed it to go through its most active phase. Even under these conditions,

TABLE III
In Vitro Degree of Hydrolysis of Protein Fractions of Risø 86 Barley by Various Proteolytic Enzymes

	Degree of Hydrolysis (%)		
Enzyme	Albumins + Globulin	Hordeins	Glutelins
Pepsin	3.80 ± 0.10	4.81 ± 0.08	0.12 ± 0.08
Trypsin	4.31 ± 0.16	3.30 ± 0.07	ND[a]
α-Chymotrypsin	4.61 ± 0.25	6.36 ± 0.06	1.98 ± 0.09
Peptidase	0.91 ± 0.07	5.06 ± 0.03	ND[a]
Total	13.63 ± 0.58	19.53 ± 0.24	2.10 ± 0.17

[a]Enzyme activity not detected.

TABLE IV
Amino Acid Composition (mol %) of Protein Fractions of Carlsberg II and Risø 86 Barleys[a]

	Carlsberg II			Risø 86		
Amino Acid	Albumins + Globulins	Hordeins	Glutelins	Albumins + Globulins	Hordeins	Glutelins
Alanine	9.1	2.6	9.0	9.6	3.3	9.1
Arginine	6.1	2.0	5.4	5.8	2.0	5.7
Aspartic acid	10.3	2.0	9.2	10.7	2.0	9.5
Glutamic acid	13.5	37.5	14.1	13.2	35.6	13.6
Glycine	11.3	2.8	9.8	12.2	3.3	9.8
Histidine	2.4	1.4	2.5	2.2	1.3	2.5
Isoleucine	3.6	3.9	4.5	3.9	4.0	4.6
Leucine	7.0	5.9	8.6	7.1	6.7	8.6
Lysine	5.5	0.6	5.7	5.9	0.6	5.9
Phenylalanine	3.1	5.9	4.4	3.1	5.8	4.4
Proline	7.2	22.7	6.4	6.7	22.7	6.1
Serine	6.0	4.5	6.0	6.2	4.2	5.8
Threonine	4.9	2.4	4.6	5.1	2.4	4.8
Tryosine	1.8	1.0	1.8	0.8	1.1	1.9
Valine	6.8	4.5	7.8	7.0	4.9	7.7

[a]Ammonia excluded for the calculation of molar ratios.

(experiments 2–4, Table VI), the barley proteins were poorly utilized by *Tetrahymena* compared to its growth in the medium containing BSA. The best growth was obtained when the barley protein fractions, like the BSA, were predigested with pronase for 3 hr at 55° C (experiment 3). In this case, the organism grew 23-fold in the medium containing BSA but less than threefold in media containing the barley protein fractions. Heating the protein fractions and BSA at 100° C for 30 min (experiment 4) produced lower growth of the organism. Of the three barley protein fractions, the hordein fraction was utilized better than the albumin plus globulin or the glutelin fractions both in the parent and the mutant barleys predigested with pronase. It is not possible to speculate on the influence of different levels of carbohydrates present in the protein fractions on the growth of *Tetrahymena*. However, predigestion of the proteins with pronase for subsequent utilization by *Tetrahymena* may not give true indication of their protein digestibility. The enzyme hydrolysis converts the proteins into smaller fragments. Furthermore, pronase may hydrolyze the three barley protein fractions at different rates which may further confound true differences of their digestibility.

CONCLUSIONS

The present study showed that the high-protein, high-lysine mutants of barley had lower protein digestibility determined both in vivo (mouse feeding) and by the in vitro pepsin digestibility procedure of Mertz et al (1984). It has not been possible to suggest reasons for the lower digestibility of the mutant protein other than those resulting from alterations in the proportions of the albumin plus globulin and hordein fractions, in their amino acid compositions, and possibly to the deposition of the enhanced levels of the albumin plus globulin fraction in the aleurone layer of the mutants. Protein fractions isolated from one pair of parent and mutant barleys confirmed the higher in vivo (*Tetrahymena* bioassay) and in vitro (multiple enzyme assays) digestibilities of the hordein fraction compared to those of the other two protein fractions. However, in vitro digestibility of the glutelin fraction by pepsin and trypsin was greatly improved by prior treatment of the protein with α-amylase, suggesting that carbohydrates were partly responsible for the lower digestibility of this protein fraction. However, other factors may also be involved, as the in vitro digestibility of the glutelin fraction was still lower, particularly with trypsin, than the two other fractions. It is unlikely, although not impossible, that the glutelin fraction plays a role in lowering the digestibility of the mutant protein. This requires additional study. Wide variations have been reported in the literature on the glutelin contents of regular and mutant barleys. In the present study, the mutant Risø 86 contained less glutelin protein than its parent, Carlsberg II.

Barley proteins in general, and the albumin plus globulin and the glutelin protein fractions in particular, promoted extremely poor growth of *Tetrahymena thermophila*. These proteins either inhibited the extracellular proteolytic enzymes of the protozoan or, for unknown reasons, proved refractory to ingestion by this organism.

