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Biological evaluation of protein quality of barley

Snehil Kalra & Sudesh Jood*

Department of Foods and Nutrition, CCS Haryana Agricultural University, Hisar 125 004, India

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Protein quality of barley cultivars, namely BH-331 (hulled), DL-88 (hullless) and Dolma (hullless) having 2.18, 4.60 and 6.23% β-glucan content, respectively, was biologically evaluated by rat growth and nitrogen balance studies. Feeding of BH-331 barley diet resulted in marked decrease of food intake, body weight gain, food efficiency ratio, protein efficiency ratio, nitrogen absorption, biological value, net protein utilization and utilizable protein. The diet containing Dolma cultivar (hullless and high β-glucan) caused by intermediate effect on all the protein quality parameters. DL-88 (hullless) gave better results than BH-331 and Dolma. Therefore, β-glucan content may not be affecting protein quality parameters in barley. However, hulled barley (BH-331) showed adverse effects on biological parameters in which husk seems to contribute towards unpalatability of the diet, thereby leading to a reduced food intake and other related parameters.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop after wheat, rice and maize. Apart from having a nutritive value comparable to wheat, it is unique among cereals containing high concentrations of mixed linked (1→3), (1→4) β-D glucans (β-glucan) which are known to have cholesterol lowering effects (Newman *et al.*, 1991; Ranhotra *et al.*, 1991; McIntosh *et al.*, 1991). Antinutritional properties of β-glucan, particularly in poultry, were also reported to produce sticky droppings due to its higher viscosity (Herselman, 1983; Herselman and Aman, 1986). Hulled barley contains 3–7% β-glucan (Aman and Newman, 1986; Yoon *et al.*, 1995) while hullless may have as high as 16% β-glucan (Hofer, 1986; Wood *et al.*, 1991). Feed value of hulled barley is lower due to its high content of fibre but hullless barley has a feed value equal to wheat and corn (Salunkhe *et al.*, 1985). At present, very little barley is used as human foods because of increased consumption of wheat and rice in developed and developing countries. It is desirable to develop low β-glucan barley cultivars for feeding to poultry and high β-glucan barley for use in human foods.

Chemical analysis of barley is very important for assessing its suitability as a human food but the real nutritional value of protein is not reflected in chemical analysis. In such a situation, biological evaluation of protein quality is more desirable. This paper reports the effect of high, medium and low β-glucan contents of screened hullless and hulled cultivars on protein quality through rat growth and nitrogen balance studies.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Thirteen hulled cultivars, namely Alfa-93, BCU-73, BH-75, BH-85, BH-331, BH-332, Clipper, DL-88, K-508 Malti, PL-419, RD-2035, RD-2503 and three hullless cultivars, namely HL-335, Karan-16 and Dolma of barley were procured from the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar and Himachal Pradesh Agricultural University, Palampur. The grains were cleaned and powdered in a cyclotec mill to pass through a 60 mesh sieve and then stored in air-tight polyethylene bottles for further chemical analysis. The barley cultivars were then screened to identify low, medium and high β-glucan contents. To estimate β-glucan, megazyme mixed linkage β-glucan and glucose test kits were used as per the method of McCleary and Glennie (1985). Three cultivars with differing β-glucan contents were selected to study the protein quality through rat growth and nitrogen balance studies.

Test animals

Young white male albino Wistar rats weighing 25 ± 5.0 g were obtained from the Disease and Germ Free Small Animal House, CCS Haryana Agricultural University. Hisar, India. The rats were randomly divided into four groups, each consisting of eight rats. The rats were housed individually in polypropylene cages kept in an air-conditioned room maintained at 22 ± 1°C with a 12 h light and dark cycle. Food and water were given *ad libitum*.

Composition of diets

Diets contained flours of three barley cultivars, viz. Dolma, DL-88 and BH-331, having 6.23, 4.60 and 2.18% β-glucan contents, respectively. One group of the rats was fed on a purified diet (casein). This group served as a standard check. All the four diets, having 8% protein level, are listed in Table 1. For the preparation of the diets, ingredients were homogenized and passed through a 60 mesh sieve to ensure uniform distribution of vitamins and minerals. The diets were prepared for one week at a time and kept in the refrigerator. All the diets were analysed for their moisture and protein contents (AOAC, 1990).

