# Interrelationships of Leucine with Lysine, Tryptophan, and Niacin As They Influence Protein Value of Cereal Grains for Humans[1]

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**ABSTRACT**

The most striking difference between the typical amino acid proportionality patterr. of wheat and corn grains is the high leucine content of corn. A series of three studies has been completed on possible leucine interactions with nutrients supplied by these cereals in low amounts in proportion to human needs or for which availability is questioned. In all three studies, adult human subjects were fed diets containing 4.0 g. nitrogen (N) per day from whole-ground wheat grain or whole-ground wheat grain plus L-leucine to total the level commonly found in corn grain. The specific objectives were to compare the effectiveness on protein nutriture of supplementation (with lysine, tryptophan, and niacin) of wheat diets containing high and normal amounts of leucine. Mean N balances (grams per day, respectively) were: of the eight subjects fed wheat alone, wheat plus lysine, wheat plus leucine, or wheat plus leucine and lysine, −0.66, −0.12, −1.08, and −0.69; of the nine subjects fed wheat alone, wheat plus tryptophan, wheat plus leucine, or wheat plus luecine and tryptophan, −0.90, −1.00, −1.19, and −1.39; of the ten subjects fed wheat alone, wheat plus niacin, wheat plus leucine, or wheat plus leucine and niacin, −0.95, −0.87, −1.20, and −0.92. These results suggest that high-level leucine content of corn is an important factor in the relatively poorer nutritional performance of corn grain, in comparison to wheat grain, in human feeding trials. Lysine supplementation of wheat was shown to be effective regardless of leucine content, although the amount of lysine added was not completely able to overcome the adverse effect of the increased amount of leucine. Niacin supplementation was effective only at high leucine-intake levels. Tryptophan supplementation was ineffective or had a slight negative effect at both levels of leucine intake.

The niacin-tryptophan-deficiency disease pellagra is commonly associated with corn-eating peoples of the world. Although corn contains appreciable amounts of niacin, it is seemingly unavailable to the human because of its bound form (1). Evidence suggests that this is also true of the niacin contained in wheat grain (2,3). Lysine and tryptophan have been found to be the first and second limiting amino acids in most lines of both corn and wheat grains under usual conditions for maintenance of adequate protein nutriture of the human adult (4,5). However, wheat grain has been found to have a higher protein value for the human adult than corn grain (6). A comparison of amino acid proportionality patterns of typical lines of wheat and corn grains (7) with each other and with the minimum amino acid requirement pattern for young men (8) indicates that the most striking difference among these patterns is the high leucine content and lower lysine and tryptophan contents of corn grain in comparison to wheat.

The principal objective of the current project was to study the effect on protein nutriture of human adults of interactions between high and moderate levels of

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dietary leucine and niacin, tryptophan, and lysine utilization. This major objective was subdivided into the following working objectives:

1) To compare the protein nutritive value for the human adult of wheat grain to wheat grain with leucine added to simulate that level of leucine commonly found in corn grain.

2) To compare the protein nutritive value of lysine-supplemented wheat grain to lysine-supplemented wheat grain with added leucine (simulating corn).

3) To compare the protein value of tryptophan-supplemented wheat to tryptophan-supplemented wheat grain with added leucine (simulating corn).

4) To compare the protein nutritive value of niacin-supplemented wheat grain to niacin-supplemented wheat grain with added leucine (simulating corn).

## EXPERIMENTAL

### Design

The project consisted of three independent studies, run simultaneously but with different experimental subjects. Each 33-day study was composed of a 2-day nitrogen (N)-depletion period, a 3-day N-adjustment period, and four experimental periods of 7 days each. The experimental designs are given in Table I.

