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## Protein Nutritive Value of Wheat and Triticale Grain for Humans, Studied at Two Levels of Protein Intake<sup>1</sup>

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### ABSTRACT

The protein nutritive value for adult humans of wheat grain (composite of high-protein Atlas 66 X Comanche lines) and of triticale grain (Rosner) has been compared at two levels of protein intake. Average nitrogen balances of nine human adults maintained on 4.0 or 6.0 g. N per day from the wheat or the triticale were -0.62 (4.0 g. N wheat diet), -0.44 (4.0 g. N triticale diet), -0.16 (6.0 g. N wheat diet), and +0.01 (6.0 g. N triticale diet), g. N per day, respectively. Differences between wheat and triticale were statistically significant at each level of nitrogen intake. A decrease in body nitrogen loss is generally interpreted as indicative of improved protein nutritive status. Thus, the decrease in nitrogen loss obtained at both levels of protein intake with the triticale diets as compared to the wheat diets suggest a slightly higher protein nutritive value for the tested triticale grain as compared with the tested wheat diets.

Obtaining protein to meet human needs is a current and growing problem in the world today (1). Direct consumption of plant proteins by humans is the most economical use of available resources, even though these proteins are, in general, of lower nutritional value for the human than proteins of animal origin (2). Triticale, a man-made amphiploid cereal, has been suggested as a potentially important food crop (3). Analyses of amino acid composition patterns and results of feeding trials with animals suggest that this cereal may have a higher protein value than wheat (3,4,5)<sup>2</sup>. Obviously, laboratory and field feeding trials with humans are necessary for valid application to human nutrition, because of species differences and because of lack of basic knowledge on human protein-amino acid requirements. The object of the present study was to compare the protein nutritive value of wheat grain<sup>3</sup> and triticale grain<sup>4</sup> at two levels of protein intake for humans on the basis of nitrogen balances and several other clinical measurements.

### MATERIALS AND METHODS

#### Experimental Plan

The experimental plan for the study is given in Table I. The study was 33 days in length and consisted of an introductory 3-day nitrogen-depletion period, two

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<sup>2</sup>Lartner, E. N. Personal communication, 1968.

<sup>3</sup>The wheat grain used in this study was a composite of high-protein Atlas 66 X Comanche lines supplied gratis by V. Johnson, J. W. Schmidt, and P. Mattern, Department of Agronomy, University of Nebraska, Lincoln. It was raised at the University of Nebraska and was whole-ground in a hammer mill.

<sup>4</sup>The triticale grain (Rosner) was supplied gratis by E. N. Lartner, Department of Plant Science, University of Manitoba, Winnipeg, Canada. It, too, was whole-ground in a hammer mill on the campus of the University of Nebraska.

TABLE I. EXPERIMENTAL PLAN

Period	No. of Days	Intake of Protein g. N/day	Source of Protein <sup>a</sup>	Total N Intake <sup>b</sup>
Depletion	3	0	...	0.8
Part 1 <sup>c</sup> , adjustment A	5	4.0	wheat or triticale	4.8
Randomly arranged:				
Experiment 1	5	4.0	wheat	4.8
Experiment 2	5	4.0	triticale	4.8
Part 2 <sup>c</sup> , adjustment B	5	6.0	wheat or triticale	6.8
Randomly arranged:				
Experiment 3	5	6.0	wheat	6.8
Experiment 4	5	6.0	triticale	6.8
Total	33			

<sup>a</sup>Source of nitrogen in adjustment period same as that for experimental period 1 or 3 as determined for each subject.

<sup>b</sup>Includes nitrogen provided by basal diet (see text footnote 6).

<sup>c</sup>Part 1 followed by part 2 for subjects 289 to 294. Part 2 followed by part 1 for subjects 285 to 300.

5-day adjustment periods, and four experimental periods of 5 days each. The experimental periods were arranged at random for each subject, as shown in Table II, to eliminate the variables of time and order of presentation.

During the preliminary nitrogen-depletion period, nitrogen intake per subject per day totaled 0.80 g. as provided by the basal diet composed of wheat starch<sup>5</sup>, fat, instant coffee and tea, nonprotein bouillon, and a few low-protein fruits and vegetables. Purposes of this period included introducing subjects to their duties and responsibilities, determining individual caloric requirements for weight maintenance, and use of a very low nitrogen diet to speed adjustment of subjects to the later experimental diets.