Growth experiment

Protein efficiency ratio (PER) was determined by the method of Chapman *et al.* (1959). The rats were initially weighed and then weighed on every alternate day. The weighed diet was given daily and the unconsumed diet was collected, dried and weighed. Food and water were given *ad libitum*. The rats fed on different experimental and control diets were finally weighed after four weeks and the gain in weight of rats during this period was recorded. The amounts of food and protein intake during this period were calculated on a dry matter basis. Food efficiency ratio (FER) and protein efficiency ratio (PER) were calculated by the following formulae:

$$\mathrm{PER} = \frac{\text{gain in body weight (g)}}{\text{protein consumed (g)}}$$

$$\mathrm{FER} = \frac{\text{gain in body weight (g)}}{\text{food consumed (g)}}$$

Table 1. Composition of the experimental diets (g kg^{-1} diet)

Ingredients	Control (casein)	Group I (Dolma)	Group II (DL-88)	Group III (BH-331)
Casein	100	—	—	—
Barley flour	—	715.0	747.66	761.9
Sucrose	50	50	50	50
Groundnut oil	100	75.79	76.96	77.67
Mineral mixture	40	40	40	40
Vitamin mixture	10	10	10	10
Cellulose	50	—	—	—
DL-methionine	3.0	—	—	—
Choline bitartarate	2.0	2.0	2.0	2.0
Corn starch	645.0	87.21	53.38	38.43

Nitrogen balance studies

For nitrogen balance studies, adult male Wistar rats were housed individually in polypropylene metabolic cages. One group of rats was fed on a protein-free diet and another on a casein diet. Three groups of rats were fed on test diets (containing flours of grains of barley cultivars having high, medium and low β-glucan contents). The experiment was conducted for nine days, which included an initial conditioning period of four days. During the last five days, urine and faeces of each rat were collected separately. Food and water were given *ad libitum* and the change in body weight was recorded. The concentration of nitrogen in urine and faeces was estimated by the Microkjeldhal method (AOAC, 1990). The data obtained from this experiment were used to calculate true digestibility (TD), biological value (BV) (Chick *et al.*, 1935), net protein utilization (NPU) (Platt *et al.*, 1961), net protein retention (NPR) and protein retention efficiency (PRE) (Bender and Doell, 1957) and utilizable proteins (UP) (Gupta *et al.*, 1979) by employing the following formulae:

$$\mathrm{TD} = \frac{\mathrm{Ni} - \mathrm{NF}_1 - \mathrm{NF}_2}{\mathrm{Ni}} \times 100$$

$$\mathrm{BV} = \frac{\mathrm{Ni} - (\mathrm{NF}_1 - \mathrm{NF}_2) - (\mathrm{NU}_1 - \mathrm{NU}_2)}{\mathrm{Ni} - (\mathrm{NF}_1 - \mathrm{NF}_2)} \times 100$$

Ni = Nitrogen intake of animals fed test diet

NF_1 = Nitrogen excreted in faeces of animals fed test diet

NF_2 = Nitrogen excreted in faeces of animals fed protein free diet

NU_1 = Nitrogen excreted in urine of animals fed test diet

NU_2 = Nitrogen excreted in urine of animals fed protein-free diet

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

$$\mathrm{NPR} = \frac{\text{Weight gain of test group} + \text{weight loss of protein-free group}}{\text{Weight of test protein consumed}}$$

$$\mathrm{PRE} = \mathrm{NPR} \times 16$$

$$\mathrm{UP} = \frac{\mathrm{NPU} \times \mathrm{N}\ (\text{\% of dry matter}) \times 6.25}{100}$$

Statistical analysis

The data were subjected to analysis of variance (ANOVA) in a completely randomized design to determine the significant differences among various cultivars (Snedecor and Cochran, 1968).

