Z. Ernährungswiss. **18**, 275–285 (1979)

ISSN 0044-264 X

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Evaluation of the protein quality of wheat grains (Grizza 155) and eight related products by the dose-response bioassay

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With 4 figures and 3 tables

(Received June 22, 1979)

Cereals, especially wheat and rice, constitute the bulk of the human diet in many areas of the world. The nutritive value of their proteins and their specific amino-acid deficiencies long have been of interest (4, 19). Cereals intended for human consumption are cooked or processed into products of higher acceptability and greater market stability. It is well established that the processing of wheat may have a profound effect on its protein quality (14, 16). The report by *Beaudoin* et al. (3) clearly indicated that the cooking of whole wheat to stimulate shredded wheat produced an improvement in nutritive value. *Kon* et al. (15) found that the crust of the bread which receives more severe heat treatment during baking than crumb has a lower nutritive value than the crumb. Lysine destruction during baking is a factor contributing to the poorer nutritive value of the crust (21). Many of the breakfast food cereals have been subjected to rather severe heat treatment during the course of processing, thus reducing the protein quality by destroying or by making some of the amino acids unavailable.

The methodology for the biological assessment of dietary-protein quality has been reviewed by several authors (1, 5, 8). It is clear nowadays that assays involving only a single test-protein level are usually inadequate measures of protein quality. Assays involving more than one test protein was suggested by *Hegsted* (11, 12) and are found necessary for the critical determination of dietary protein quality (23, 20).

The present study concerns with the amino acid composition of wheat and eight common wheat derivatives. Their protein qualities were also assessed by measuring the growth response and change in carcass water of weanling rats fed diets containing different levels of the experimental protein.

Materials and methods

The wheat sample used in the course of this work is a soft winter variety (Triticum vulgaris, Gizza 155) obtained from Cereal Department, Ministry of Agriculture, Egypt. It was milled at 16% moisture content in a semi plant Buhler Cylinder Mill, whereby white (first-clear) flour was obtained at long extraction of 87.5%. Four different bread types were analyzed: baladi bread, prepared from either white or low-grade flour (a flat circular loaf composed of two layers with no crumb); french bread, prepared from white flour. Dietetic bread and biscuits were discussed in separate reports (under publication). Belila was prepared in the laboratory according to the traditional batch procedure using open-pot method. The wheat grains were cooked in water (1:5) until they cracked, and an expanded structure was created.

Preparation of sample for analysis:

Samples were prepared for analysis by drying in a slow stream of unheated air. They were then ground in an electric mill and kept in an air-tight containers until used. Milled samples were analyzed for crude protein by the semi-micro *Kjeldahl* method (2). The samples were analyzed for amino acids by the method of *Heese* et al. (7). The results were expressed as mg/g nitrogen. Chemical scores were calculated on the basis of the lysine content of 344 mg/g nitrogen in the 1973 reference amino-acid pattern (25).

Rat feeding experiment:

Male Sprague-Dawley rats aged 21 ± 2 days at arrival were housed environmental control. The experiments correspond nearly with the experimental procedure described by the German group on Protein Evaluation (18). For the first three days the rats were fed equal parts of all experimental diets. After three days the food was removed for 6 hours, and the rats were weighed and distributed over 10 blocks of 6 rats of equal weights. Within each block, the rats were randomised for diet and cage. A control group was killed for carcass analysis. In each experiment one group received a protein-free diet which consisted of the following (in %): cotton seed oil, 5; salt mix (18), 6; vitamin mix (18), 2; cellulose, 4; cornstarch, 83. Three groups of six animals each received diets which contained three levels of casein + dl-methionine. At the two lower dietary protein levels (3.5, 7.0%), the casein diets were supplemented with 0.3% dl-methionine; whereby at 10% dietary protein level, the casein diets were supplemented with 0.5% dl-methionine.

Three groups of six animals each received diets containing 3.5, 7.0 and 10.0% protein derived from the wheat product under test. The diet was prepared by substituting wheat or its processed product for the starch in the protein-free diet; cellulose was also omitted in this case. The dietary protein levels were selected to cover the widest range possible, but with the highest level below that which allowed maximum growth.

