



The composition and nutritional value of a high lysine, high sugar barley (*Hordeum vulgare* L.)
by Petrea Jolain Hofer

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Home Economics

Montana State University

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Abstract:

Compana (CP) barley mutant isolines, Prowashonupana (PWSNP) 1, 2 and 3 grown in 1981 were compared to the normal isolate, Washonupana (WSNP), for chemical composition and nutritional value. PWSNP 1, 2 and 3 were higher than WSNP on a dry matter basis in protein, lysine, ether extract, fiber, ash, phosphorus, total sugar, reducing sugar and 8-glucan content. PWSNP 1, 2 and 3 were lower in starch, alkaline and acid extract viscosity and kernel weight and PWSNP 2 and 3 had fewer plump kernels when compared to WSNP.

Chicks were fed diets of maize, WSNP and PWSNP 2 and 3, balanced for 23 % protein and 1.20 % lysine with β -glucanase added. Chicks fed PWSNP 3 were lower ($P < 0.01$) in body weight gain, higher, in feed/gain ratio and had heavier, more moist excreta than chicks fed other diets.

Rats consuming 10 % isonitrogenous diets of WSNP, PWSNP 1, 2 and 3 and casein were compared in nitrogen balance (NB) and digestible energy (DE) trials. Rats fed the PWSNP 3 diet had lower ($P < 0.01$) true protein digestibility (TPD) and higher biological value (BV) scores than rats fed other isolate diets. Net protein utilization (NPU) was similar for all barley isolines but differed ($P < 0.01$) from that of casein. DE of rats had lowest values ($P < 0.01$) in PWSNP 3 and highest values ($P < 0.01$) in WSNP, when comparing barley isolines. A second DE rat trial consisted of 95 % barley diets made from PWSNP 1, 2 and 3, WSNP and CP. The PWSNP 3 diet had lowest DE ($P < 0.01$) in the two trials.

Analysis of variance with orthogonal comparisons was used to determine effects of maturity at four stages on 1983 normal Compana isolines Nupana (NP), Shonupana (SNP) and WSNP compared with mutant isolines PWSNP 1 and 3 and Pronupana (PNP). Sensory evaluation tested 1983 PWSNP 1, PWSNP 3 and WSNP at the second and fourth stages of maturity for taste, sweetness and preference differences. Panel members were able to differentiate between all three isolines at both stages of maturity. Preference and sweetness data were subjected to log-linear contingency table analysis. Preference was found for PWSNP 3 at the second stage but not at the fourth stage. All panel members rated PWSNP 3 as the sweetest and WSNP as the least sweet at the second stage of maturity but could not differentiate sweetness at the fourth stage.

Major nutrient proportions are most unusual in FWSNP 3, followed by PNP, showing high sugar, protein, lysine and dietary fiber but low starch levels. This barley may be beneficial to populations trying to lower calorie intake, increase fiber consumption yet obtain quality protein.

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A thesis submitted in partial fulfillment
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ABSTRACT

Compana (CP) barley mutant isolines, Prowashonupana (PWSNP) 1, 2 and 3 grown in 1981 were compared to the normal isolate, Washonupana (WSNP), for chemical composition and nutritional value. PWSNP 1, 2 and 3 were higher than WSNP on a dry matter basis in protein, lysine, ether extract, fiber, ash, phosphorus, total sugar, reducing sugar and β -glucan content. PWSNP 1, 2 and 3 were lower in starch, alkaline and acid extract viscosity and kernel weight and PWSNP 2 and 3 had fewer plump kernels when compared to WSNP.

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Rats consuming 10 % isonitrogenous diets of WSNP, PWSNP 1, 2 and 3 and casein were compared in nitrogen balance (NB) and digestible energy (DE) trials. Rats fed the PWSNP 3 diet had lower ($P < 0.01$) true protein digestibility (TPD) and higher biological value (BV) scores than rats fed other isolate diets. Net protein utilization (NPU) was similar for all barley isolines but differed ($P < 0.01$) from that of casein. DE of rats had lowest values ($P < 0.01$) in PWSNP 3 and highest values ($P < 0.01$) in WSNP, when comparing barley isolines. A second DE rat trial consisted of 95 % barley diets made from PWSNP 1, 2 and 3, WSNP and CP. The PWSNP 3 diet had lowest DE ($P < 0.01$) in the two trials.

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Major nutrient proportions are most unusual in PWSNP 3, followed by PNP, showing high sugar, protein, lysine and dietary fiber but low starch levels. This barley may be beneficial to populations trying to lower calorie intake, increase fiber consumption yet obtain quality protein.

INTRODUCTION

Cereals are the most important food staple of the world for meeting caloric and other nutritional needs of humans and animals. Barley (Hordeum vulgare L.) composes about 12 % 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 increasing steadily (Montana Agricultural Statistics, 1984). Barley production in the nation totaled 519 million bushels in 1983; rose to 597 million bushels in 1984 and was forecasted to increase to 600 million bushels in 1985.

Montana was second among the states in barley production in 1983 (77.7 million bushels); it dropped to fifth in 1984 (59 million bushels) and will possibly rank even lower in 1985 because of severe drought. Despite lower production here in Montana because of climatic conditions, barley production is up throughout the United States. The top five producing states in 1984, ranked from high to low, were North Dakota, Minnesota, Idaho, Washington and Montana.

Since 1968, intensive worldwide research and breeding programs have produced new barleys with a high percentage of lysine in the protein. Lysine is the first limiting amino acid in most cereals, therefore a high lysine grain offers a more valuable source of protein. Unfortunately, an increase in lysine has been associated with small kernels due in part to a shrunken endosperm. This results in lower yields, and the long-term benefit is masked by apparent

economic disadvantages. Factors which cause reduced kernel weight have not been completely resolved. Suggested causes include reduced starch formation due to inhibition of the starch synthesizing enzymes (Solomonsson, 1983), reduction in the rate of grain growth due to phytohormonal changes (Mounla et al., 1980) and lower potassium content (Hagberg et al., 1979).