During the adjustment and experimental periods of part 1 and part 2 of the study, nitrogen intake was maintained at 4.8 or 6.8 g. N per day, respectively, 4.0 or 6.0 g. N provided by wheat or triticale, 0.80 g. N from the basal diet. Half of the subjects followed the regime of part 1 followed by part 2; for the other half, the order was reversed. During the two experimental periods composing each part, either wheat (see footnote 3) or triticale (see footnote 4) provided the dietary protein at the level of intake indicated in randomized order. The source of dietary nitrogen during the adjustment period was determined for the individual subject to be the same as that in the experimental periods immediately following.

#### Experimental Subjects

The nine young adults who volunteered to be subjects for these studies were all either college students or employees of the University of Nebraska; description data are given in Table III. All maintained their usual daily activities in regard to work or

<sup>5</sup>The wheat starch used in these studies was furnished gratis by the Hercules Powder Co., Inc., Harbor Beach, Michigan.

TABLE II. DIET ARRANGEMENT

Subject No. and Dates	Period and Diet <sup>a</sup> Fed						
	Depletion Period	Adjustment Period	1	2	Adjustment Period	3	4
289	D	T-4	T-4	W-4	W-6	W-6	T-6
290	D	W-4	W-4	T-4	T-6	T-6	W-6
292	D	W-4	W-4	T-4	T-6	T-6	W-6
293	D	T-4	T-4	W-4	W-6	W-6	T-6
294	D	W-4	W-4	T-4	T-6	T-6	W-6
295	D	W-6	W-6	T-6	T-4	T-4	W-4
297	D	W-6	W-6	T-6	T-4	T-4	W-4
298	D	T-6	T-6	W-6	W-4	W-4	T-4
300	D	T-6	T-6	W-6	W-4	W-4	T-4
	April 22, 23, 24	April 25, 26, 27, 28, 29	April 30, May 1, 2, 3, 4	May 5, 6, 7, 8, 9	May 10, 11, 12, 13, 14	May 15, 16, 17, 18, 19	May 20, 21, 22, 23, 24

<sup>a</sup>Diet code definitions (amount N per subject per day): D, depletion diet providing 0.8 g.; T-4, triticale, 4.8 g.; W-4, wheat, 4.8 g.; T-6, triticale, 6.8 g.; W-6, wheat, 6.8 g.

TABLE III. VITAL STATISTICS OF SUBJECTS

Subject No.	Age years	Sex	Nationality and Race	Height cm.	Weight kg.
289	28	M	U.S., Caucasian	187	97
290	18	F	U.S., Caucasian	164	58
292	44	M	U.S., Caucasian	178	71
293	20	F	U.S., Caucasian	161	51
294	21	F	U.S., Caucasian	168	68
295	23	M	India (lacto-vegetarian)	180	60
297	23	F	U.S., Caucasian	168	54
298	25	M	U.S., Caucasian	192	90
300	19	M	U.S., Caucasian	176	74

study but reported to the human metabolism unit for meals. Health records of all were reviewed by a physician of the Student Health Division of the University of Nebraska to ascertain the desirability or safety, or both, of their participation in studies of this type.

#### Diets

Caloric intake for each subject was kept constant during the experimental periods of each study at the level required for weight maintenance by varying the intake of maltose, wheat starch (see footnote 5), jelly, and butter oil among the subjects. Wheat starch was used to equalize differences in intake of wheat flour or triticale flour during the experimental periods, as dictated by the experimental design. A few low-protein fruits and vegetables were part of the daily basal diets<sup>6</sup>.

<sup>6</sup>Composition of basal diet: wheat or triticale flour (varied as to experimental plan), wheat starch (varied), hydrogenated vegetable oil (varied), jelly (varied), maltose (varied), apple sauce (100 g.), tomato juice (100 g.), instant decaffeinated coffee (2.5 g. dry powder), green beans (100 g.), pears (100 g.), peaches (100 g.), maltose (varied), lemon juice (30 ml.). All goods were purchased at the beginning of the study in case lots to eliminate brand and lot variability.

Vitamin and mineral supplements<sup>7,8</sup> were also included. Wheat and triticale flours were fed as baked, yeast-risen rolls. The daily allotment of wheat or triticale flour for each subject as determined by the experimental design was combined with wheat starch in amounts to total 240 g. from all sources. To these were added 40 g. of hydrogenated vegetable fat, 8 drops of glycerine, 2 g. glycerol monostearate, 24 g. sugar, 7 g. yeast, 4.3 g. mineral mix, 12 g. sodium chloride, and 175 ml. water, and ingredients were mixed basically according to the method of Steele et al. (6). These yeast-risen rolls were fed in equal amounts at each of the three daily meals. Extra-calorie wheat-starch rolls were prepared basically by the same method (6) for consumption by subjects with high caloric needs.