TABLE I. DIET PLANS

| Period[a] | No. of Days | N Intake wheat g. N/day | Diet Supplement[b] Kind | Amount mg./day |
|---|---|---|---|---|
| Study A | | | | |
| Depletion | 2 | 0 | None | ... |
| Adjustment | 3 | 4 | None | ... |
| Expt. 1 | 7 | 4 | None | ... |
| Expt. 2 | 7 | 4 | Lysine | 960 |
| Expt. 3 | 7 | 4 | Leucine | 1,676 |
| Expt. 4 | 7 | 4 | Leucine + lysine | 960 + 1,676 |
| Study B | | | | |
| Depletion | 2 | 0 | None | ... |
| Adjustment | 3 | 4 | None | ... |
| Expt. 1 | 7 | 4 | None | ... |
| Expt. 2 | 7 | 4 | Tryptophan | 212 |
| Expt. 3 | 7 | 4 | Leucine | 1,676 |
| Expt. 4 | 7 | 4 | Tryptophan + leucine | 212 + 1,676 |
| Study C | | | | |
| Depletion | 2 | 0 | None | ... |
| Adjustment | 3 | 4 | None | ... |
| Expt. 1 | 7 | 4 | None | ... |
| Expt. 2 | 7 | 4 | Niacin | 25 |
| Expt. 3 | 7 | 4 | Leucine | 1,676 |
| Expt. 4 | 7 | 4 | Niacin + leucine | 25 + 1,676 |

[a]The following number of subjects were used in each study: eight, study A; nine, study B; ten, study C. Order of experimental periods was randomized for each subject in each study.

[b]During the adjustment and experimental periods the diet consisted of wheat yeast-rolls; applesauce, 100 g.; green beans, 100 g.; peaches, 100 g.; pears, 100 g.; tomatoes, 100 g.; dry bouillon, 3.5 g.; and variable (dependent on caloric need) amounts of sucrose, butteroil, jelly, hard candy, and soft drinks. Purified, crystalline L-form of leucine, lysine, and tryptophan were used as supplements.

During the introductory N-depletion period of all three studies, total N intake per subject per day was 0.8 g., as provided by the basal diet (Table I). This procedure of preliminary feeding of a very low protein diet has been found in this laboratory to hasten the attainment of N equilibrium by subjects to later, moderately-low-protein experimental diets. Total N intake during the 3-day N-adjustment period of each study was 4.8 g. per subject per day – 4.0 g. N from ground wheat grain[2] and 0.8 g. N from the basal diet. Objectives of this period included adjustment of subjects to the level of total N intake to be used during the experimental periods, estimation of individual caloric requirements for approximate weight maintenance, and introduction of subjects to their duties and responsibilities.

Total N intake during the four experimental periods comprising each of the three studies was 4.8 g. per subject per day – 4.0 g. N from wheat and supplemented wheat, and 0.8 g. N from the basal diet. The four experimental periods of each study were randomly arranged for each subject.

In study A, the experimental-diet variables consisted of the unsupplemented wheat diet, wheat diet plus leucine, wheat diet plus lysine, and wheat diet plus leucine and lysine. In study B, the experimental-diet variables were the unsupplemented wheat diet alone, the wheat diet plus tryptophan, the wheat diet plus leucine, and the wheat diet plus leucine and tryptophan. For study C, the experimental-diet variables were the unsupplemented wheat diet, the wheat diet plus leucine, the wheat diet plus niacin, and the wheat diet plus leucine and niacin. Levels of supplementation per subject per day were as follows: leucine, 1.676 g.; tryptophan, 0.212 g.; lysine, 0.960 g.; and niacin (as nicotinamide), 25 mg. The level of leucine was selected to raise the total leucine content of the wheat to that commonly found in corn. The levels of lysine and tryptophan supplementation were determined by comparison of the levels of these two essential amino acids in 4.0 g. wheat-protein N to the Recommended Essential Amino Acid Proportionality Pattern for Young Men (8). The niacin-supplementation level was selected to give slightly more than the recommended niacin intake for human adults as set by the National Research Council (9).

