Determination of the First-Limiting Amino Acid of Wheat and Triticale Grain for Humans[1]

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ABSTRACT

The objective of the studies was to determine the first-limiting amino acids in ground whole triticale grain and in ground whole-wheat grain for maintenance of nitrogen retention in human adults. Mean nitrogen balances of ten human adults fed 4.0 g. N per day from the wheat grain plus no amino acid supplement, plus L-lysine, plus L-tryptophan, plus L-methionine, or plus a combination of the listed amino acids, were −0.68, +0.05, −0.64, −0.69, and +0.10 g. N per day, respectively. Nitrogen balances of nine human adults fed 4.0 g. N per day from the triticale grain plus no amino acid supplement, plus L-lysine, plus L-tryptophan, plus L-methionine, or plus a combination of all three listed amino acids, were −0.59, +0.11, −0.24, −0.41, and +0.27 g. N per day, respectively. The significant improvement in nitrogen retention that resulted from lysine supplementation of both wheat and triticale diets indicates that lysine is the first-limiting amino acid in both these cereal grains for protein nutriture of the adult human.

Concern about human population growth has resulted in greater interest in the use of plant proteins to meet human needs (1,2). The several projects in which the protein nutritional value of certain plant proteins for humans has been studied with human subjects have used a great variety of experimental designs and procedures (3). Therefore, comparisons of these data are difficult. A project was initiated in this laboratory to study a wide variety of cereal and other plant proteins in a series with identical experimental designs. The general objective of the current study was to expand this project to include a high-protein wheat grain and a triticale grain. Specific objectives were to determine the first-limiting amino acid in whole ground grain of triticale and wheat for maintenance of nitrogen equilibrium in human adults.

PROCEDURES

Experimental Plans

Experimental plans for the two studies composing the project are given in Table I.

Studies I and II were each 33 days in length; each had an introductory 3-day nitrogen-depletion period, a 5-day nitrogen-adjustment period, and five experimental periods of 5 days each. The experimental periods were arranged at random for each subject during both studies to eliminate the variables of time and order of presentation. Thus, order of presentation of dietary periods differed for each subject.

During the preliminary nitrogen-depletion periods of both studies, nitrogen

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TABLE I. EXPERIMENTAL PLAN

Period[a]	No. of Days	N Intake, Wheat or Triticale[b] g. N/day	Amino Acid Supplement[c]	Total Nitrogen Intake[d] g. N/day
Depletion	3	0	None	0.8
Adjustment	5	4.0	None	5.0
Experiment				
1	5	4.0	None	5.0
2	5	4.0	L-lysine	5.0
3	5	4.0	L-tryptophan	5.0
4	5	4.0	L-methionine	5.0
5	5	4.0	L-lysine + L-tryptophan + L-methionine	5.0

[a]Experiment periods 1 through 5 randomly arranged for each subject.

[b]Different groups of subjects received wheat or triticale.

[c]In amounts needed to supplement cereal so that total intake would meet Rose's recommendations of amino acid intake for young men.

[d]Includes N provided by basal diet. Urea used to maintain diets isonitrogenous.

intake per subject per day totaled 0.68 g. as provided by the basal diet composed of wheat starch, 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 using a diet very low in nitrogen to speed adjustment of subjects to the later experimental diets.

During all experimental and adjustment periods of studies I and II, nitrogen intake was maintained at 5.0 g. N per subject per day–4.0 g. N from flour of whole-ground wheat grain[2] (study I) or triticale grain[3] (study II) plus 0.68 g. N from the basal diet[4], and systematically variable amounts of nitrogen from the amino acid supplements. Urea was used to maintain diets isonitrogenous at the 5.0 g. N intake level.

These levels of total nitrogen intake and total test-protein intake have been selected in this laboratory as standards in studies designed to determine the first-limiting amino acids in food proteins for human adults. If subjects are not in negative balance while receiving nonamino acid-supplemented diets, the effects of amino acid additions are difficult to demonstrate.

No amino acid supplements were used during the nitrogen-adjustment period. The purpose of this period was to allow the subjects time to adjust to both level

[2]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 grown at the University of Nebraska and was whole-ground in a hammer mill.

[3]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.