#### Laboratory Methods

Evaluation of protein nutriture of the subjects was primarily by the nitrogen balance technique. Nitrogen content of the wheat and triticale flours, other dietary items, and excreta was determined according to the boric acid modification of the Kjeldahl method (7). Creatinine in urine was analyzed by the method of Folin (8) to check the accuracy of collections. Urinary nitrogen and creatinine excretions were determined daily on each 24-hr. collection, and fecal nitrogen was analyzed from 5-day composite collections for each individual. Urine collections were made in glass bottles and preserved under toluene until daily composites were made. Collections for each subject for each 24-hr. period were acidified to 1% HCl and diluted with distilled water to standard amounts, usually 2,000 ml. Each was thoroughly mixed before sampling was done for the various daily analyses. Fecal collections were made in plastic-lined, cardboard freezer containers and immediately frozen. Carmine was used as a marker to divide collections for each individual into 5-day-period lots. Fecal collections for each individual for each period were mixed and digested with HCl before being sampled for analyses. Composites of foods used daily were prepared by weighing allotments and mixing thoroughly in a Waring Blendor before they were sampled for analysis.

Venous blood samples (fasting condition) were drawn from subjects on the first day of each study and at the end of each experimental period for a variety of clinical analyses by a commercial laboratory, to ascertain general maintenance of health and gross protein nutriture. These methods are defined in Table IV.

#### RESULTS AND DISCUSSION

Mean nitrogen balances of each individual subject during each experimental period as well as collective means for all subjects of each study are shown in Fig. 1.

Comparison of nitrogen retentions of subjects dependent on triticale-flour or wheat-flour protein as their near sole source of dietary protein indicates that triticale is the better source at both levels of intake. At the lower level (4.0 g. N per

<sup>7</sup>Composition of vitamin supplement (per subject per day, capsule form); 5,000 USP units of vitamin A, 600 USP units of vitamin D, 2 mg. thiamine, 2.5 mg. riboflavin, 20 mg. niacinamide, 50 mg. ascorbic acid, 1 mg. pyridoxine, 1  $\mu$  cyanocobalamin, 1 mg. calcium pantothenate.

<sup>8</sup>Composition of mineral supplement per subject per day, in g. (part capsule form, part mixed with cereal diet: Ca, 1.00; P, 1.00; Mg, 0.199; Fe, 0.015; Cu, 0.002; K, 0.00005; I, 0.00015; Mn, 0.002; and Zn, 0.0009. NaCl was allowed ad libitum.