ACKNOWLEDGMENTS

R. S. Bhatty is grateful to the Director, Crop Development Centre, for grant of the sabbatical leave and to Dr. J. R. Whitaker for laboratory facilities during the sabbatical. We thank C. W. Newman of Montana State University, Bozeman, for a generous supply of barley samples, J. M. Bell, Department of Animal and Poultry Science, University of Saskatchewan, for assistance in conducting the mouse bioassay, and B. Ng for technical assistance.

TABLE V
Effect of Heat and α-Amylase Treatment on the Degree of Hydrolysis of Barley Protein Fractions by Pepsin and Trypsin

Enzyme Treatment	Degree of Hydrolysis (%)		
	Albumin + Globulin	Hordeins	Glutelins
Pepsin			
Untreated[a]	3.91 ± 0.48	4.93 ± 0.47	ND[b]
Dry heat	3.38 ± 0.03	4.94 ± 0.14	ND
Wet heat	5.31 ± 0.23	5.92 ± 0.04	0.19 ± 0.09
α-Amylase	7.46 ± 0.12	6.75 ± 0.19	6.19 ± 0.08
Trypsin			
Untreated[a]	4.86 ± 0.10	3.68 ± 0.32	0.70 ± 0.08
Dry heat	3.79 ± 0.08	3.33 ± 0.01	ND
Wet heat	3.92 ± 0.07	2.64 ± 0.01	ND
α-Amylase	8.33 ± 0.10	10.03 ± 0.42	5.83 ± 0.27

[a]Mean of four separate determinations.
[b]Enzyme activity not detected.

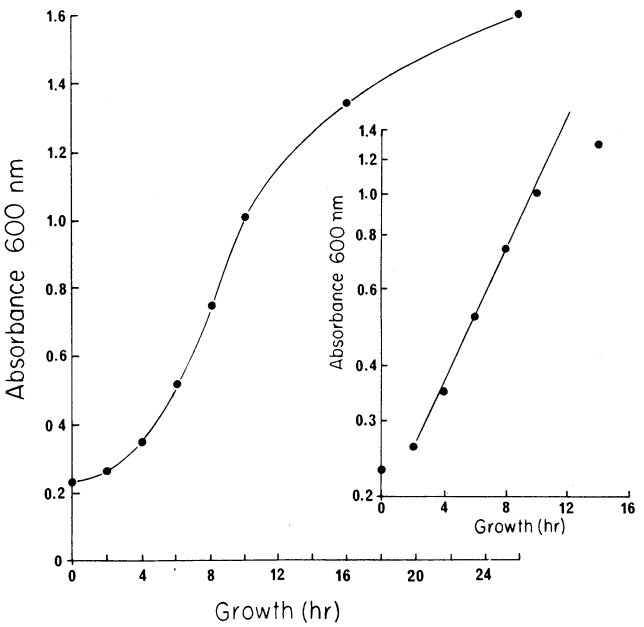

Fig. 2. The rate of growth of *Tetrahymena thermophila* in complete defined medium (Rasmussen and Modeweg-Hansen 1973) at 30° C for 24 hr.

TABLE VI
Relative 24 hr Growth (100 at Zero Time) of *Tetrahymena thermophila* in Growth Media Containing Bovine Serum Albumin (BSA) or Protein Fractions of Carlsberg II and Risø 86 Barleys

Experiment No.[a]	BSA	Carlsberg II			Risø 86		
		Albumins + Globulins	Hordein	Glutelin	Albumins + Globulins	Hordeins	Glutelins
1	273	101	84	102	100	74	91
2	217	107	113	93	108	116	101
3	2375	147	261	121	138	271	112
4	1155	114	81	107	116	71	102

[a]The following protein concentrations were used in each experiment in a total volume of 20 ml of the defined medium: 1) 20 mg; 2) 12 mg; 3) 10 mg predigested with pronase in 0.05 *M* phosphate buffer, pH 7.0 for 3 hr at 55° C at an enzyme-to-substrate ratio of 1:20; 4) 20 mg in 1.0 ml of water denatured by heating at 100° C for 30 min.

LITERATURE CITED

ALEXANDER, P. N., NEWMAN, C. W., EL-NEGOUMY, A. M., and ESLICK, R. F. 1979. Protein quality evaluation of a high lysine barley mutant. Proc. West. Sect. Am. Soc. Anim. Nutr. 30:126.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1980. Official Methods of Analysis, 13th ed. The Association: Washington, DC.

BANSAL, H. C. 1970. A new mutant induced in barley. Curr. Sci. (India) 39:494.

BANSAL, H. C., SRIVASTAVA, K. N., EGGUM, B. O., and MEHTA, S. L. 1977. Nutritional evaluation of high protein genotypes of barley. J. Sci. Food Agric. 28:157.

BHATTY, R. S. 1986. The potential of hull-less barley—A review. Cereal Chem. 63:97.

BOLIN, D. W., and LOCKHART, W. C. 1960. The use of perchloric acid as the sole oxidizing agent for the organic matter and chromium oxide. N. Dakota Acad. Sci. 14:102.