### Diets

Caloric intake for each subject was kept relatively constant at the amount required for weight maintenance by adjusting the intake of starch, fat, sucrose, hard candy, and soft drinks. In order to better simulate field-study conditions, no added vitamin and mineral preparations were used.

The ground wheat grain was prepared for consumption in the form of a yeast-risen roll. The following ingredients were mixed, basically in the manner suggested by Steele et al. (10) for preparation of this product: 118 g. ground wheat, 132 g. wheatstarch, 24 g. sucrose, 40 g. oil, 2 g. glycerol monostearate, 8 drops glycerine, salt, mucilose flakes, and water. Ingredients were weighed individually for the daily allotment. This allotment, after mixing, was divided into three equal portions for consumption at the three daily meals, and baked.

Daily allotments of vitamin and purified essential amino acid supplements determined by the experimental design were given to the subjects in water

[2] Wheat grain (composite of high-protein Atlas 66 and Comanche lines) was grown and supplied gratis by the Department of Agronomy, University of Nebraska. It was prepared as a whole-ground flour.

solutions. These were equally divided among the three daily meals. Other items composing the diet were as listed in Table I.

**Subjects**

A total of 27 young adult men and women were subjects for the three studies: eight for study A, nine for study B, and ten for study C. All these volunteers were students of the University of Nebraska or Nebraska Wesleyan University. All were in good health as determined by physical examinations and health records evaluated by medical physicians of the Student Health Service of the University of Nebraska. During the study, subjects consumed meals in the metabolism laboratory of the Department of Food and Nutrition, University of Nebraska. However, they maintained their usual study, work, and other living routines. Descriptive data are given in Table II.

**Analyses**

The N-balance technique was used as the main criterion of evaluation of level of protein nutriture. Nitrogen determinations by the boric acid modification of the

TABLE II. VITAL STATISTICS OF SUBJECTS

| Subject No.[a] | Age | Sex | Height cm. | Weight kg. |
|---|---|---|---|---|
| Study A | | | | |
| 350 | 19 | M | 184 | 88 |
| 351 | 21 | M | 176 | 89 |
| 352 | 21 | M | 170 | 69 |
| 353 | 19 | M | 178 | 86 |
| 354 | 21 | M | 185 | 77 |
| 356 | 19 | F | 159 | 50 |
| 358 | 18 | F | 173 | 74 |
| 359 | 20 | M | 185 | 84 |
| Study B | | | | |
| 361 | 19 | M | 170 | 95 |
| 362 | 19 | M | 183 | 85 |
| 363 | 20 | M | 187 | 78 |
| 364 | 21 | M | 178 | 66 |
| 365 | 20 | M | 180 | 76 |
| 366 | 34 | M | 168 | 68 |
| 367 | 21 | M | 176 | 76 |
| 368 | 20 | M | 170 | 75 |
| 369 | 25 | M | 179 | 76 |
| Study C | | | | |
| 370 | 22 | F | 170 | 54 |
| 371 | 19 | F | 155 | 52 |
| 372 | 18 | F | 168 | 66 |
| 373 | 18 | F | 164 | 61 |
| 374 | 19 | F | 173 | 61 |
| 375 | 21 | F | 163 | 57 |
| 376 | 19 | F | 169 | 66 |
| 377 | 20 | F | 168 | 66 |
| 378 | 18 | F | 173 | 65 |
| 379 | 25 | M | 185 | 93 |

[a]The ethnic group for all subjects was white/American.

Kjeldahl method (11) were made on samples of food, amino acids, urine, and feces. These materials were collected and prepared for analysis according to methods previously described (12). Daily N excretion in urine was based on 24-hr. collections, and daily N excretion in the feces was based on the average values of 7-day composites. Daily urine creatinine excretions, used as an index of accuracy of 24-hr. urine collections, were determined by a modification of the procedure described by Folin (13).