The barley cultivar *Compana* (C.I. 5438), a two-rowed feed barley, has been used to produce many isogenic lines. The waxy gene was incorporated into *Compana* by backcrossing it to *Waxy Oderbrucker*, (C.I. 7563), followed by six backcrosses to the recurrent parent (Fox, 1981). The short-awn and hulless seed genes were donated by the cultivar *Sermo* (C.I. 7776). The short-awn, the hulless and the short-awn, hulless isolines were selected while backcrossing to *Compana* six times (Hockett, 1981) yielding *Washonupana*.

Washonupana was treated with diethyl sulfate, producing three isoline mutants designated *Prowashonupana* 1, 2 and 3. These barleys are high protein, high lysine and contain high levels of low molecular weight sugar when compared to the shrunken endosperm, high lysine barleys such as *Risø* mutants and *Hiproly* or normal barley. High sugar barleys may be useful in human food products because of possible sweeter flavor and the high protein, high lysine content. Information on the nutritional value of the sugar/starch and protein/lysine relationships of these barleys has not been reported in the literature.

The purpose of this study was to compare the chemical characteristics and nutritional value of three *Prowashonupana* barley isolines,

containing high levels of sugar and lysine, with those of related isogenic lines and normal barley. General objectives of the research included:

- 1) determination of chemical composition of the selected barleys at four stages of development,
- 2) determination of growth, feed consumption and feed efficiency of rats and chickens fed these barleys,
- 3) measurement of energy and nitrogen balances in rats fed these barleys, and
- 4) evaluation of barleys for sweetness differences in the kernels using taste panels.

LITERATURE REVIEW

The nutritional quality of a barley is dependent on many factors which can be categorized into the general areas of protein, fat, carbohydrate and fiber. Barley is traditionally thought of as being low in protein. Newman and McGuire (1986) reported a range of 8.1 - 24.1 % protein with an average of 12.8 % protein in barleys grown at the Montana Agricultural Experiment Station (MAES), Bozeman. The same study reported a range of 1.8 - 4.9 % in ether extract and 1.9 - 9.9 % in crude fiber with averages of 2.1 and 5.3 %, respectively. Torp (1980) reported starch content in forty barley varieties to range from 55 - 62 % and low molecular weight carbohydrates to average 2.3 % of the dry matter.

High Lysine Barley

Lysine, methionine, threonine and tryptophan are essential amino acids found in deficient amounts in barley (Howe et al., 1965). Lysine, methionine and threonine are reported in fifty-four western grown barleys to have means and ranges of 0.36 % (0.22 - 0.48 %), 0.18 % (0.09 - 0.27%) and 0.33% (0.17 - 0.46 %), respectively (Ullrich et al., 1984). Lysine is the first limiting essential amino acid in barley and is therefore the most important among amino acids when evaluating the nutritional quality of barley protein.

High lysine barley was discovered in 1968 in the Hiproly cultivar (C.I. 3947) (Munck et al., 1970). Breeding programs have since produced high lysine Hiproly, Risó, sex, and Notch mutants as well as crosses on normal cultivars (Bansal, 1970; Jarvi and Eslick, 1975;

Balaravi et al., 1976; Karlsson 1976, 1977; Ullrich and Eslick, 1978; Jensen, 1979; Jensen and Doll, 1979; Doll, 1980). Animal nutrition studies have demonstrated the superior nutritional value of high lysine barley mutants over normal barley cultivars (Munck et al., 1970; Eggum, 1973; Newman et al., 1974, 1977, 1978; Thomke et al., 1978; Tallberg and Eggum, 1982).

High lysine barleys have characteristically produced low grain yields to date. There is some indication that high lysine barleys differ in composition from normal barleys. A study by Thomke and Widströmer (1975) indicated that carbohydrate composition differed between normal and high lysine varieties. Køie and Doll (1979) found the starch yield of eleven Risø mutants to be 38 - 88 % the yield of the parents while Solomonsson et al. (1980) reported no consistent chemical differences between the major constituents of high lysine and normal, Risø 1508 and four Hiproly SV mutant barleys produced at Svalöf, A. B., Sweden.

Explanations for Reduction in Grain Weight

Reduced kernel size is characteristic of high lysine barley genotypes and can be readily detected by visual inspection in some cases. The biochemical and physiological causes have not been clarified for this reduction in grain weight. Suggested causes include lowered starch formation due to inhibition of the starch synthesizing enzymes (Solomonsson, 1983), reduction in the rate of grain growth due to phytohormonal changes (Mounla et al., 1980), and lower potassium content (Hagberg et al., 1979).

Bhatia and Rabson (1976) showed that high lysine genes are only responsible for a maximal 2.5 % reduction in grain weight, unless the photosynthates were impaired. The remainder of loss in grain weight is thought to be due to reduced starch formation and grain growth. When Bomi Risø 1508 and SV 73608 (Hagberg et al., 1979) were compared, only the rate of grain growth was impaired in SV 73608 but in the Risø 1508, both the rate of growth and starch formation were impaired. Coles (1979) reported that when the seed reached less than 47 % moisture, starch synthesis was reduced or stopped. Fastnaught (1982) suggested that reduction of dry matter accumulation may not be due to a lower moisture content, but rather that the seed proceeded to reach maturity due to physiological processes. The sex mutants (Ullrich and Eslick, 1978) reached 40 % moisture seven to eight days later than their normal isotypes, but the dry matter accumulation was not prolonged. It was suggested that the higher moisture content was a result of the high sugar content because of the strong positive correlation between total sugar in the kernel and mean moisture percentage. Water may be pulled into the cell, maintaining a higher osmotic state. The sex type mutants appear as plump as a normal seed until dry down. A block may occur in the physiological pathway from sucrose to starch, causing higher than normal total sugar content. Starch and protein synthesis may be linked to an intermediate or end product of one pathway being required by the other.

Higher potassium levels were also found in the normal genotypes, Mona and Bomi, throughout the entire grain filling period (Hagberg et al., 1979). Hartt (1970), Mengel and Viro (1974) and Ashley and

Goodson (1972) all found that potassium enhances the transport of photosynthates to the place of storage. Greater potassium levels also increased the rate of photosynthesis (Mengel and Haeder, 1976). By contrast, the calcium content was greater in the high lysine SV 73608 and Risø 1508 showing the opposite pattern as that of potassium (Hagberg et al., 1979). Forster and Mengel (1976) found that higher potassium levels produced more flag leaf area in five spring wheat varieties, which in return showed a correlation with increased grain yield.