TABLE IV. EFFECT ON LEVEL AND SOURCE (WHEAT OR TRITICALE) OF DIETARY PROTEIN ON SEVERAL HUMAN BLOOD CONSTITUENTS

Blood Constituent	Diets and Mean Fasting Blood Values <sup>a</sup> of Subjects While Receiving the Diet				
	Normal	4.0 g. N Wheat	4.0 g. N Triticale	6.0 g. N Wheat	6.0 g. N Triticale
Hematocrit, ml./100 ml.	47 (42-51)	45.4 (39-51)	46.1 (39-52)	45.5 (37-53)	45.6 (38-53)
Hemoglobin, g./100 ml.	15.9 (13.7-18.5)	15.0 (13.3-17.3)	15.2 (13.3-17.0)	15.1 (12.6-18.0)	15.2 (12.6-18.0)
Sedimentation rate	8 (2-15)	6.7 (0-16)	6.6 (1-22)	6.7 (1-19)	7.2 (1-26)
White blood cell count, thousand/cmm.	7.4 (4.4-10.4)	7.3 (5.3-9.7)	7.4 (5.9-11.3)	6.9 (5.1-9.3)	7.3 (6.0-10.4)
White blood cell platelet, mm.	<1	<1	<1	<1	<1
Icterus index, units	<10	<10	<10	<10	<10
Differential % <sup>b</sup>					
Bands	0.7 (0-2)	0.1 (0-1)	0.2 (0-2)	0.1 (0-1)	0.1 (0-1)
Segs	57 (45-67)	45.3 (33-58)	44.6 (36-61)	46.7 (31-62)	47.3 (36-65)
Eosins	3 (0-5)	5.1 (0-14)	3.5 (0-13)	4.3 (1-14)	4.3 (0-10)
Lymphs	36 (27-46)	44.9 (32-57)	45.6 (29-55)	42.3 (21-56)	41.7 (22-58)
Monos	4 (1-7)	5.4 (4-10)	5.2 (2-9)	6.4 (1-16)	6.8 (1-11)
Glucose, mg./100 ml.	101 (90-137)	92.4 (82-101)	91.9 (82-97)	96.0 (85-119)	93.7 (80-126)
Blood urea N, mg./100 ml.	14 (9-18)	9.7 (6-17)	8.7 (6-13)	9.5 (9-13)	9.0 (8-12)
Total protein, g./100 ml.	7.1 (6.6-6.4)	6.9 (6.4-7.2)	6.8 (6.3-7.3)	6.9 (6.6-7.3)	7.0 (6.4-7.3)
Albumin, g./100 ml.	4.6 (4.1-4.8)	4.4 (4.2-4.8)	4.4 (4.2-4.6)	4.5 (4.0-4.9)	4.5 (4.2-4.9)
Globulin, g./100 ml.	2.6 (2.3-2.7)	2.4 (2.2-2.7)	2.4 (2.0-2.7)	2.4 (2.2-2.9)	2.5 (2.2-2.7)
Albumin/globulin ratio	1.78 (1.59-1.96)	1.82 (1.67-1.96)	1.86 (1.68-2.15)	1.90 (1.48-2.45)	1.86 (1.61-2.35)
Calcium, mg./l.	4.8 (4.6-5.0)	4.9 (4.4-5.4)	4.8 (4.3-5.4)	4.6 (4.2-4.9)	4.8 (4.3-5.1)
Cholesterol, mg./100 ml.	203 (148-244)	163 (141-210)	166 (143-235)	165 (122-238)	173 (146-253)
Acid phosphatase, B. R. units	2.2 (2.1-2.5)	2.1 (1.8-2.4)	2.1 (1.7-2.3)	2.1 (1.9-2.5)	2.1 (1.8-2.3)
Serum glutamic pyruvate transaminase, Babson units	13 (7-50)	27.9 (15-83)	25.6 (15-73)	27.0 (14-61)	31.7 (15-83)

<sup>a</sup>Determinations were by standard laboratory procedures of the St. Elizabeth Hospital Laboratory, Lincoln, Nebraska. Additional information regarding methodology and quality control (within 1% for method used) is available from this laboratory.

Hematology methods: (a) erythrocyte sedimentation rate and hematocrit by the Wintrobe Hematocrit tube method under standard conditions as described in Wintrobe Clinical Hematology, ed. 4, Lea and Febiger, Philadelphia; (b) leukocyte and erythrocyte counts by Coulter counter (Model F); (c) hemoglobin determination with cyanmethemoglobin-stable reagent (NiH and Sunderman Standards) read in Coleman Junior spectrophotometer at 55-nm. setting; and (d) differential counts by examination of 100 cells in representative fields stained by the Wright stain procedure.

Autoanalyzer methods: (a) glucose by a modification of the W. S. Hoffman method (J. Biol. Chem. 120:51; 1937); (b) urea nitrogen by a modification of the L. T. Skeggs method (Am. J. Clin. Pathol. 28:311; 1957); (c) total protein by the T. E. Weichselbaum modification of the D. L. Stevens method (Am. J. Clin. Pathol. 7:40; 1946); and (d) albumin by the method of Rutstein et al. (J. Clin. Invest. 33:211; 1954).

<sup>b</sup>100 Cells counted.