[4]Composition of basal diet: wheat or triticale flour (varied as to experimental plan), wheat starch (varied), corn oil (varied), jelly (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.).

and source of dietary protein used in each of the studies. During the five randomly arranged experimental periods of each study, the following purified crystalline L-amino acids were used individually as supplements: lysine (0.960 g. per day, wheat study; 0.891 g. per day, triticale study); methionine (1.152 g. per day, wheat study; 1.092 g. per day, triticale study); or tryptophan (0.212 g. per day, wheat study, 0.236 g. per day, triticale study). During one experimental period of each study no amino acid supplements were given (negative control), and during one period, a combination of all three amino acids was given (positive control). Determination of level and kind of amino acid supplements used in these two studies was made by comparison of the amino acids provided by 4.0 g. wheat or triticale nitrogen with human daily need as listed by Rose's provisional recommended intake pattern of essential amino acids for young men (4).

Experimental Subjects

The 19 young adults who volunteered to be subjects for these studies were all either college students or employees of the University of Nebraska. Subject description data are given in Table II. All maintained their usual daily activities regarding work or 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 such studies.

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, 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[4]. Vitamin and mineral supplements[5,6] were also included. Wheat and triticale flours were fed as baked, yeast-risen rolls. Methods used in preparation and administration of the purified nitrogen mixtures, wheat and triticale flours, starch, and other food items were basically the same as described in an earlier paper (5).

Laboratory Methods

Protein nutriture of the subjects was evaluated 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 (6). Creatinine in urine was analyzed by the method of Folin (7) to check the accuracy of collections. Urinary nitrogen and creatinine excretions were determined daily on each 24-hr. collection, and fecal nitrogen data were obtained from 5-day composite collections for each individual. Details are given in

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[5]Composition of vitamin supplement (subject per day, capsule form): vitamin A, 5,000 USP units; vitamin D, 600 USP units; thiamine, 2 mg.; riboflavin, 2.5 mg.; niacinamide, 20 mg.; ascorbic acid, 50 mg.; pyridoxine, 1 mg.; cyanocobalamin, 1 γ. calcium pantothenate, 1 mg.

[6]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 given ad libitum.

TABLE II. VITAL STATISTICS OF SUBJECTS

Subject	Age years	Sex	Nationality and Race	Height cm.	Weight kg.
Study I					
201	24	M	U.S., Caucasian	185	78
202	19	F	U.S., Caucasian	162	56
203	18	F	U.S., Caucasian	171	68
204	27	F	U.S., Caucasian	160	54
206	20	M	U.S., Caucasian	175	67
207	23	M	U.S., Caucasian	173	67
208	19	M	U.S., Caucasian	173	69
209	25	M	Mongolian	170	62
210	23	M	Indian	162	53
211	19	M	U.S., Caucasian	185	81
Study II					
212	18	F	U.S., Caucasian	168	64
213	18	F	U.S., Caucasian	162	60
214	23	F	U.S., Caucasian[a]	168	57
215	19	M	U.S., Caucasian	170	68
216	24	M	U.S., Caucasian	192	80
218	24	M	Turkish, Caucasian	170	59
219	47	M	Mongolian	162	60
220	18	M	U.S., Caucasian	188	91
221	21	M	U.S., Caucasian	178	78

[a]Lacto-vegetarian.

an earlier paper (5). Amino acid compositions (except tryptophan) of the triticale and wheat flours were determined by analysis with a Technicon Amino Acid Auto-Analyzer. Handbook values were used for estimations of tryptophan content (8–11)[7].

Blood samples 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 Tables III and 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 Figs. 1 and 2.

In study I, subjects showed increases in nitrogen retention when lysine was used as a supplement to the wheat-flour diet, either singly or in combination with tryptophan and methionine, as compared with nitrogen balances achieved with single supplements of tryptophan or methionine or when no supplement was employed. The average increment in nitrogen retention achieved between lysine-supplemented and unsupplemented diets was 0.73 g. N per day. Analyses of variance gave an F value greater than the 1% level of probability. Thus, lysine is shown to be the first-limiting amino acid in wheat for maintenance of nitrogen equilibrium in human adults under the experimental conditions described.

[7] Lartner, E. N. Personal communication, 1958.