The genes that cause high lysine mutants may also be responsible for changes on the hormone-based growth-regulating system of the barley (Hagberg et al., 1979). Both Hagberg et al. (1979) and Mounla et al. (1980) have reported significantly higher gibberellin-like activity in high lysine mutants than in normal isotypes.

Starch

Starch exists in two molecular forms, linear or branched, and is composed entirely of α -glucan or D-glucopyranose units. It can either be joined in straight chains linked α 1,4 called amylose or exist as amylopectin where the α 1,4 units are branched through α 1,6 linkages (Briggs, 1978). 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. Mutants such as high-amylose Glacier (Merritt, 1967) contain equal amounts of amylose to amylopectin. Barleys referred to as waxy contain only 0 - 3 % amylose (Briggs, 1978). Waxy starch granules are

pitted or eroded, whereas the normal starch granule is spherical (Eslick, 1979).

Starch deposition parallels the increase in dry matter. About 75 - 80 % of barley starch is found in the endosperm (Munck, 1981). Starch accumulates progressively following a logarithmic pattern (Cerning and Guilbot, 1973). MacGregor et al. (1971) reported starch synthesis to begin eleven days after ear emergence with synthesis continued rapidly for fourteen days after which it slowed down until deposition stopped on day thirty-two. This western Canadian study reported that 95 % of the barley starch was synthesized in seventeen days. Growing season also dictates length of time needed for similar starch accumulation in other environments, as other researchers have recorded from a requirement of thirty-five to forty days for starch deposition in the United Kingdom (Harris and MacWilliam, 1957; and Merritt and Walker, 1969)

Low molecular weight carbohydrates are negatively correlated to starch values; therefore when soluble sugars are high, starch content is lowered. Lowered starch values of high lysine mutants are not due to any limitation in the supply of soluble sugar precursors. Batra et al. (1982) compared parent NP-113 with its high lysine Notch-2 mutant and found the mutant contained substantially higher sugar and lower starch content throughout all stages of development.

The Risø 1508 mutant shows lower starch values but contains more free sugars than its normal parent Bomi (Køie & Doll, 1979; Kreis and Doll, 1980). Torp (1980) found Hiproly to contain less starch than the average normal barley whereas Balaravi et al. (1976) did not find

a starch reduction. Solmonsson (1980) compared Bomi and its mutant Risó 1508 along with five Hiproly SV mutants and normal counterparts. The results showed no marked difference in carbohydrates types between high lysine and normal barley genotypes. A common mechanism is thought to be responsible for the lowered starch synthesis regardless of the differences in the type of high lysine genes involved (Torp, 1980).

Sugar

Low molecular weight or ethanol extractable carbohydrates are referred to as sugars. Sugars can be classified as reducing or non-reducing. Reducing sugars such as glucose, fructose and maltose have a free carbonyl group which can be either aldehydic or ketonic. Reducing sugars take up an oxygen atom donated by a mild oxidizing agent at the carbonyl carbon to carboxylic acids. An oligosaccharide is considered reducing if one of the anomeric centers is not involved in glycosidic linkages. When all the anomeric centers of the oligosaccharide are substituted with glycosidic linkages, the sugar is considered non-reducing (Shallenberger, 1982). Glucose and fructose comprise the majority of the reducing sugars and together with sucrose the total free sugars (Smith, 1973). Sucrose, the main sugar found in barley is a non-reducing sugar. During barley development, total sugars and reducing sugars decline, while non-reducing sugars remain relatively constant (Briggs, 1978). Carbohydrate composition in developing barley is constantly changing with starch being synthesized at the expense of both reducing and non-reducing sugars.

In comparing low molecular weight carbohydrates in forty high-yielding barley varieties and mutant lines, Torp (1980) found the total sugar to average 2.3 % with a 50 % deviation range. During the period of anthesis to maturity, LaBerg et al. (1973) found glucose and fructose to peak early, followed by a rise in sucrose and later a rise in raffinose shortly before maturity.

Harris and MacWilliam (1954) reported fructose, sucrose, fructosans, and glucodiffructose present in the barley kernel at the beginning of development, with raffinose appearing only at a later stage. The late appearance of raffinose was confirmed by Cerning and Guilbot (1973), not only in barley, but also in wheat. Harris and MacWilliam (1954) found sucrose and fructosans in maximum concentration four weeks after ear formation whereas glucose showed two peaks. The first peak was five weeks after ear formation and the second appeared three weeks later. MacGregor et al. (1971) reported that sugars rose to a maximum of sixteen days after ear emergence and then declined.

In tests conducted by Gohl et al. (1977) on the cultivar Ingrid, distribution of the low molecular carbohydrates consisted mainly of sucrose, together with stachyose, raffinose, glucose, fructose, glucitol and myoinositol. A greater percentage of these sugars was found at late maturity and a greater percentage of them on the outer part of the kernel. Sugars rose from 1.93 % in early yellow-ripeness maturity to 2.27 % in combine-ripeness maturity (dry matter basis). The concentration of ethanol-extractable carbohydrates was more than 3 % in the outer 30 % of the kernel and between 1 - 2 % in the remaining part of the kernel (Gohl et al., 1978b).

In the early harvested barley, sucrose accounted for about 81 % of the low molecular carbohydrates in the outer 30 % of the kernel and decreased to 46 % in the center. Stachyose appeared only in the inner part of the kernel and made up 10 % of the ethanol extracts. Glucose ranged from 7 - 17 % and was found in all fractions. Amounts of raffinose increased toward the center of the kernel. This sugar existed in all fractions with maximum concentration reaching 28 %. The bran fraction held 23 % of the fructose with the amount decreasing toward the inner part of the kernel. Glucitol, first reported by Gohl as a major contributor to the content of low molecular carbohydrates, constitutes about 40 % in the outer kernel layers. Myoinositol occurred in small amounts throughout the kernel and xylose in small amounts in the outer kernel fractions. The presence of free maltose was questioned. The presence of fructosans could not be determined due to the specific gas liquid chromatography analysis used (Gohl et al., 1977).