Food consumption was measured, and nitrogen intake was calculated for each animal from the nitrogen content of the diet. The animals were weighed twice weekly although only the final weights have been utilized in the analysis. They were killed on the 21st day, weighed and dried at 95 °C until constant weight was obtained. The carcasses were then milled and the percentage dry matter was obtained by drying at 105 °C.

Body water gain with a protein source was calculated. The body water values of a group of animals killed at the start of the experiment were used to calculate the original body water of each animal, and then calculate the gain in body water of each animal during the experiment, thus improving the precision of the assay using body water (12).

Nitrogen intakes versus weight gain or change in body water within a protein source were used for the subsequent computation of regression equations.

Results and discussion

Protein content and amino acid composition of wheat grains and processed wheat products are presented in table 1. The results of the amino acid analysis indicate that lysine should be the first limiting amino acid in the wheat and all its products, according to the reference amino acid pattern of 1973 (25). The corresponding chemical score ranged from 21 to 49% in the biscuits and baladi bread from second-class flour respectively. The latter value is slightly higher than its parent wheat (44%), and is attributed to the bran dust, which is usually high in lysine (4 g/16 g

Table 1. Amino-acid composition of whole wheat and related processed products (mg/g nitrogen) (Determined by the colorimetric method of *Spies* [1967]).

Amino acid	Whole Wheat	Belila	2nd Clear flour	2nd Clear baladi bread	White flour	White baladi bread	French bread
Essentials							
Lysine	168.5	173.4	182.7	186.6	152.6	162.5	153.2
Methionine	92.8	96.5	94.1	86.8	90.8	93.1	91.9
Cystine	150.1	148.0	159.3	149.8	157.1	152.0	165.9
Phenyl alanine	263.4	271.4	280.8	267.1	268.8	284.1	283.8
Leucine	412.5	414.6	431.1	412.8	408.0	434.8	437.9
Isoleucine	200.7	204.1	207.6	204.8	201.0	210.1	214.3
Valine	262.0	265.0	268.0	265.5	242.0	260.4	266.7
Threonine	171.6	171.6	171.2	168.5	153.0	163.8	165.5
Tryptophan	70.2	66.0	68.7	66.6	55.2	57.6	62.3
Total	1791.8	1809.6	1863.5	1808.5	1728.5	1818.4	1841.5
Non-essentials							
Aspartic acid	337.0	236.0	314.4	323.0	257.8	298.3	267.1
Glutamic acid	1763.0	1814.3	1853.4	1833.3	1984.5	1969.3	1957.8
Alanine	217.6	220.6	211.7	212.6	172.5	191.8	187.8
Proline	286.6	278.2	298.9	288.2	281.6	293.1	290.5
Serine	606.8	621.3	631.1	628.1	674.6	705.4	665.7
Glycine	249.9	248.8	236.5	234.4	215.0	217.8	215.9
Arginine	281.5	275.0	278.1	278.9	207.0	241.6	232.0
Histidine				145.6			
NH_3	242.1	254.1	248.2	259.5	292.1	261.5	251.3
Total	3984.5	3948.3	4072.3	4203.6	4085.1	4178.8	4063.1

Table 2. Mean change in body weight, body water, and PER of weanling rats fed wheat and its processed products at different protein-dietary levels.

Protein in diet %	Intake				Change in body				PER	
	Food		Nitrogen		Weight		Water			
	$X_{(g)}$ (G)	S_x	$X_{(g)}$ (MG)	S_x	$X_{(g)}$ (G)	S_x	$X_{(g)}$ (G)	S_x	X	S_x
Experiment 1	Baladi bread – 87.5% extraction									
3.5	109	8.4	690	500	0.6	2.4	−1.2	1.6	0.07	0.164
7.0	142	8.2	1750	420	3.3	3.3	4.0	1.3	1.40	0.10
10.0	157	5.7	2530	420	28.1	3.1	12.8	1.9	1.80	0.46
	Wheat flour – 87.5% extraction									
3.5	120	7.7	680	50	2.3	1.0	−0.3	0.6	0.62	0.25
7.0	125	4.8	1340	60	12.2	2.0	6.5	1.6	1.58	0.23
10.0	119	5.2	2170	140	20.2	3.1	15.8	3.6	1.61	0.18
	Reference casein + methionine									
3.5	109	7.6	570	30	1.2	0.8	0.7	1.1	0.35	0.23
7.0	150	4.1	1530	70	26.7	3.4	15.5	2.9	2.74	0.47
10.0	168	1.2	2430	140	45.2	1.6	28.9	1.8	3.26	0.38
Experiment 2	Whole wheat flour									
3.5	165	4.7	1050	0	7.6	1.5	4.7	0.7	1.21	0.2
7.0	175	11.7	2290	60	15.2	1.8	10.8	0.6	1.15	0.09
10.0	186	9.6	3230	210	29.7	2.5	23.7	2.0	1.61	0.41
	Baladi bread – 72% extraction									
3.5	164	6.2	1120	50	4.2	0.3	3.7	0.6	0.68	0.01
7.0	138	3.1	1840	50	12.3	1.3	9.5	0.6	1.16	0.10
10.0	177	12.2	3030	180	21.5	1.8	15.2	0.6	1.24	0.05

