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## Lysine Absorption from Chickpea (*Cicer arietinum*) and Milk-Supplemented Wheat Diets at Two Levels of Energy

Inderjit K. Hundal and Charanjeet K. Hira\*

Nine young adult girls were fed diets containing wheat, wheat and chickpea, or wheat and milk at each of the two energy levels of 1600 and 1900 kcal/day. Lysine was estimated in food and feces. From a balance study, nitrogen balance, true digestibility (TD), biological value (BV), and net protein utilization (NPU) were calculated. Results indicated that lysine excretion was negatively correlated with lysine intake. Percent lysine absorption was significantly higher ( $P < 0.01$ ) from high-energy diets. Percent lysine absorption was significantly related to dietary protein sources. At the lower, but not at the higher, level of energy consumption lysine absorption was significantly related to N balance, BV, and NPU of the diets.

Diets consumed in developing countries are predominantly cereal based, and lysine is usually the first limiting amino acid in these diets. The indispensable nature of lysine in human diets has been well established (Rose et al., 1954). In addition to protein synthesis for growth and maintenance, lysine plays an important role in several bodily functions (Jansen, 1962; Kurup et al., 1983).

The amino acid composition of a protein is usually studied to determine its protein quality. However, amino acid availability to the individual is also important. Since lysine is the first limiting amino acid of cereal-based diets, its absorption from common dietary regimes becomes all the more important. Many studies relating to effect of lysine supplementation on nitrogen balance improvement

Table I. Characteristics of the Subjects

subject	wt, kg	ht, cm	age, years
AD	47.5	155.0	21
AN	41.5	156.3	22
BA	45.0	158.7	21
IN	50.0	165.0	21
KB	50.0	162.5	21
LA	48.0	156.2	21
ME	46.0	158.7	22
PS	46.0	152.5	22
VS	46.5	156.2	21

have been reported in the literature (Rice et al., 1970; Clark et al., 1962; Jones et al., 1956). However, absorption of lysine from different dietary regimes has not been well studied. A recent study of Meredith et al. (1986) showed that consumption of meals increased plasma lysine only when it was amply supplied by the diet and when subjects consumed less than 20 mg/kg per day decreased plasma

\*Department of Foods and Nutrition, Punjab Agricultural University, Ludhiana 141004, India.

**Table II. Composition of the Diets (g/day)<sup>a</sup>**

ingredient	E <sub>19</sub> W	E <sub>19</sub> C	E <sub>19</sub> M	E <sub>16</sub> W	E <sub>16</sub> C	E <sub>16</sub> M
whole wheat flour	228	173	173	223	168	168
chickpea		50			50	
milk	40	40	300	40	40	300
sugar	38	38	48	33	33	43
potatoes	120	95	120	120	95	120
butter	10	10	10	5	5	5
hydrogenated oil	50	45	40	35	30	25

<sup>a</sup> Equal amounts of fruits and vegetables were used in each diet. Key: E<sub>19</sub> = diet providing 1900 kcal of energy, E<sub>16</sub> = diet providing 1600 kcal of energy, W = wheat diet, C = chickpea-supplemented diet, M = milk-supplemented diet.

lysine was observed. However, information on absorption of lysine from commonly consumed cereal-based diets is scanty. In the present investigation an attempt has been made to study the availability of dietary lysine in young girls from cereal diets supplemented with chickpea and milk at two levels of energy.

#### MATERIALS AND METHODS

Nine girls who were free from infections and infestations and whose heights and weights were within the normal limits prescribed by ICMR (1981) were selected for the study (Table I). On the basis of normal consumption patterns of the subjects, two levels of energy, 1600 and 1900 kcal, were selected for the formulation of the diets at 40 g of protein (N × 6.25) intake. Energy content of the diets was changed mainly by varying the intake of fat, sugar, and potatoes. Three diets were formulated at each level of energy: i.e., W (wheat), C (wheat and chickpea), and M (wheat and milk) diets. Intake of individual food items per day by each subject for different diets is given in Table II. Food was consumed in the form of three major meals: breakfast, lunch, and dinner. Tea was served with snacks between lunch and dinner. All foods were cooked and served in the laboratory.

Six feeding trials of 11-days (adjustment, 7 days; collection, 4 days) duration each were carried out. High-energy diets were fed first in the order of wheat diet, chickpea-supplemented diet, and milk-supplemented diet followed by low-energy diets in the same sequence. In chickpea-supplemented diets, *dhal* was served with lunch and dinner in equal proportions. In milk-supplemented diets milk with sugar was served after the dinner to each subject. Charcoal tablets were used as a marker for each of the feeding trials. Three tablets were given to each subject before breakfast on the first day of the collection period and after dinner on the last day of the collection period. The samples of food, feces, and urine were collected for the last 4 days of each feeding trial and kept in deep freezer. Food and feces were homogenized and dried in enamelled trays in an hot-air oven at 60 ± 1 °C. Urine samples were preserved under toluene. Food, urine, and

feces were analyzed for nitrogen by the macro-Kjeldahl method (AOAC, 1980). Lysine in food and feces was estimated by the method of Carpenter (1960). Nitrogen balance was calculated on the basis of food, urinary, and fecal nitrogen values. Biological value and net protein utilization were calculated using FAO/WHO (1973) values of 37 and 12 mg N/kg of body weight for urine and feces, respectively, as endogenous losses. These values were used because a nitrogen-free diet could not be fed to the subjects. Lysine-free diets could not be fed to the subjects as the diets were from natural food sources.

The results were statistically analyzed by analysis of variance, and critical differences were calculated. Coefficients of correlations were worked out wherever applicable.