Gohl et al. (1978b) reported that the only significant differences in low molecular weight sugar between early and late harvested barley was in raffinose and stachyose. Stachyose increased considerably during maturation especially in the kernel center while raffinose decreased at the expense of stachyose. Raffinose was hydrolyzed to fructose and melibiose under mild conditions.

A comparison of sugar composition of various cereals (Table 1) shows that sucrose is the major sugar component with sorghum reaching higher levels than barley. Barley and pearl millet show similar raffinose composition, which is higher than other cereal grains. Maltose levels are usually low, but if high, are likely due to the

Table 1. Sugar composition of wheat, maize, barley, oat, sorghum and millet (g/100 g sample).

Cereal	Raffinose	Sucrose	Maltose	Stachyose	Fructose	Glucose	% Total Sugars	References
Wheat flour	0.05-0.43	0.16-0.60	0.07-0.09	-	0.02-0.03	0.04-0.06	1.3-2.8	MacArthur & D'Appolonia (1979)
Maize	0.20-0.30	0.70-1.25	-	-	0.20-0.26	0.03		Cerning (1970)
Barley	0.14-0.83	0.34-1.69	0.14	-	0.03-0.16	0.02-0.09		Kent (1975)
Oat flour	0.16-0.26	0.40-0.63	0.01-0.03	-	0.02-0.05	0.06-0.07	0.9-1.3	MacArthur & D'Appolonia (1979)
Sorghum	0.10-0.39	0.92-3.90	-	-	0.06-0.74 ^a		1.30-5.19	Subramanian et al. (1980)
Millet	0.65-0.84	1.32-1.82	-	0.06-0.13	0.08-0.16 ^a		2.16-2.78	Subramanian et al. (1981)

^aFructose + glucose

Source: Subramanian et al., 1981.

enzymatic breakdown of starch or raffinose arising from poor harvesting conditions or from alteration during storage (Cerning and Guilbot, 1973).

Fructosans

Fructosans, also known as glucofructosans, represent a series of oligosaccharides possessing increasing numbers of fructose residues with a single terminal glucose unit. Fructosans occur in two forms, inulins and levans. Inulins are β 2,1 linked D-fructofuranose polymers and levans are β 2,6 linked D-fructofuranose polymers (Smith, 1973). Fructosans make up barley's second most predominant sugar, after sucrose (MacGregor et al. 1971; Harris and MacWilliam, 1954, 1957). Both sucrose and fructosans are readily hydrolyzed to their constituent monosaccharides by dilute acid (MacGregor et al., 1971; Smith, 1973).

MacGregor et al. (1971) found that the synthesis of fructosans to be the reason for the rapid sugar increase between the seventh and sixteenth days after barley ear emergence. Maximum sugar content was on day eleven if expressed on a dry matter basis and on day sixteen if expressed on a per kernel basis with a decline occurring thereafter.

Non-Starch Polysaccharides

Hydrocolloids composed basically of sugar units or polysaccharides are known for their thickening or gel-forming ability. The term hydrocolloids is used because they rarely give true solutions (Gohl, 1977). Gums contain a variety of sugars and upon fractionation yield a β -glucan and an arabinoxylan (Briggs, 1978). These buffer

extracted carbohydrates are dominated by glucose, and include arabinose and xylose with smaller amounts of mannose, galactose (Solomonsson et al., 1980; Gohl et al., 1978b), rhamnose (Gohl et al., 1978b), glucaronic acid and galacturonic acid (Solomonsson et al., 1980). The glucose originated mainly from cellulose and β -glucans, whereas xylose was derived from arabinoxylans (Fincher, 1975; Forrest and Wainwright, 1977; McNeil et al., 1975). Barley endosperm cell walls consist of 75 % β -glucan and the rest consists mainly of arabinoxylan (Munck, 1981).

Compounds containing two or more glucose molecules linked together with a β -bond are given the general name β -glucan. These glucans, found in both barley and oats consist of approximately a 7:3 ratio of β 1,4 and β 1,3-D-glucopyranosyl units respectively (Bathgate et al., 1974; Bourne and Pierce, 1972). The structure consists of two or three 1,4 bonds separated by 1,3 bonds but the number of adjacent 1,3 bonds are few (Chanda et al., 1957; Parrish et al., 1960).

Barley β -glucan content has been found to range from 1 - 6 % (Eslick, 1979) and 4 - 8 % (Prentice et al., 1980) of the kernel weight. Oats have a β -glucan range of about 1 - 3 % (Wood et al., 1977), whereas wheat and rye have high levels of pentosans but little β -glucan (Perlin and Suzuki, 1965; Preece and Hobkirk, 1954).

The β -glucans are a major contributor to the viscosity of barley extracts (Preece and MacKenzie, 1952). Greenberg (1974) found a high correlation ($r=0.89$) between β -glucan and barley extract viscosity. Extract viscosity in barley increased during ripening until the yellow-ripeness stage (Gohl, 1977). Viscosity was affected by dry

matter content of the kernel especially when dry matter exceeds 50 % with maximum values at 70 % of the dry matter content. Maximum values were reached at about 70 % dry matter, after which the viscosity decreased (Gohl, 1977). The water-soluble arabinoxylan may also be involved in the viscosity of barley (Gohl, 1978b). A difference in content and distribution of buffer extracted carbohydrates has been seen between early and late stages. The highest concentration of viscous materials was found in a layer immediately under the aleurone layer at early harvest and more in the center of the kernel at combine ripeness (Gohl et al., 1977).

A very rapid accumulation of pentosans and crude fiber (cellulose, hemicellulose and lignin) occurs in barley. Unlike starch, pentosans and crude fiber show parallel accumulation occurring in two stages (Cerning and Guilbot, 1973). Cellulose is also a β -glucan linked at the 1,4 positions of straight-chained D-glucopyranose units containing an average of 3000 units per molecule. Cellulose occurs as a flat molecule whereas the mixed linked 1,3:1,4 β -glucans are spirals, thus having different solubilities. Hemicellulose is a combination of β -D-glucopyranose and D-pentose units (Briggs, 1978).