				Reference casein + methionine						
3.5	152	4.9	900	30	4.8	1.3	4.0	0.9	0.84	0.18
7.0	221	5.4	2870	120	44.0	3.1	22.0	1.8	2.39	0.12
10.0	244	11.9	4140	230	79.2	5.4	51.7	2.4	3.00	0.14
Experiment 3				Whole wheat belila						
3.5	94	7.2	630	50	3.8	0.9	3.9	0.2	1.05	0.21
7.0	98	6.3	1150	70	10.2	1.1	8.7	1.9	1.55	0.08
10.0	104	7.0	1690	110	17.3	1.4	17.1	0.8	1.81	0.14
				Reference casein + methionine						
3.5	80	2.2	400	10	2.6	1.1	5.5	0.2	1.02	0.43
7.0	117	14.7	1260	160	20.2	3.9	16.0	1.1	1.00	0.21
10.0	153	6.7	2270	120	45.0	3.1	32.1	2.0	3.10	0.08
Experiment 4				French bread						
3.5	94	7.6	570	50	0.0	0.0	–0.9	0.5	–	–
7.0	114	2.8	1350	30	8.3	1.2	4.8	0.4	1.08	0.14
10.0	115	8.1	2440	150	20.7	1.7	12.2	1.3	1.50	0.09
				Wheat flour – 72% extraction						
3.5	92	5.4	560	40	–2.2	5.0	–2.1	1.3	–	–
7.0	90	11.8	1050	60	3.3	0.5	6.6	0.5	0.72	0.09
10.0	101	6.7	1460	90	8.3	1.7	11.0	0.4	0.97	0.17
				Reference casein + methionine						
3.5	78	4.4	390	20	2.0	1.0	0.9	1.2	–	–
7.0	143	8.7	1620	90	34.2	3.4	18.4	1.7	3.33	0.31
10.0	154	6.4	2320	40	44.2	3.2	26.0	1.4	3.00	0.11

Mean value of 6 male rats/dietary group; feeding period 24–45 days.

nitrogen; 6), and which is commonly placed on the board used for flattening the dough prior to baking.

Cooking had no adverse effect on the proteins of the belila as indicated from its amino acid pattern.

Threonine followed by isoleucine appear to be the next limiting amino acids in the wheat proteins after lysine. Casein contained more lysine, threonine and tryptophan, but less total sulfur amino acids than wheat proteins.

The data obtained in the four feeding experiments are presented in table 2. This table shows the mean weight gain, gain in body water and protein efficiency ratio (PER). The (PER) values reveal that the wheat belila (PER = 1.8) and the baladi bread from the second-class flour (PER = 1.8) had better protein quality at the 10% dietary protein level than the wheat grains (PER = 1.6). The difference did not yet reach a statistical level ($P > 0.05$). All types of bread prepared from white flour (72% extraction) had lower (PER) values, (1.24 ± 0.05 and 0.98 ± 0.17 for baladi bread and french bread respectively) compared to the corresponding values of the wheat grains ($P > 0.01$).

Net protein utilization (NPU) and Protein-produktiver Wert (PPW) values were presented in figures 1 and 2. Correction for maintenance need in the calculation of the (NPU) values (represented by the values obtained from rat fed the protein-free diet) was done as suggested by *Miller* and *Bender* (17). Determination of the protein value below maintenance (3.5, 7.0% dietary protein level) by the protein utilization gave erroneously high estimates, because of the error involved in subtracting two small numbers; namely change in body nitrogen from body nitrogen lost by the group on the protein-free diets; each with a possible experimental error.