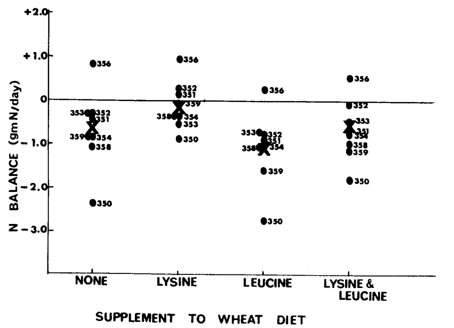
Fasting venous blood samples collected from each subject at the beginning of each study and at the end of each experimental period were analyzed by a hospital laboratory via routine clinical procedures, as described in an earlier paper (14), for possible changes in nutritional and general physical status.

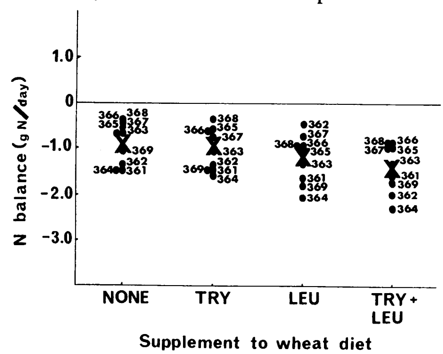
Statistical analyses, including analyses of variances and Duncan's Multiple Range Test, were done by the College of Agriculture Statistical Laboratory, University of Nebraska.

## RESULTS AND DISCUSSION

Nitrogen-balance data obtained from the three studies are given in Figs. 1, 2, and 3.

Mean N balances of subjects in study A while receiving the wheat diet alone, the wheat diet plus lysine, the wheat diet plus leucine, and the wheat diet plus leucine



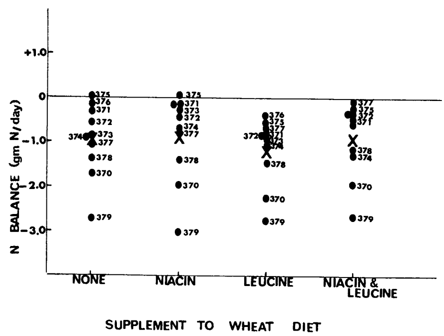




**Fig. 1 (top left). Effect of leucine and lysine additions to wheat diets on the N balances of human adults. Fig. 2 (top right) shows the effect of additions of leucine and tryptophan; and Fig. 3 (bottom), of additions of leucine and niacin. For each figure, the dots represent the average N balances of each individual for the 7 days composing each experimental period, and crosses represent the mean balances of all subjects while receiving each diet.**

and lysine, were -0.66, -0.12, -1.08, and -0.69 g. N per day, respectively. Analyses of variance indicated differences in values at greater than the 5% level of significance. Application of the Duncan's Multiple Range Test indicated that the value for leucine supplement alone was different from all other values, that the value for lysine supplementation alone was different from all other values; but whereas the values for no supplementation and for leucine-plus-lysine supplements were different from the lysine- or the leucine-value supplements, they were not significantly different from each other.

In study B, mean N balances of subjects fed wheat diet alone, wheat diet plus tryptophan, wheat diet plus leucine, and wheat diet plus leucine and tryptophan, were -0.90, -1.00, -1.19, and -1.39 g. N per day, respectively. Analyses of variance indicated differences significant at greater than the 5% level. Application of the Duncan's Multiple Range Test indicated that results obtained with leucine supplementation and with leucine and tryptophan supplement were significantly different from the results obtained with the tryptophan-supplemented and the nonsupplemented diets (greater than 5% level).

In study C, mean N balances of subjects fed wheat alone, wheat plus niacin, wheat plus leucine, and wheat plus leucine and niacin were -0.95, -0.87, -1.20, and -0.92 g. N per day, respectively. Analyses of variance indicated differences among treatments to be present (greater than 5% level). Application of the Duncan's Multiple Range Test indicated that results with leucine supplementation were significantly different (5% level) from results obtained with niacin supplementation, leucine-plus-niacin supplementation, or no supplementation.