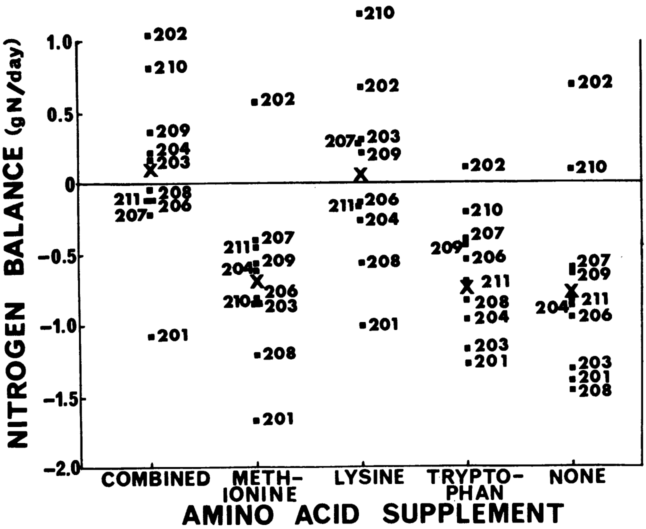

Fig. 1. Determination of first-limiting amino acid in wheat grain protein for maintenance of nitrogen equilibrium in human adults. Dots represent average nitrogen balances of each individual subject for the 5 days composing each experimental period. Crosses represent mean balances for all subjects while receiving each experimental diet.

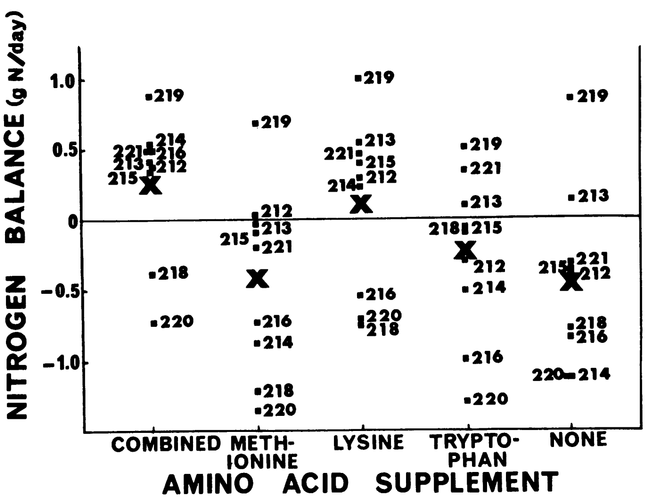

Fig. 2. Determination of first-limiting amino acid in triticale grain protein for maintenance of nitrogen equilibrium in human adults. Dots and crosses, same as in Fig. 1.

TABLE III. EFFECT OF AMINO ACID SUPPLEMENTATION OF WHEAT DIETS ON SEVERAL BLOOD CONSTITUENTS OF HUMAN ADULTS

Blood Constituent	Diet and Mean Fasting Blood Values[a] of Subjects While Receiving the Diet					
	Normal	Wheat	Wheat, Lysine	Wheat, Tryptophan	Wheat, Methionine	Wheat, Combined A A
Glucose, mg./100 ml.	99.8 (86–118)	89.7 (79–102)	95.9 (88–110)	91.5 (80–102)	91.0 (73–104)	94.5 (73–124)
Blood urea N, mg./100 ml.	16.5 (7–24)	7.4 (4–12)	5.5 (2–10)	6.5 (3–11)	7.0 (3–12)	5.8 (4–11)
Total protein, g./100 ml.	6.83 (6.6–7.1)	6.57 (6.2–7.0)	6.64 (6.4–6.8)	6.63 (6.2–7.1)	6.56 (6.2–6.8)	6.60 (6.2–7.0)
Albumin, g./100 ml.	4.42 (4.3–4.6)	4.34 (4.1–4.6)	4.32 (4.1–4.6)	4.29 (4.1–4.5)	4.29 (4.1–4.6)	4.33 (4.2–4.8)
Globulin, g./100 ml.	2.4 (2.1–2.6)	2.2 (1.9–2.5)	2.3 (2.0–2.6)	2.3 (1.8–2.8)	2.3 (1.9–2.5)	2.3 (2.0–2.6)
Albumin/globulin ratio	1.84 (1.69–2.05)	1.96 (1.61–2.36)	1.87 (1.58–2.19)	1.88 (1.66–2.50)	1.91 (1.64–2.30)	1.92 (1.61–2.15)
Serum glutamic pyruvate transaminase, Babson units	14.5 (10–22)	33.2 (18–61)	32.8 (15–50)	32.8 (17–56)	30.7 (15–55)	30.8 (17–76)
Hematocrit, ml./100 ml.	45.9 (39–52)	45.2 (38–51)	44.7 (38–51)	45.4 (39–52)	44.6 (40–49)	44.8 (39–49)
Hemoglobin, g./100 ml.	15.1 (11.6–17.3)	14.9 (12.0–17.2)	14.9 (12.0–17.0)	15.1 (12.4–17.5)	14.7 (12.4–17.3)	15.1 (12.2–16.7)
Sedimentation rate	5.8 (1–15)	7.0 (1–23)	6.5 (0–15)	6.8 (0–20)	6.4 (1–17)	7.0 (1–17)
White blood cell count, 1,000/cmm.	8.3 (5.3–11.3)	8.8 (6.0–13.0)	8.0 (4.9–12.4)	8.0 (5.8–12.9)	8.1 (4.7–13.7)	8.7 (5.7–14.6)
White blood cell pack, mm.	<1 (all)	<1 (all)	<1 (all)	<1 (all)	<1 (all)	<1 (all)
Icterus Index, units	<10 (all)	<10 (all)	<10 (all)	<10 (all)	<10 (all)	<10 (all)
Differential % [b]						
Segs	53.8 (37–70)	47.1 (30–62)	45.6 (31–60)	44.9 (30–62)	46.4 (30–62)	47.6 (24–59)