Weight gain is plotted against nitrogen intake in figure 3, which also shows the regression equations of the lines. The slopes obtained with the casein + methionine diets were set equal 100. The relative nutritive value (RNV) which is the slope of the test protein expressed as the percentage of the slope obtained with casein + methionine. It can be noted also that several of the wheat products yielded higher intercepts (weight gain predicted at zero intake from the regression line) than the intercepts obtained with casein or the value found by feeding protein-free diets. This is characteristic for proteins deficient in their lysine content (12).

The relative nutritive values of the processed wheat rank baladi bread from second-class flour (87.5% extraction) and belila as the highest, with values of 53 and 55% of the casein diets. In the last column the utilizable protein (in %) is also given. This index was obtained by computing the % relative nutritive value of the test highest value of 7.6 followed by belila, which had a value of 7.0%.

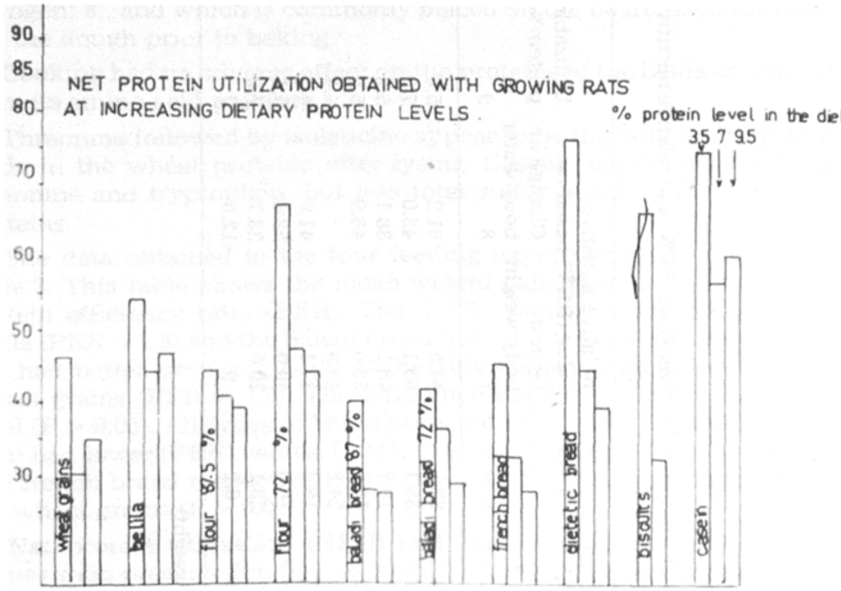
The changes in body water were treated similarly and plotted versus nitrogen intake (fig. 4). The regression lines were drawn, and the corresponding slopes of the lines were obtained for the calculation of the relative nutritive value of the wheat products, table 3; column 8.

The correlation between RNV and chemical score is highly significant (RNV based on changes in body weight, RNV = 0.8466, $P < 0.01$).

Table 3. Egg ratio, protein-efficiency ratio (PER), net protein utilization (NPU), Protein-produktiver Wert (PPW) and relative nutritive value (RNV) of wheat and its products.