Mean N balances of subjects participating in the three studies while receiving the unsupplemented wheat control diet varied; however, results among the three studies were not found to be significantly different. These differences emphasize the necessity of presenting all experimental variables to each subject in laboratory-controlled N-balance studies, rather than attempting to use comparisons among different, small groups of humans. While spread of the N-balance data was great among subjects on any one diet, the pattern responses of individuals in most cases duplicated the directional change of mean values for each study as a whole. Although not specifically examined in this study, earlier work from this laboratory shows only poor correlation of protein requirement with body weight. Spreads of N-balance data from humans are typically large.

Results of other clinical measurements are given in Table III. There were no significant changes in these data owing to experimental treatment, and all values for all subjects were within "normal" range.

The results of this study demonstrate that inclusion of excessive amounts of the essential amino acid leucine has a negative effect on protein nutriture of adult humans. This then suggests that evaluation of cereal products (and other products as well) for protein value should consider amino acid balance in terms of amino acids, such as leucine, supplied in super-optimal amounts as well as those supplied at less than ideal levels. The objective of the current project was not to compare the protein values of corn and wheat grains, but rather to define some factors which may be interacting to affect protein value or to account for possible differences in predicted and demonstrated values.

Leucine interaction with niacin seemed in this study to be an important

TABLE III. EFFECT OF EXPERIMENTAL VARIABLES ON SEVERAL HUMAN BLOOD CONSTITUENTS

| Determination[a] | Mean Fasting Blood Value for Group While Receiving[b] | | | | |
|---|---|---|---|---|---|
| | Normal diet | No suppl. | Plus Leu | Plus Lys (A) Try (B) Nia (C) | Plus Leu and Lys (A) Try (B) Nia (C) |
| Blood urea N, mg. /100 ml. | | | | | |
| Study A | 15 | 5 | 5 | 5 | 5 |
| Study B | 15 | 6 | 6 | 6 | 6 |
| Study C | 14 | 8 | 8 | 8 | 8 |
| Glucose, g./100 ml. | | | | | |
| Study A | 104 | 89 | 90 | 90 | 89 |
| Study B | 94 | 90 | 90 | 88 | 89 |
| Study C | 96 | 86 | 86 | 78 | 86 |
| Total plasma protein, g./100 ml. | | | | | |
| Study A | 7.3 | 7.0 | 7.0 | 7.0 | 6.9 |
| Study B | 7.4 | 7.2 | 7.1 | 7.1 | 7.2 |
| Study C | 7.3 | 7.1 | 7.2 | 7.1 | 7.1 |
| Plasma albumin, g./100 ml. | | | | | |
| Study A | 4.7 | 4.6 | 4.5 | 4.6 | 4.5 |
| Study B | 4.7 | 4.6 | 4.5 | 4.5 | 4.5 |
| Study C | 4.5 | 4.5 | 4.5 | 4.4 | 4.5 |
| Plasma globulin, g./100 ml. | | | | | |
| Study A | 2.6 | 2.4 | 2.6 | 2.4 | 2.4 |
| Study B | 2.7 | 2.6 | 2.6 | 2.6 | 2.7 |
| Study C | 2.8 | 2.6 | 2.7 | 2.6 | 2.7 |
| Plasma A/G ratio | | | | | |
| Study A | 1.8 | 1.9 | 1.7 | 1.9 | 1.9 |
| Study B | 1.7 | 1.8 | 1.8 | 1.8 | 1.7 |
| Study C | 1.6 | 1.7 | 1.7 | 1.7 | 1.7 |
| Hemoglobin, g./100 ml. | | | | | |
| Study A | 15.9 | 15.0 | 15.0 | 14.9 | 15.0 |
| Study B | 16.2 | 15.7 | 15.7 | 15.4 | 15.7 |
| Study C | 13.9 | 13.4 | 13.2 | 13.2 | 13.4 |
| Hematocrit, ml./100 ml. | | | | | |
| Study A | 46 | 44 | 44 | 44 | 44 |
| Study B | 46 | 45 | 44 | 44 | 45 |
| Study C | 42 | 39 | 39 | 39 | 40 |
| Cholesterol | | | | | |
| Study A | 182 | 179 | 176 | 172 | 167 |
| Study B | 190 | 172 | 173 | 184 | 174 |
| Study C | 190 | 172 | 171 | 174 | 181 |
| Serum glutamic pyruvate transaminase, Babson units | | | | | |
| Study A | 30 | 35 | 35 | 34 | 35 |
| Study B | 31 | 40 | 51 | 55 | 42 |
| Study C | 26 | 29 | 26 | 31 | 26 |
| Potassium | | | | | |
| Study A | 4.4 | 4.4 | 4.2 | 4.2 | 4.3 |
| Study B | 4.2 | 4.3 | 4.2 | 4.4 | 4.3 |
| Study C | 4.4 | 4.1 | 4.2 | 4.2 | 4.3 |