Energy Value of Barley

Although barley can provide a major part of the protein an animal or human needs, it is primarily used as an energy source. A grain's ability to supply energy is of great importance in determining nutritive value. Gross energy values of food constituents vary with foods averaging about 4.4 kcal/g of dry matter. The gross energy of glucose

and starch is 3.74 and 4.23 respectively (McDonald et al., 1973). The difference in energy value is due to the ratio of carbon plus hydrogen to oxygen. Fats have a lower state of oxidation and therefore yield more energy when oxidized. Fats average about 9.2 kcal/g dry matter, where as proteins contain about 5.3 kcal/g gross energy.

Based on data from 258 digestibility and nitrogen balance experiments with growing pigs, Jørgensen (1980) found the correlation between soluble carbohydrates and the digestibility of gross energy to be positive ($r=0.45$). Sibbald and Price (1976) reported significant correlations between true metabolizable energy and starch ($r=0.83$), starch and sugar ($r=0.84$), ash ($r=-0.76$) and crude fiber ($r=-0.90$) for poultry. Therefore a higher content of soluble carbohydrates, which is nearly 100 % digestible, will indicate a higher digestibility of gross energy. Jørgensen (1980) also showed crude fiber, containing cellulose, hemicellulose and lignin, to be negatively correlated to digestibility coefficients of all chemical fractions. A linear decrease was seen in gross energy with increasing crude fiber ($r=0.92$). A 2.4 unit decrease in digestibility of gross energy was found for every percent increase in crude fiber. Crude fiber showed the highest predictive value of the digestibility of gross energy when compared to the Van Soest (1963) method of carbohydrate fractionation. However, King and Taverner (1975) found neutral detergent fiber to give a more accurate estimate of the digestibility values than either acid detergent fiber or crude fiber.

A hulled barley should have lowered digestibility compared to hullless varieties because the husk contains almost all the lignin of

the barley (Briggs, 1978; Munck, 1981). Hulless barleys should have higher digestible energy values than hulled barleys as they are higher in fat, protein and starch content due to the reduction in crude fiber represented by the absence of hulls (McGuire and Hockett, 1981). When the hulls, representing about 10 % of the kernel, are left in the field the remaining kernel has a corresponding increase in starch level and energy value (Munck, 1981).

Anderson et al. (1961) reported no significant differences in performance of broiler chicks fed either covered or hulless barleys. In comparison, chicks fed a maize diet in this study gained 17 % faster and were 17 % more efficient than chicks fed the hulless barley. Another study by Sibbald (1982) found similar true metabolizable energy values for normal and hulless barley when fed to adult chicks. Newman et al. (1968) also reported covered and hulless barley isolines to be equal for weanling pigs in digestible energy value. However, higher digestible energy values were reported for hulless barley by Bhatti et al. (1979) and Truscott (1980) when compared to covered barleys.

MATERIALS AND METHODS

Barleys

The barleys studied consisted of isogenic lines derived from the two-rowed cultivar Compana (C.I. 5438). The Prowashonupana isolines 1, 2 and 3 were produced by treating Washonupana with diethyl sulfate. The isoline Pronupana was produced by crossing Nupana with the spontaneous mutant gene sex1 in Compana (R. F. Eslick, personal communication). Barleys included in this study were grown at the Montana Agricultural Experiment Station, Bozeman, MT and the Arizona Agricultural Experiment Station, Mesa, AZ in 1980, 1981, 1982 and 1983.

Barley cultivars and lines studied were as follows:

	<u>Hull type</u>	<u>Starch type</u>	<u>Awn</u>
1. Compana	covered	normal	normal
2. Nupana	hulless	normal	normal
3. Shonupana	hulless	normal	short
4. Washonupana	hulless	waxy	short
5. Prowashonupana 1	hulless	waxy	short
6. Prowashonupana 2	hulless	waxy	short
7. Prowashonupana 3	hulless	waxy	short
8. Pronupana	hulless	normal	normal

Barleys grown in 1981 were harvested at maturity from one field plot whereas barleys grown in 1983 were harvested at four stages of maturity and from two separate field plots (A and B). Harvest began July 29, 1983 with collection times at weekly intervals. A fifth harvest (approximately one week after the fourth harvest) was collected by the Plant and Soils Department from only plot A. Data from this harvest are presented in appendix tables 40 through 52.

Barleys from 1983 were cleaned and a sample of each air dried to be used for chemical analysis. The husk from these immature dry

kernels was removed by hand before analysis. The husk adhered to the kernel throughout the combining and cleaning process even though these were hulless lines. Another sample from the second and fourth stages of maturity were appropriated for use in future taste panels. Because of the immaturity of stage two, the barley was rinsed, blanched and frozen for use in taste panels. Blanching time totaled four and one-half minutes broken down into one and one-half minutes for 1.5 km of altitude, two minutes for the steam method utilized (McWilliams and Paine, 1977) and one minute as an arbitrary length of time chosen for the barley itself. The barley was stored in sealed plastic freezer bags at -17.8° C.

Chemical Analyses

Proximate analyses were determined according to the Association of Official Analytical Chemists (AOAC) methods (1980). Neutral detergent fiber (NDF) was measured according to the Robertson and Van Soest (1977) method as modified by Roth et al. (1982). Acid detergent fiber (ADF) was measured according to AOAC (1980). Estimated dietary fiber (EDF) was calculated using the method described by Aman 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).

Soluble carbohydrate levels were determined on all barleys. Free sugars were extracted using Method 80-60 as described in the American Association of Cereal Chemists Methods Handbook (AACC, 1969). Reducing and total sugars were then determined from this extract (Bruner,

1964) utilizing modifications made by Fastnaught (1982). Starch values were determined using the method described by Aman and Hesselman (1984).

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).

All barleys were analyzed for β -glucan, acid viscosity and alkaline viscosity by the Plant Science Department of Washington State University at Pullman (Lance, 1984). Greenberg and Whitmore's (1974) method was used to determine acid viscosity, and alkaline viscosity was measured using the method of Wood et al. (1977). Alkaline extract viscosity was also measured by Bendalov's falling ball technique as described by Coon et al. (1978). All analyses were duplicated with the exception of viscosity measurements and amino acids which were single determinations.

Data were analyzed by analysis of variance in a split plot design using the method of Newman-Kuel to separate significant differences between the 1983 barley isolines and stage of maturity mean differences (Snedecor and Cockran, 1980). Orthogonal comparisons were made to determine linear, quadratic and cubic effects on parameters at the four stages of maturity of the isolines (Snedecor and Cockran, 1980).