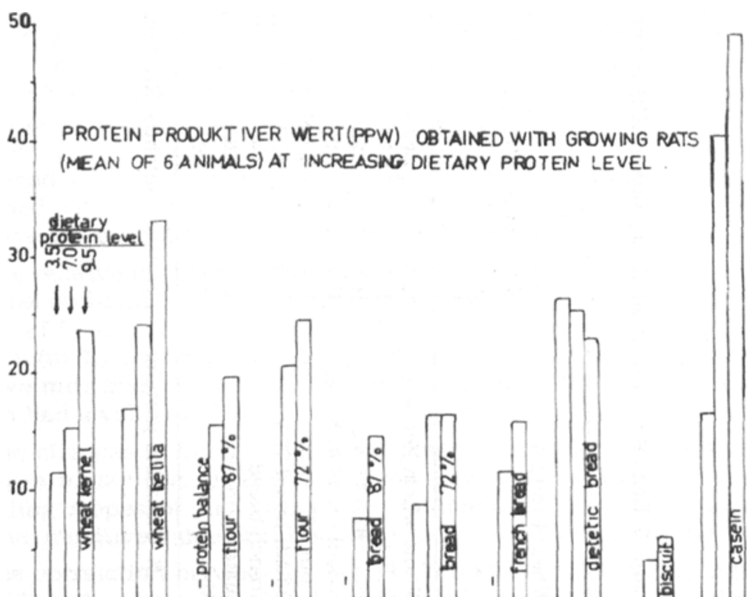
Wheat Product	Protein N × 6.25%	Lysine g/16 g N	Egg ratio[a]	PER[b]	NPU[b]	PPW[b]	Relative nutritive value (RNV) based on Gain in body weight	Change in body water	Utilizable protein%
	1	2	3	4	5	6	7	8	9
Whole wheat kernel	12.6	2.92	44	1.61	34.8	23.8	44.5	61.2	5.6
Belila	12.7	2.77	45	1.81	46.7	33.1	55.1	43.0	7.0
Flour 87.5% extr.	10.9	2.92	48	1.61	39.3	19.7	48.1	38.1	5.2
Baladi bread 87.5% extr.	10.8	3.00	49	1.80	27.2	14.5	53.0	48.9	5.9
Flour 72% extr.	10.8	2.44	40	0.97	43.8	24.6	40.9		4.4
Baladi bread 72% extr.	11.0	2.60	42	1.24	28.7	16.4	37.4	41.0	4.1
French bread	10.4	2.45	40	1.51	27.6	16.0	49.0	52.4	5.1
Diatetic bread	38.1	2.66	43	1.34	39.2	23.1	20.0	23.7	7.6
Biscuits	6.8	1.31	21	–	32.4	6.2	0	12.6	0

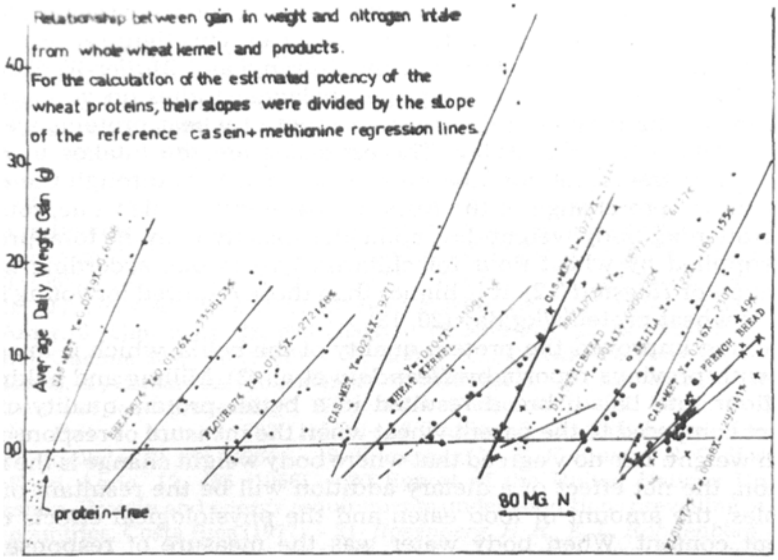
% Utilizable protein = % protein content × RNV (based on gain in body weight)



The correlation between gain in weight and net increase in body water was highly significant, it ranged between 0.77 for dietetic bread and 0.95 for the groups fed wheat grains or bread.