CHIANG, B. Y., and JOHNSON, J. A. 1977. Measurement of total and gelatinized starch by glucoamylase and *o*-toluidine reagent. Cereal Chem. 54:429

DOLL, H. 1973. Inheritance of the high lysine character of a barley mutant. Hereditas 74:293.

DOLL, H., and EGGUM, B. O. 1974. Induced high lysine mutants in barley. Radiation Bot. 14:73.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

EGGUM, B. O. 1978. Protein quality of induced high lysine mutants in barley. Page 317 in: Nutritional Improvement of Food and Feed Protein. M. Friedman, ed. Plenum Press: New York.

EGGUM, B. O. 1985. Digestibility of plant proteins. Page 275 in: Digestibility and Amino Acid Availability in Cereals and Oilseeds. J. W. Finley and D. T. Hopkins, eds. American Association of Cereal Chemists: St. Paul, MN.

FIELDS, R. 1972. The rapid determination of amino groups with TNBS. Page 464 in: Methods in Enzymology. Vol. 25. C. H. W. Hirs and S. N. Timasheff, eds. Academic Press: New York.

HSU, H. W., VAVAK, D. L., SATTERLEE, L. D., and MILLER, G. A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42:1269.

HURRELL, R. F., and FINOT, P. A. 1985. Digestibility of plant proteins. Page 233 in: Digestibility and Amino Acid Availability in Cereals and Oilseeds. J. W. Finley and D. T. Hopkins, eds. American Association of Cereal Chemists: St. Paul, MN.

INGVERSEN, J., KOIE, B., and DOLL, H. 1973. Induced seed protein mutant of barley. Experientia 29:1151.

KAKADE, M. L. 1974. Biochemical basis for the differences in plant protein utilization. J. Agric. Food Chem. 22:550.

MERTZ, E. T., HASSEN, M. M., CAIRNS-WHITTERN, C., KIRLEIS, A. W., TU, L., and AXTELL, J. D. 1984. Pepsin digestibility in sorghum and other major cereals. Proc. Natl. Acad. Sci. USA 81:1

MISIR, R., and SAUER, W. C. 1982. Composition, amino acid digestibilities and protein quality of high-lysine barley lines for growing rats. Can. J. Anim. Sci. 62:1149.

MUNCK, L., KARLSSON, E. E., HAGBERG, A., and EGGUM, B. O. 1970. Gene for improved nutritional value in barley seed proteins. Science 168:985.

NEWMAN, C. W., ALEXANDER P. N., and ESLICK, R. F. 1979. Comparison of a high-lysine barley mutant to normal barley in swine diets. Proc. West. Sect. Am. Soc. Anim. Sci. 30:122.

OH, H. I., HOFF, J. E., ARMSTRONG, G. S., and HAFF, L. A. 1980. Hydrophobic interaction in tannin-protein complexes. J. Agric. Food Chem. 28:394.

PRICE, M. L., SCOYOC, S. V., and BUTLER, L. G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26:1216.

RASMUSSEN, L., and MODEWEG-HANSEN, L. 1973. Cell multiplication in *Tetrahymena* cultures after addition of particulate material. J. Cell Sci. 12:275.

ROSSNAGEL, B. G., BHATTY R. S., and HARVEY, B. L. 1981. Developing high energy hulless feed barley for Western Canada. Page 293 in: Proc. 4th Int. Barley Genet. Symp.: Edinburgh, U.K.

SAUNDERS, R. H., and KOHLER, G. O. 1972. In vitro determination of protein digestibility in wheat millfeeds and monogastric animals. Cereal Chem. 49:98.

SAUNDERS, R. H., WALKER, H. G., and KOHLER, G. O. 1969. Aleurone cells and the digestibility of wheat millfeeds. Poultry Sci. 48:1497.

SHEWRY, P. R., HILL, J. M., PRATT, H. M., LEGATT, M. M., and MIFLIN, B. J. 1978. An evaluation of techniques for the extraction of hordein and glutelin from ...rest of citation missing from manuscript—please provide.

SILANO, V. 1977. Factors affecting digestibility and availability of proteins in Cereals. Page 13 in: Nutritional Evaluation of Cereal Mutants. IAEA: Vienna, Austria.

TAMIR, M., and ALUMONT, E. 1969. Inhibition of digestive enzymes by condensed tannins from green and ripe carobs. J. Sci. Food Agric. 20:199.

TANKSLEY, T. D., and KNABE, D. A. 1985. Direct measurements of amino acid digestibility in swine. Page 259 in: Digestibility and Amino Acid Availability in Cereals and Oilseeds. J. W. Finley and D. T. Hopkins, eds. American Association of Cereal Chemists: St. Paul, MN.

WANG, Y. Y. D., MILLER, J., and BEUCHAT, L. R. 1979. Comparison of four techniques for measuring growth of *Tetrahymena pyriformis* with rat PER bioassay in assessing protein quality. J. Food Sci. 44:540.

[Received June 18, 1986. Accepted January 5, 1987.]