[a]Determinations were by standard laboratory procedures of the St. Elizabeth's Hospital Laboratory, Lincoln, Nebr., as described in an earlier paper (14). Additional information regarding methodology and quality control (within 1% for method used) is available from this laboratory.

[b]Values obtained while subjects were maintained on their ordinary pre-experimental diets (Normal diet); on the unsupplemented wheat diet (No suppl.); on the wheat diet plus L-leucine (Leu); on the wheat diet plus L-lysine (Lys), L-tryptophan (Try), or niacin (Nia); or the leucine-supplemented wheat diet plus lysine, tryptophan, or niacin.

possibility. Niacin supplementation evidently had no demonstrated effect at "normal" intakes of leucine; however, when leucine was added to the diet, niacin supplementation resulted in restoration of N retention to the same level achieved when no additional leucine was included. No interactions between tryptophan and leucine were demonstrated. Lysine was evidently the first limiting amino acid in both leucine-supplemented, and nonsupplemented wheat, which suggests that lysine and leucine were operating as independent variables.

Niacin supplementation has been demonstrated to result in improvement in protein nutriture of adult human subjects maintained on corn diets (15). Niacin-tryptophan interrelationships, particularly pertaining to factors influencing tryptophan-to-niacin conversion, have received considerable research attention (16,17). Interrelations among isoleucine, leucine, and valine affecting protein nutriture of humans have also been investigated (18). Feeding excess leucine has been demonstrated to influence excretion of niacin-tryptophan metabolites in both humans and in adult rats (19,20,21). An adverse effect on N retention of feeding high levels of leucine to adult rats has also been reported (21). The results of the current project are in agreement with these earlier reported results.

That *opaque-2* lines of corn have protein nutritional qualities superior to "usual" corn lines has been demonstrated (22). This improvement has been usually credited to the relatively higher lysine content of *opaque-2* line corn grains; however, these lines also are characterized by a lower than usual leucine content. Dogs maintained on non-*opaque-2* corn rations, or on *opaque-2* corn rations with leucine additions, developed the niacin-tryptophan-deficiency disease blacktongue, whereas dogs maintained on unsupplemented *opaque-2* corn rations did not (23). These results suggest that the relative excess of leucine in ordinary corn diets may be important in development of the human niacin-tryptophan-deficiency disease pellagra, supporting but not explaining the apparent effects of niacin-leucine-tryptophan interrelationships on human protein nutrition demonstrated in the present study.

Additional data is needed to explain the biochemical mechanisms involved in these demonstrated leucine-niacin interrelationships. However, from a practical viewpoint, the current project stresses the need to define total nutritional environment in indicating quantitative needs for any single nutrient or in assessing nutritional value of a food product.

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