Basos	0	0.5 (0–2)	0.3 (0–2)	1.1 (0–2)	0.3 (0–2)	0.7 (0–2)
Lymphs	40.7 (22–57)	44.4 (34–63)	46.6 (30–63)	43.9 (30–58)	43.0 (32–52)	41.3 (26–60)
Eosins	2.5 (1–5)	3.5 (0–7)	2.7 (0–7)	3.5 (0–9)	3.3 (0–9)	2.7 (1–4)
Monos	2.7 (1–8)	4.3 (2–7)	4.4 (2–9)	6.0 (1–11)	6.3 (2–12)	7.3 (3–12)
Bands	0.3 (0–2)	0.2 (0–1)	0.4 (0–3)	0.5 (0–2)	0.7 (0–2)	0.4 (0–1)

[a]Determinations were by standard laboratory procedures of the St. Elizabeth Hospital Laboratory, Lincoln, Nebr. 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 (4th ed.), Lea and Febiger, Philadelphia; (b) leukocyte and erythrocyte counts by Coulter counter, Model F; (c) hemoglobin determination using cyanmethemoglobin-stable reagent (NIH and Sunderman Standards) read in Coleman Junior spectrophotometer at 550 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 Skeggs' method (Am. J. Clin. Pathol. 28: 311 (1957)); (c) total protein by 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)).

[b]100 Cells counted.

TABLE IV. EFFECT OF AMINO ACID SUPPLEMENTATION OF TRITICALE DIETS ON SEVERAL BLOOD CONSTITUENTS OF HUMAN ADULTS

Blood Constituent	Diet and Mean Fasting Blood Values[a] of Subjects While Receiving the Diet					
	Normal	Triticale	Triticale, Lysine	Triticale, Tryptophan	Triticale, Methionine	Triticale, Combined A A
Glucose, mg./100 ml.	96.7 (82–108)	90.2 (78–100)	91.2 (83–98)	93.7 (86–104)	92.3 (81–108)	90.9 (78–102)
Blood urea N, mg./100 ml.	13.6 (8–18)	6.3 (5–11)	4.9 (3–8)	6.1 (4–9)	6.8 (6–9)	4.7 (2–7)
Total protein, g./100 ml.	6.9 (6.4–7.2)	6.6 (6.2–6.8)	6.7 (6.0–7.1)	6.5 (6.1–6.9)	6.6 (6.2–7.0)	6.6 (6.1–7.3)
Albumin, g./100 ml.	4.4 (4.2–4.6)	4.3 (3.9–4.7)	4.4 (4.1–4.7)	4.4 (4.1–4.7)	4.4 (4.1–4.7)	4.4 (4.2–4.9)
Globulin, g./100 ml.	2.5 (2.2–2.8)	2.3 (2.0–2.6)	2.3 (1.7–2.7)	2.3 (2.0–2.7)	2.2 (1.8–2.6)	2.2 (1.8–2.8)
Albumin/globulin ratio	1.82 (1.67–2.09)	1.88 (1.62–2.24)	1.97 (1.69–2.46)	1.97 (1.63–2.30)	1.98 (1.72–2.46)	2.07 (1.61–2.60)
Serum glutamic pyruvate transaminase, Babson units	16.2 (13–28)	36.6 (22–88)	31.4 (18–64)	35.3 (17–68)	35.2 (24–56)	31.6 (18–58)
Hematocrit, ml./100 ml.	45.5 (41–49)	44.0 (40–47)	43.9 (38–49)	43.9 (40–48)	44.6 (38–49)	43.6 (37–49)
Hemoglobin, g./100 ml.	15.0 (13.2–16.8)	14.5 (12.8–16.0)	14.4 (12.2–15.8)	14.5 (12.8–16.0)	14.9 (12.6–16.4)	14.6 (12.3–16.2)
Sedimentation rate	5.0 (1–11)	5.7 (1–15)	6.6 (0–15)	6.0 (0–15)	5.8 (0–18)	6.2 (1–18)
White blood cell count, 1,000/cmm.	8.0 (4.0–9.7)	7.7 (4.7–10.7)	7.6 (5.0–10.3)	8.0 (5.0–11.5)	7.2 (4.4–9.5)	7.6 (5.6–10.8)
White blood cell pack, mm.	<1 (all)	<1 (all)	<1 (all)	<1 (all)	<1 (all)	<1 (all)
Icterus Index, units	<10 (all)	<10 (all)	<10 (all)	<10 (all)	<10 (all)	<10 (all)