Physical Measurements

Percent plump and thin kernels and thousand kernel weight were determined by the Department of Plant and Soil Sciences Cereal Quality Laboratory at Montana State University. Percent plump was the amount

of barley that stayed on top of a 2.4 x 19 mm sieve and percent thin was the amount of barley that fell through a 2.2 x 19 mm sieve. Kernel weight determinations were made on 100 g samples of each isolate.

Animal Experiments

Chick Experiment

A chick growth trial was conducted to determine the relative feed value of Prowashonupana 2 and 3, and Washonupana. The barley diets and a maize control diet were prepared at a 23 % protein level and balanced for lysine at 1.20 % (Table 2). β -glucanase was added to all barley diets to reduce wet and sticky droppings. One day old cockerel Hubbard broiler chicks from Fors Hatchery in Puyallup, Washington, were number banded and allowed a two day adjustment period. After the adjustment period, chicks were randomly allotted to each of four treatments. Six replications within each diet treatment containing six chicks per cage were fed for seventeen days. The chicks were housed in metal woven cages with thermostatically controlled temperature (35 - 26.7° C) decreasing with age in a temperature controlled room (26.7° C) and given water ad libitum.

Data were analyzed by analysis of variance and differences between means were determined by the method of Newman-Kuels (Snedecor and Cockran, 1980).

Rat Experiments

All three rat trials utilized Holtzman Sprague-Dawley male weanling rats. Individual wire woven cages housed the rats in an environmentally controlled (23.3° C) room with twelve hours of light and

twelve hours of dark. Non-softened water was provided ad libitum. Rats were stratified to diet-treatments by initial weight except in the nitrogen-free trial where all rats were on the same diet.

Table 2. Chick diets for growth feeding trial using 1981 Compana barley isolines and maize, trial 1.

Diet	Prowashonupana		Washonupana	Maize control
	2	3		
	-----%			
Barley	63.80	68.70	59.80	---
Maize	---	---	---	47.40
Soybean meal	26.20	21.30	30.20	43.20
Oil ^a	5.50	5.50	5.50	4.90
Dicalcium phosphate	2.80	2.80	2.80	2.80
Limestone	0.55	0.55	0.55	0.55
Vitamin premix ^b	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50
Antibiotic ^c	0.10	0.10	0.10	0.10
DL-methionine	0.41	0.46	0.61	---
L-Lysine HCl	0.10	0.15	0.12	---
β -glucanase ^d	0.05	0.05	0.05	0.05

^a50% Mazola corn oil, 50% Crisco vegetable oil.

^bFurnishes the following (per kg diet): 7716 USPS vitamin A acetate, 2205 ICU vitamin D₃, 6.61 USPS vitamin E, 11 μ g vitamin B₁₂, 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 μ g 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.

^cOxytetracycline, 110.2 g/kg.

^dEnzeco (R) β -glucanase, 200 units/g, Enzyme Development Corporation, Keyport, NJ 07735.

Endogenous fecal and urinary nitrogen excretion was determined on thirty-two rats utilizing a nitrogen-free diet (Eggum, 1973). The

rats were adjusted to the diet for three days prior to a four day collection period of urine and feces. Their diet consisted of 85 % cornstarch, 5 % corn oil, 5 % alphacel, 2 % vitamin mixture, 2 % mineral mixture, 0.8 % CaCO_3 and 0.2 % antibiotic. The vitamin mixture, mineral mixture and antibiotic sources are described in Table 3.

Table 3. Rat diets for growth, nitrogen balance and digestible energy trials using 1981 Compana barley isolines and casein, trial 1.

Diet	Prowashonupana			Washonupana	Compana
	1	2	3		
	-----%				
Barley	55.25	54.94	50.00	60.61	---
Cornstarch	39.75	40.06	45.00	34.39	79.74
Casein	---	---	---	---	11.76
Corn oil	---	---	---	---	3.50
Vitamin mix ^a	2.00	2.00	2.00	2.00	2.00
Mineral mix ^b	2.00	2.00	2.00	2.00	2.00
CaCO_3	0.80	0.80	0.80	0.80	0.80
Antibiotic ^c	0.20	0.20	0.20	0.20	0.20

^aVitamin Diet Fortification Mixture, ICN-NBC, Cleveland, OH 44128.

^bBernhart Tomarvelli Salt Mixture, J. Nutr. 89:495 (1966).

^cOxytetracycline, 110.2 g/kg.

Four 1981 Compana barley isolines, Prowashonupana 1, 2 and 3, were compared with casein in a nitrogen balance trial (Eggum, 1973) on rats to determine biological value (BV), true protein digestibility (TPD) and net protein utilization (NPU). Isonitrogenous, 10 % protein diets were fed (10 g diet/rat/day) to thirty rats with six rats per diet (Table 3). A four-day diet adjustment period preceded the four-day collection of feces and urine.

The same rats utilized in the nitrogen balance trial were fed 15 g of the same diet for five days immediately following the nitrogen balance collection. Fecal matter was then collected for four days in order to determine digestible energy of the diets (Eggum, 1973).

An extended growth trial was not completed due to a shortage of barley. Calculations on rat data collected from the beginning of the nitrogen balance trial and beyond the digestible energy trial were used to determine feed/gain ratios (F/G) and protein efficiency ratios (PER). This twenty-two day period of time included the limited feeding of 10 g diet/rat/day for the first nine days, 15 g diet/rat/day for nine days and four days where rats were fed ad libitum.

Two consecutive energy balance trials were completed using diets prepared from 1981 Compana, Washonupana and Prowashonupana 1, 2 and 3 barley isolines (Table 4). A six-day adjustment period preceded the two consecutive five-day collection periods. Rats were fed 12 g diet/rat/day with eight rats assigned per diet.

Data were analyzed by analysis of variance and differences between means were determined by the method of Newman-Kuel (Snedecor and Cockran, 1980).

Taste Panels

Sensory evaluation was used to determine if the high sugar content found in the two Prowashonupana mutants could be detected. Washonupana was used as a control as it was the most similar genetically and its sugar level equaled that in most normal barleys. The barley isolines Prowashonupana 1 and 3 and Washonupana at the

second and fourth stages of maturity were evaluated by a trained twelve-member panel consisting of nine females and three males. Panel member ages ranged from twenty-one to thirty-seven with a mean age of twenty-seven years.