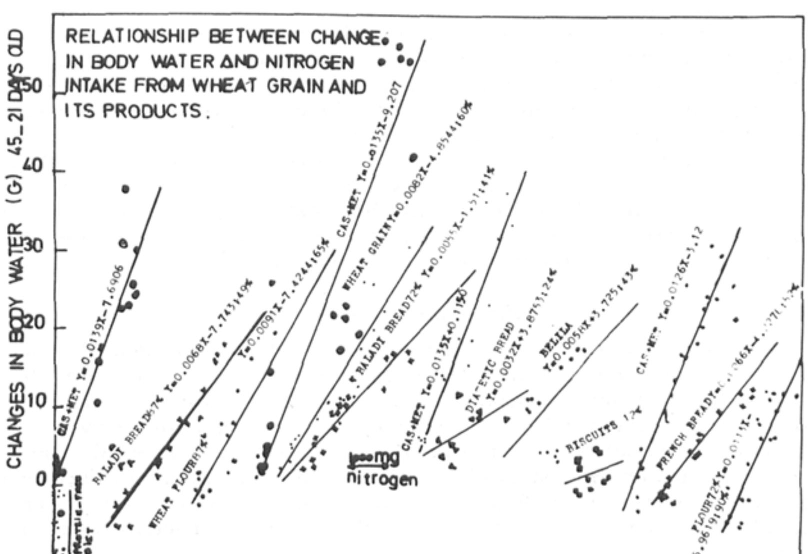
The slope-ratio bioassay was used in the present study to assess the protein quality of wheat and its processed products. The scatter diagrams





of the data for individual animals suggested that the dose-response curves are linear. There was no curvature in the growth of animals fed the proteins under study.

The (NPU) values were constant for casein, fed at different dietary protein levels, compared with NPU values of the wheat and its products which were erroneously high at the low dietary protein levels, and tended to decrease dramatically once the dietary protein level was raised to about 10%.



Based on the reference amino acid pattern (25), and from the rat growth data, the wheat grains had a protein quality of 44. This figure agrees quite well with the values of 37–45 found by *Kofranyi* and *Muller-Wecker* (13) and by Young et al. (24) from nitrogen balance studies on young men. Estimates of the minimum daily requirement of wheat proteins were in average 180 mg protein/rat/day. The estimates are the intakes in milligrams where the regression line intersects the X axis through the point representing zero change in the response parameter. This value, roughly 3.6 g protein/kg/body weight/day, coincides exactly with the total protein need supplied by wheat flour for children 7 years old, according to the estimation of *Hegsted* (12). It is higher than those required for young men; 1.0–1.1 g wheat proteins/kg/day (20, 13).

Cooking improved the protein quality of the belila, which is in agreement with previous reports by *Beaudoin* et al. (3). Milling and baking of dark flour into baladi bread resulted in a better protein quality of the product compared to the parent wheat when the measure of response was gain in weight. It is now agreed that where body weight change is the main criterion, the net effect of a dietary addition will be the resultant of two variables, the amount of food eaten and the physiological effects of its nutrient content. When body water was the measure of response, the protein quality of whole wheat ranks highest, compared to its processed products.

It is now becoming more evident that animals can adapt themselves to low lysine diets, since the mean estimate of lysine for maintenance is 0.09% of the diet, and for growth 0.9% of the diet (22).

In conclusion, the slope-ratio bioassay lends itself for routine evaluation of food proteins and for screening the protein quality of new foods before they are introduced in the market.

Summary

The amino acid analysis revealed that wheat grains, white and dark flour, baladi bread prepared from white or dark flour, bread prepared from formulae enriched with gluten and biscuits are poor in lysine with chemical scores ranging between 20 and 49. The assessment of the protein quality of wheat and related products was done by slope ratio bioassay. Results based on slopes relative to those of reference casein + methionine ranked bread prepared from dark flour and cooked wheat (belila) as the highest in their protein quality, followed by their parent; wheat (RNV = 44). Dietetic bread with gluten had RNV = 20–24; owing to its high protein content (38%), its utilizable protein approached that of good proteins (8%). Very high significant correlation existed between the two measures of response; gain in weight and net increase in body water as response of nitrogen intake.

Zusammenfassung

Das Aminosäurenmuster von Weizenkernen, ungekocht und gekocht (Belila), Weiß- und Braunweizenmehl und Baladibrot, hergestellt aus Weiß- oder Braunmehl, Kaviarbrot, eiweißreichem Glutenbrot und Biskuit wurde ermittelt. Proteine der Weizenkerne und deren Produkte sind arm an Lysin, mit Werten zwischen 20 und 49. Der Proteinnährwert wurde im Rattenversuch mittels der Methode der Dosierung durch den Biotest ermittelt. Kaseindiät + DL-Methioninsupplement diente als Referenzsubstanz. Baladibrot und gekochte Weizenkerne haben die höchsten biologischen Proteinwerte gezeigt (53–55% im Vergleich zu Kasein-Referenz-Diät).

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