Differential %[b]						
Segs	48.1 (39–64)	47.4 (29–61)	48.8 (35–66)	48.3 (41–59)	46.1 (32–63)	46.1 (31–62)
Basos	0.3 (0–1)	0.5 (0–1)	0.3 (0–1)	0	0.4 (0–2)	0.5 (0–2)
Eosins	2.2 (1–4)	4.0 (0–9)	3.8 (0–11)	3.2 (0–7)	4.2 (1–8)	2.9 (0–12)
Lymphs	45.2 (31–59)	40.6 (27–55)	41.0 (28–52)	43.9 (34–53)	42.9 (33–52)	45.7 (28–61)
Monos	3.8 (1–7)	5.2 (2–9)	5.6 (1–12)	4.4 (1–13)	5.1 (1–10)	4.0 (1–9)
Bands	0.3 (0–3)	2.0 (0–9)	0.5 (0–3)	0.1 (0–1)	0.1 (0–1)	0.7 (0–6)

[a]See footnote a, Table III.

[b]100 Cells counted.

In study II, subjects again showed higher nitrogen retention when lysine was used as a supplement to the triticale flour diet, either singly or in a combination with tryptophan and methionine, as compared with nitrogen balances achieved with these two single amino acid supplements, or no supplement. The average improvement in nitrogen retention between the lysine-supplemented and nonsupplemented diets was 0.54 g. N (significant at 1% level).

Decreases in blood urea values when wheat or triticale was supplemented with lysine support the contention that lysine is the first-limiting amino acid in these cereal grains,as shown in Tables III and IV; other clinical parameters listed in these tables were all in normal range. The significance of the increases in blood transaminase values of subjects while receiving experimental diets is unknown, but might suggest an increase in amino acid synthesis necessitated by the low level of dietary protein.

Triticale grain, a new wheat-rye hybrid, has been suggested as a potentially important food for humans in that the lysine content of triticale protein has generally been found higher than that of wheat protein (9–11) (see footnote 7). However, even though the lysine content of the triticale grain was higher than that of wheat, results of the current study indicate that lysine is still the first-limiting amino acid in this cereal for maintenance of nitrogen equilibrium in human adults. Knipfel (12), using a serum amino acid proportionality technique, reported results indicating that lysine is the first-limiting amino acid in triticale (Rosner) to meet the protein needs of the growing rat. The results of these studies are supportive in spite of species-age variations in the test animals used (adult human, growing rat).

Even though it has been commonly accepted for many years that lysine is the first-limiting amino acid in wheat cereal for protein nutriture of humans, controlled feeding trials with humans, particularly those involving children, have given mixed, inconclusive results (1–19).Variations in these results may be due, at least in part, to any one or a combination of the following factors: 1) physiological condition of the subjects; 2) amino acid chemical composition of the wheat grain; 3) digestibility of the wheat grain; 4) amino acids supplied by other foods in the diet; and 5) intake of other nutrients. Data from this study indicate clearly that under the experimental conditions used, lysine is the first-limiting amino acid of the protein of the evaluated wheat grain for maintenance of protein nutriture of the human adult. Thus, when either wheat or triticale provides the chief source of dietary protein for humans, the desirability of lysine enrichment is demonstrated.

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