Table 4. Rat diets for digestible energy trial using 1981 Compana and Compana barley isolines, trial 2.

Diet	Prowashonupana			Washonupana	Compana
	1	2	3		
	-----%				
Barley	95.0	95.0	95.0	95.0	95.0
Vitamin mix ^a	2.0	2.0	2.0	2.0	2.0
Mineral mix ^b	2.0	2.0	2.0	2.0	2.0
CaCO ₃	0.8	0.8	0.8	0.8	0.8
Antibiotic ^c	0.2	0.2	0.2	0.2	0.2

^aVitamin Diet Fortification Mixture, ICN-NBC, Cleveland, OH 44128.

^bBernhart Tomarvelli Salt Mixture, J. Nutr. 89:495 (1966).

^cOxytetracycline, 110.2 g/kg.

Training of taste panel members was conducted according to American Society for Testing and Materials (ASTM, 1981) recommendations. Prospective panel members were screened for their ability to detect sweetness and differentiate between sweetness intensity utilizing the triangle and sweetness ranking tests (ASTM, 1968). Sucrose solutions of 2 % were identified with 100 % accuracy by the candidate before inclusion in the panel. The successful candidates then used the triangle and sweetness ranking tests in training with each stage of the immature barley before final data were collected. Training sessions for each of the two panels took place the day prior to the

actual data collection. Evaluation of each stage was done on different days and separated by two weeks.

Equal amounts of barley from each of the two plots were mixed together to give a representative sample. The barleys from each stage of maturity were presented to the taste panel members in different forms. Green kernels from the second stage of maturity were served with the husk on because it adhered throughout the cleaning and blanching process. The blanched, frozen barley kernels were thawed and served at room temperature in white paper cups. The dry barley kernels from the fourth harvest were ground through a UDY cyclone mill (1 mm screen) and made into a 7 % dispersion using distilled water. Samples of the solutions were placed in white paper cups and warmed to 60° C in ovens before serving.

In both panels, the paper cups with samples were placed on coded paper plates. Panelists were presented three triangle tests followed by a sweetness ranking test and asked to record their responses (Appendix Tables 33 and 34). Room temperature distilled water was provided at each session.

Significance of triangular tests was determined using the appendix table E from Amerine et al. (1965). The method of log-linear contingency table analysis (Fienberg, 1980) was used to choose the best fitting model for both barley varietal preferences and ranking of barley sweetness. Three criteria were used in selecting a model for each table. First, the p-value for the fit of the model to the data had to be 0.10 or larger. Second, the model contained no effects which significantly ($P < 0.05$) decreased the fit to the data if left out

of the model. Third, there were no effects not in the model that, if added to the model, would increase the fit significantly.

RESULTS

1981 Barleys

Barley Isoline Composition

Proximate analyses and carbohydrates

Protein, lipid and all fiber levels were higher in the 1981 Prowashonupana mutants than in Washonupana (Table 5). Protein levels of Prowashonupana 1, 2 and 3 were 109, 110 and 120 % of that of Washonupana. Ether extract levels of the Prowashonupana mutants were 163, 179 and 221 % of that of Washonupana. Considering fiber, Prowashonupana 1, 2 and 3 were 110, 110 and 170 % for crude fiber; 125, 120 and 152 % for NDF; 121, 150 and 179 % for ADF; and 122, 116 and 196 % for EDF, respectively, of that of Washonupana.

Table 5. Protein, ether extract and fiber analyses of 1981 Compana barley isolines at maturity, dry matter basis.

Barley isolate	PROT ^a	EE	CF	NDF	ADF	EDF
Prowashonupana 1	19.0	3.9	1.1	19.2	1.7	25.7
Prowashonupana 2	19.1	4.3	1.1	18.3	2.1	24.3
Prowashonupana 3	20.9	5.3	1.7	23.3	2.5	41.1
Washonupana	17.4	2.4	1.0	15.3	1.4	21.0

^aPROT = protein, EE = ether extract, CF = crude fiber, NDF = neutral detergent fiber, ADF = acid detergent fiber, EDF = estimated dietary fiber.

Percentage of ash, phosphorus, total sugars and reducing sugars of the Prowashonupana mutants were higher than that of Washonupana, whereas the opposite was true for starch plus free glucose values (Table 6). Ash was 121, 132 and 126 % of that of Washonupana and phosphorus was 126, 130 and 121 % of that of Washonupana for the three Prowashonupanas, respectively.

Table 6. Ash, calcium, phosphorus and soluble carbohydrate analyses of 1981 Compana barley isolines at maturity, dry matter basis.

Barley isolate	ASH ^a	Ca	P	T SUG	R SUG	STAR
Prowashonupana 1	2.3	.00	.54	4.5	2.4	44.6
Prowashonupana 2	2.5	.02	.56	7.2	2.2	42.6
Prowashonupana 3	2.4	.02	.52	9.5	1.6	20.8
Washonupana	1.9	.00	.43	1.9	.45	55.4

^aT SUG = total sugar, R SUG = reducing sugar, STAR = starch plus free glucose.

Major differences were seen in total sugar with Prowashonupana 1, 2 and 3 containing values of 237, 379 and 500 % of that of Washonupana, respectively. The Prowashonupana mutants displayed 533, 489 and 356 % the reducing sugar levels, respectively, of that of Washonupana whereas in starch, 19, 23 and 62 % lower values occurred.

Amino acids

Except for phenylalanine, glutamic acid and proline, 1981 Prowashonupana mutants had higher values in all amino acids measured than

Washonupana whether expressed as a percentage of the grain or a percentage of the protein (Tables 7 and 8). Lysine, expressed as percent of the grain was 131, 138 and 150 % of that of Washonupana for Prowashonupana 1, 2 and 3, respectively. When expressed as percent of the protein, Prowashonupana 1, 2 and 3 contained 119, 125 and 130 %, respectively, the lysine of Washonupana.

Table 7. Amino acid content of 1981 Compana barley isolines, dry matter basis.