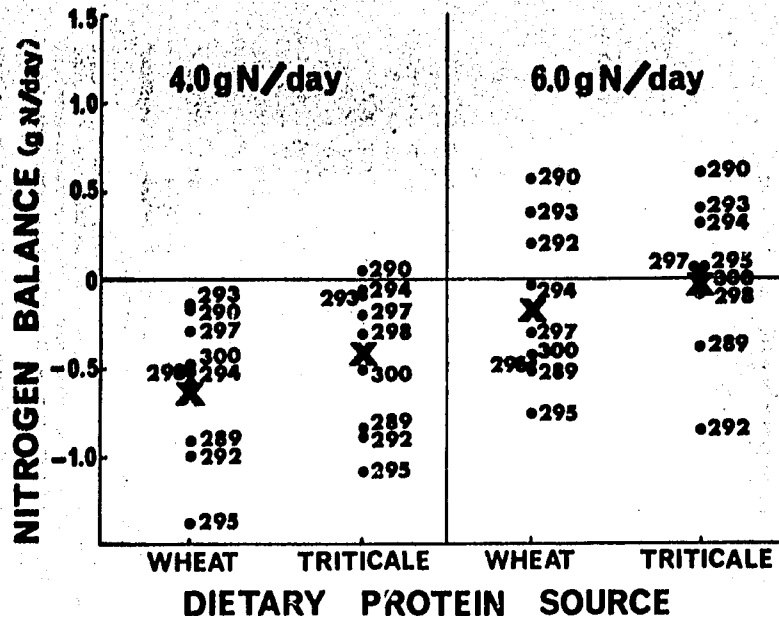


Fig. 1. Comparison of protein nutritive value of wheat grain and triticale grain when fed to provide 4.0 g. or 6.0 g. N per subject per day. Dots represent average nitrogen balances of each individual for the 5 days composing each experimental period. Crosses represent mean balances of all subjects on each experimental diet.

day) the average difference in nitrogen balance achieved was 0.18 g. N per day, and at the higher level the average increment was 0.17 g. N per day. These changes, although small, were statistically significant at the 5% level (4.0 g. N diets) and the 1% level (6.0 g. N diets). On either the triticale or the wheat diet, subjects showed significantly improved nitrogen retention when receiving the 6 g. N intake-level diets than when receiving the 4 g. N intake-level diets.

Although these changes in nitrogen balance are small, all subjects except subject 300 on the 4.0-g. N level and subject 292 on the 6.0-g. N level showed higher nitrogen retention while receiving triticale grain than when receiving wheat grain at similar nitrogen intake levels. Failure of isolated subjects to conform to a pattern response set by a group is not unusual in nitrogen-balance studies. Possible causes may include failure to follow dietary regime, individual variations in nutrient requirements, changes in physical well-being of the subject, and emotional stress.

Female subjects tended to retain more nitrogen than did male subjects. Although caloric intake was determined individually for each subject so that body weight was maintained nearly constant for all subjects throughout the project, it is possible that caloric intake was marginal for some male subjects. Activity records kept by the subjects indicated a higher level of participation in strenuous exercise by male subjects than by female subjects, which may have affected protein as well as caloric need.

Of all the cereals, wheat grain has been the most extensively investigated for its

protein nutritive value in human feeding trials (9). It has been reported recently that human adults can maintain apparent positive nitrogen balances on diets in which 90 to 95% of the protein is provided by wheat flour (10). The authors concluded that amino acid requirements of subjects were met when these wheat diets provided 11.5 g. N per subject per day (479 g. white flour per day). Examination of the amino acid proportionality pattern of wheat-flour protein and results of the current study suggest that human adult needs for amino acids can be met by a much lower intake of wheat flour than that fed in the Bolourchi study (10). In addition, earlier results from this laboratory suggest that if another source of nonspecific nitrogen (nitrogen from any metabolically usable, nontoxic source) is present in the diet, protein requirements from wheat or other cereals can be further reduced (11,12,13). Since urea, glycine, and diammonium citrate were the usual choices of nonspecific nitrogen, this sparing effect was not the result of mutual supplementation effect of protein.

Bolourchi et al. (14) also observed a lowering of blood-urea nitrogen levels of subjects maintained on wheat-flour diets. As shown in Table IV, blood-urea nitrogen levels of subjects were, in general, lower on the experimental wheat and triticale diets than while subjects were receiving their usual diets (pre-experimental value). However, it has been shown that blood-urea nitrogen levels reflect the level of dietary nitrogen intake in normal human adults. Hence, the lowered blood-urea nitrogen levels in the present study cannot be directly attributed to the source of dietary protein, since the level of dietary protein was also low. All other blood clinical values shown in Table IV indicate diets sufficient to support normal values. The lowered blood-cholesterol values shown by subjects while they were receiving the experimental diet more likely can be attributed to the low cholesterol content of the diet rather than to any particular characteristics of wheat or triticale. In our laboratory, this seems to be the typical response to the usual experimental basal diet<sup>9</sup>.

Triticale grain, a new, amphiploid cereal, has been suggested as being a potentially important food for humans in that the lysine content of triticale protein has generally been found to be higher than that of wheat protein (3,4,5) (see footnote 2). "Triticale (hexaploid) is similar to bread wheat in that two-thirds of its make-up is tetraploid wheat, but differs in that the other one-third is rye (*Secale* spp.) instead of goat grass (*Aegilops squarrosa*)" (3). The results of the current study confirm that the protein of triticale grain tested has a slightly higher nutritional value than that of the tested wheat. Thus, additional human feeding trials with this cereal should be encouraged.

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<sup>9</sup>Kies, Constance, and Fox, Hazel; unpublished data.



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