Table 2. Food intake, protein intake and body weight gain of rats, and FER and PER[a] of low, medium and high β-glucan barley genotypes

Dietary group	β-glucan (% in flour)	Food intake (g)	Protein intake (g)	Body weight gain (g)	FER	PER	Corrected[b] PER
Casein	0.00	193.50 ± 4.75	17.00 ± 1.22	48.85 ± 2.13	0.20 ± 0.01	2.87 ± 0.04	2.50
BH-331	2.18	169.00 ± 3.25	13.52 ± 1.26	18.98 ± 0.72	0.11 ± 0.01	1.40 ± 0.03	1.22
DL-88	4.60	179.25 ± 3.13	14.34 ± 1.00	22.80 ± 1.58	0.13 ± 0.00	1.59 ± 0.02	1.39
Dolma	6.23	171.25 ± 3.16	13.70 ± 1.25	19.81 ± 1.04	0.12 ± 0.01	1.45 ± 0.01	1.26
	SE(m)	2.79	1.20	1.13	0.003	0.002	
	CD ($p < 0.05$)	7.89	3.60	3.29	0.007	0.006	

[a]Values are means ± SD of eight rats in each group.
[b]Based on values of 2.5 as standard for casein.

RESULTS AND DISCUSSION

FER and PER

Food intake on the casein diet (control) was the highest (193.50 g per rat) and it differed significantly ($p < 0.05$) from that of other diets where food intake ranged from 169.0 to 179.25 g per rat (Table 2). Among the barley diets, the food intake was highest in the group fed on DL-88 diet and lowest in the group fed on BH-33 1 diet. Reduced intake of BH-331 diet in rats could be due to poor palatability owing to the presence of hull. Bhatty *et al.* (1975) also reported that a proportion of the hull content of barley affects the food intake of rats. Dolma diet produced an intermediate effect on food intake. Moderate levels of food intake are difficult to relate to a high β-glucan content. β-glucan has antinutritional properties, particularly in chicken diets, where it results in sticky droppings and affects food intake and growth rate (Newman and McGuire, 1985). On the other hand, Malkki *et al.* (1992) reported no significant difference in food intake and weight gain in rats fed with a β-glucan-rich diet. Protein intake is determined from the food intake; it followed the same pattern as that of food intake. Protein intake of the control group was 17.00 g per rat which was significantly higher than the protein intake of the test groups which ranged from 13.52 to 14.34 g day^{-1}.

Gain in body weight of control group rats was highest (48.85 g) after 28 days. The gain in body weight of three test group rats ranged from 18.98 to 22.80 g per rat. This improportional gain was probably due to a combination of two factors, viz, amount of hull and β-glucan in the diet, which affect the absorption of protein by the body. Protein intake of the control (casein) group rats was maximum. No significant difference was observed in body weight gain of rats fed on oats (which are a good source of β-glucan) (Malkki *et al.*, 1992).

Food efficiency ratio (FER) was maximum on the casein diet (0.20), which differed significantly from the barley diets. The barley diets had FER ranging from 0.11 to 0.13 and did not differ significantly among themselves. The casein diet had a PER of 2.87 which was significantly higher than the other diets. PER of barley diets ranged from 1.40 to 1.59, respectively. The corrected PER values of three barley diets viz 1.22 (BH-331), 1.26 (Dolma) and 1.39 (DL-88) were lower than the standard casein diet (2.50). The lower values of FER and PER of barley diets in comparison to the casein diet may be attributed to reduction in food intake. Husk (BH-331) and high β-glucan (Dolma) contents might be responsible for lower food intake.

Nitrogen consumption, absorption, digestibility, BV and NPU

Significant reduction was noticed in nitrogen consumption in the test groups (72.50–77.80 mg day^{-1}) compared to the control group (102.67 mg day^{-1}) (Table 3). Nitrogen absorption also followed some pattern. In the control group, nitrogen absorption was 93.83 mg day^{-1} while in test groups, it ranged from 59.00 to

Table 3. TD, BV, NPU and UP values[a] of low, medium and high β-glucan barley genotypes fed to rats for five days