Amino acid ^a	Prowashonupana			Washonupana
	1	2	3	
	-----%			
Alanine	0.84	0.85	0.86	0.58
Arginine	0.98	1.00	1.16	0.81
Aspartic acid	1.04	1.03	1.19	0.86
Glutamic acid	4.67	4.62	4.84	4.77
Glycine	0.61	0.60	0.65	0.50
Histidine	0.40	0.39	0.43	0.36
Isoleucine	0.59	0.59	0.64	0.53
Leucine	1.18	1.18	1.24	1.06
Lysine	0.63	0.66	0.72	0.48
Methionine	0.29	0.28	0.30	0.24
Phenylalanine	0.88	0.84	0.91	0.84
Proline	2.14	2.10	2.12	2.20
Serine	0.89	0.88	0.92	0.79
Threonine	0.63	0.63	0.68	0.55
Tyrosine	0.57	0.55	0.58	0.50
Valine	0.86	0.84	0.78	0.66

^aCystine/2 and tryptophan were not determined.

Table 8. Amino acid content of 1981 Compana barley isolines, % of protein.

Amino acid ^a	Prowashonupana			Washonupana
	1	2	3	
	-----%			
Alanine	4.91	5.00	4.74	3.68
Arginine	5.68	5.87	6.43	5.14
Aspartic acid	6.06	6.06	6.61	5.45
Glutamic acid	27.17	27.10	26.83	30.36
Glycine	3.53	3.52	3.60	3.17
Histidine	2.33	2.29	2.39	2.27
Isoleucine	3.41	3.44	3.56	3.38
Leucine	6.85	6.90	6.90	6.77
Lysine	3.67	3.84	4.01	3.08
Methionine	1.70	1.76	1.65	1.51
Phenylalanine	5.12	4.90	5.05	5.35
Proline	12.42	12.32	11.76	14.01
Serine	5.18	5.13	5.10	5.00
Threonine	3.68	3.70	3.78	3.48
Tyrosine	3.30	3.24	3.23	3.17
Valine	4.99	4.93	4.33	4.20

^aCystine/2 and tryptophan were not determined.

β -glucan and viscosity

All Prowashonupana mutants, when compared to Washonupana, had higher levels of β -glucan and lower acid and alkaline viscosity measurements (Table 9). The percentage of β -glucan of Prowashonupana 1, 2 and 3 were 125, 124 and 250 %, respectively, of that of Washonupana.

For both alkaline and acid viscosity all Prowashonupana mutants were distinctly lower than Washonupana. Acid viscosity measurements show Prowashonupana 2 and 3 with similar values of 27 and 28 %, respectively, of that of Washonupana.

respectively, of that of Washonupana whereas Prowashonupana 1 was 32 % of that of Washonupana.

Table 9. β -glucan and viscosity measurements of 1981 Compana barley isolines at maturity.

Barley isolate	β -glucan	Viscosity ^a		
		Alkaline WSU	Alkaline MSU	Acid
	-----%-----	-----cP-----		
Prowashonupana 1	9.0	3.28	3.89	4.99
Prowashonupana 2	8.9	3.43	3.90	4.25
Prowashonupana 3	18.0	3.28	4.21	4.36
Washonupana	7.2	8.87	7.92	15.70

^aWSU = Washington State University, MSU = Montana State University.

Physical Measurements

Kernel measurements of the four 1981 isolines at maturity are presented in Table 10. Similar values are seen between Prowashonupana 1, 2 and Washonupana isolines, where Prowashonupana 1 and 2 exhibited 102 and 95 % the plump kernels found in Washonupana. Only 0.6 % of Prowashonupana 3 kernels were plump whereas 99.0 % were classed thin. Prowashonupana 1 and 2 had 83 and 104 % the thin kernels found in Washonupana.

Prowashonupana 1, 2 and Washonupana were all similar in kernel weight. Prowashonupana was lower with 81 % the kernel weight of that of Washonupana.

Table 10. Kernel measurements of 1981 Compana barley isolines at maturity.

Barley isolate	Plump kernels	Thin kernels	Kernel weight
	-----%-----		-----mg-----
Prowashonupana 1	69.6	13.8	35.1
Prowashonupana 2	64.9	16.6	33.5
Prowashonupana 3	0.6	99.0	29.6
Washonupana	68.5	16.0	36.5

Animal Experiments

Chick experiment

Proximate components of the chick diets fed in the growth trial 1 are given in Table 11.

Table 11. Proximate components of chick diets prepared with the 1981 Compana barley isolines and maize, trial 1, dry matter basis.

Diet	Protein	Ether extract	Crude fiber	Ash	Nitrogen-free extract
	-----%-----				
Prowashonupana 2	24.0	8.5	2.3	6.8	58.4
Prowashonupana 3	23.0	10.4	2.4	7.6	56.6
Washonupana	23.1	7.3	2.3	6.6	60.7
Maize	23.1	7.8	3.3	6.9	58.9

Results of the chick growth study comparing three 1981 Compana barley isolines and maize are shown in Table 12. Chicks fed

Prowashonupana 3 had the lowest final body weight and lowest weight gain, differing ($P < 0.01$) from the chicks fed all other diets.

Although final body weight and weight gain were higher on the Washonupana diet than on the Prowashonupana 2 diet, the difference was not significant. Like Prowashonupana 3, the chicks fed Prowashonupana 2 and Washonupana diets differed ($P < 0.01$) from the maize control diet in both final body weight and weight gain.

The F/G ratio for Prowashonupana 3 was higher ($P < 0.01$) than for all other isolines. The Prowashonupana 2, Washonupana and maize diets were similar in the amount of feed needed to produce gain.

Despite the β -glucanase that was added to all diets, chicks on the Prowashonupana 3 diet still had heavier ($P < 0.01$) excreta than chicks on the other diets. The subjective dropping scores found Prowashonupana 3 and Washonupana excreta to be wetter ($P < 0.01$) than the excreta from both Prowashonupana 2 and maize diets at day thirteen. Dropping collections four days later were similar to the first collection except that Washonupana and Prowashonupana 2 scores did not differ significantly from any of the other dropping scores.

Rat experiments

The content of rat diets fed in the growth, nitrogen balance and energy digestion trials are presented in Table 13.

Growth trial

Results of the rat growth trial comparing four 1981 Compana barley isolate diets and a casein diet are presented in Table 14. No