Dietary group	β-glucan (% in flour)	Nitrogen consumed (mg/rat day^{-1})	Nitrogen absorbed (mg/rat day^{-1})	TD (%)	Nitrogen retained (mg/rat day^{-1})	BV (%)	NPU (%)	UP (%)
Casein	0.00	102.67 ± 2.13	93.83 ± 1.07	91.35 ± 3.56	88.16 ± 3.00	93.96 ± 3.52	85.83 ± 3.17	67.01 ± 1.56
BH-331	2.18	72.50 ± 1.98	59.00 ± 2.00	69.20 ± 2.58	45.25 ± 2.00	76.27 ± 2.50	52.78 ± 2.53	6.42 ± 0.38[a]
DL-88	4.60	77.80 ± 3.02	63.50 ± 2.75	79.35 ± 2.26	51.52 ± 2.15	81.13 ± 3.00	64.38 ± 1.98	8.04 ± 0.98
Dolma	6.23	77.20 ± 2.98	63.33 ± 2.62	78.16 ± 3.16	55.35 ± 1.96	80.77 ± 2.91	63.13 ± 1.43	7.48 ± 0.97
	SE (m)	0.99	0.86	1.30	0.93	1.45	1.19	0.12
	CD ($P < 0.05$)	2.40	2.09	3.17	2.27	3.55	2.90	0.36

[a]Values are means ± SD of six rats in each group.

Table 4. NPR and PRE values[a] of low, medium and high β-glucan barley genotypes fed to rats for five days

Dietary group	β-glucan (% in flour)	Weight gain (g)	Weight loss (g)	Protein consumed (g)	NPR	PRE
Casein	0.00	10.80 ± 0.16	2.85 ± 0.14	2.95 ± 0.04	4.65 ± 0.16	74.40 ± 2.50
BH-331	2.18	2.82 ± 0.12	2.85 ± 0.14	2.39 ± 0.04	2.37 ± 0.13	37.92 ± 2.15
DL-88	4.60	3.95 ± 0.15	2.85 ± 0.14	2.52 ± 0.03	2.70 ± 0.23	43.20 ± 2.45
Dolma	6.23	3.60 ± 0.18	2.85 ± 0.14	2.45 ± 0.05	2.63 ± 0.20	42.08 ± 2.71
	SE (m)	0.17	—	0.07	0.08	1.31
	CD ($P < 0.05$)	0.51	—	0.20	0.22	3.93

[a]Values are means ± SD of six rats in each group.

63.50 mg day^{-1}. Amongst the test groups, DL-88 had maximum and BH-331 had minimum nitrogen absorption whereas the Dolma diet had an intermediate effect on nitrogen absorption. Water-soluble β-glucan content, due to its high viscosity has an influence on the absorption of nutrients from the small intestine (Mclntosh *et al.*, 1991).

True digestibility of the control group was also significantly ($p < 0.05$) higher than that of the test groups. Among test groups, TD was found highest in the DL-88 (hull-less) diet and lowest in the BH-331 (hulled) diet fed group. The hull of barley cultivars was reported to influence the digestibility in mouse feeding studies (Bhatty *et al.*, 1975) where the mean digestibility value of six hullless cultivars was higher (85.7%) than the mean value (79.2%) of ten hulled cultivars. Carmen *et al.* (1985) also observed poor digestibility of protein-nitrogen in rats fed on wheat and barley husk. Similarly, nitrogen retention was also significantly ($p < 0.05$) poor in rats fed on BH-331 and Dolma diets compared to casein-fed rats. Biological value was also observed to be higher in the control group (93.96%) than in the test groups (76.27–81.13%). Among the test groups, BV was found highest in rats fed on DL-88, followed by the Dolma and BH-331 diets. NPU and utilizable protein also followed a similar trend. The DL-88 diet-fed group had the highest utilizable protein among the test groups. This might be due to the low content of husk and β-glucan in the diet.

NPR and PRE

Data summarized in Table 4 indicate the distinct superiority of the casein diet over the three barley diets, with respect to NPR and PRE. The NPR and PRE values of casein diets were 4.65 and 74.40, and for barley diets these ranged from 2.37 to 2.70 and 37.92 to 43.20, respectively. The highest values of NPR (2.70) and PRE (43.20) among barley diets were reported for DL-88. This could be the result of lower contents of β-glucan and husk.

It may be inferred from the present studies that hulled barley manifested adverse effects on the biological parameters wherein husk seemed to contribute towards unpalatability of diet thereby leading to reduction in food intake and also influenced the utilization of protein and other related parameters. β-glucan had little effect on protein quality parameters in rats. Thus it is desirable to develop hullless cultivars for feeding purposes.

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