#### RESULTS AND DISCUSSION

The results of these studies showed (Table III) that lysine intake in all six diets was above the normal intake (0.4–0.5 g/day) required for positive nitrogen balance (Rose et al., 1955). The total lysine excretion was less in high-energy diets, making them superior in protein quality. The mean fecal lysine excretion changed significantly ( $P < 0.01$ ) with a change in energy intake. Lysine excretion was negatively correlated with lysine intake. The correlation coefficient ( $r = -0.65$ ) was highly significant ( $P < 0.01$ ) at a lower level of energy compared to that at high-energy intake ( $r = -0.39$ ) where it was significant at the 5% level.

A change in protein source of the diets did not affect lysine excretion significantly at a higher level of energy intake. At a low level of energy consumption, a shift from wheat to milk supplementation affected the lysine excretion significantly ( $P < 0.05$ ) whereas a shift from wheat to chickpea and from chickpea to milk supplementation did not affect lysine excretion significantly. Energy-protein interaction had no effect on lysine excretion.

Percent lysine absorption was maximum in milk-supplemented diets at both energy levels followed by chickpea-supplemented and wheat diets. Analysis of variance showed that a change in protein source had a significant effect on percent lysine absorption ( $P < 0.01$ ), probably due to a change in lysine intake. The results are supported by those of Meredith et al. (1986), indirectly indicating increased absorption with higher intake. They reported that increased dietary lysine resulted in increased plasma lysine in humans only when it was amply supplied by the diet and low lysine intake in fact decreased plasma lysine. Percent lysine absorption was significantly higher ( $P < 0.01$ ) at a higher level of energy intake compared to low energy intake. Different values of lysine availability (65–92.8%) using rats from whole wheat and flour have been reported in earlier studies (Kuiken and Lyman, 1948; Guthneck et al., 1953; Gupta et al., 1958; Calhoun et al., 1960). The present data indicating lysine absorption between 70.18 and 84.07% of intake falls between the lit-

**Table III. Protein Quality and Lysine Absorption from Different Diets<sup>a</sup>**

measurement	E <sub>19</sub> W	E <sub>19</sub> B	E <sub>19</sub> M	E <sub>16</sub> W	E <sub>16</sub> B	E <sub>16</sub> M	CD at 5% <sup>b</sup>	CD at 1%
Lys intake, g/day	1.40	1.41	1.82	1.29	1.37	1.79		
Lys, excr g/day	0.31 ± 0.02	0.32 ± 0.06	0.29 ± 0.05	0.38 ± 0.02	0.36 ± 0.04	0.34 ± 0.08	0.037	0.049
app Lys abs, %	74.21 ± 2.1	77.58 ± 4.2	84.07 ± 2.8	70.18 ± 1.6	73.62 ± 3.2	80.68 ± 4.5	2.341	3.089
N intake, g/day	6.64	6.72	6.43	6.60	6.67	6.50		
N balance, g/day	1.36 ± 0.10	1.49 ± 0.20	1.48 ± 0.15	0.54 ± 0.19	0.83 ± 0.14	1.01 ± 0.16	0.120	0.161
app N digestibility, %	78.32 ± 1.5	78.50 ± 1.9	78.84 ± 2.0	78.77 ± 2.6	78.71 ± 1.2	79.05 ± 1.6		
true N digestibility, %	86.89 ± 1.8	87.09 ± 2.7	87.67 ± 1.9	87.48 ± 2.8	87.62 ± 1.8	88.03 ± 1.7		
BV, %	36.53 ± 1.8	38.53 ± 3.0	39.43 ± 2.3	22.37 ± 3.1	29.46 ± 3.1	30.92 ± 3.6	2.232	2.975
NPU, %	31.73 ± 1.5	33.33 ± 3.1	34.53 ± 1.9	19.28 ± 2.6	24.25 ± 2.8	27.12 ± 3.1	1.926	2.568

<sup>a</sup> Key: E<sub>19</sub> = diet providing 1900 kcal of energy, E<sub>16</sub> = diet providing 1600 kcal of energy, W = wheat diet, C = chickpea-supplemented diet, M = milk-supplemented diet. Values are means ± SD. <sup>b</sup> Where the differences between treatments were significant, CD values were calculated. When the differences between two treatments were more than the CD value, differences were statistically significant.

erature values. However, a wide range existing in the literature cannot be compared with the present data because the present study was in human beings compared to earlier studies with animals. Moreover, the nature of the diets is different; in earlier studies, individual food items were used while in the present study mixed foods were used. Moreover, the present study indicated that the source of protein and a change in energy levels has a significant effect on lysine absorption.

Lysine absorption from various diets was found to be positively related to nitrogen balance of the subjects, and the correlation coefficient ( $r = 0.69$ ) was found to be highly significant ( $P < 0.01$ ) at a lower level of energy. Though an increase in lysine absorption showed an improvement in nitrogen balance at a higher level of energy, it could not attain significance. Similarly, lysine absorption at a lower level of energy was significantly ( $P < 0.01$ ) related to biological value ( $r = 0.55$ ) and net protein utilization ( $r = 0.54$ ), but only a trend was found at higher level of energy intake. Thus, the effect of lysine absorption on nitrogen balance, biological value, and net protein utilization was more pronounced only for lower levels of energy intake, thus stressing the need for supplementation of wheat diets at low energy intake common in Indian diets.

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## Cereal Products as a Source of Polycyclic Aromatic Hydrocarbons

Jari P. Tuominen,\* Heikki S. Pyysalo, and Marketta Sauri

Gas chromatography-mass spectrometry was used for the determination of 25 polycyclic aromatic hydrocarbons (PAH) in cereal products available in Finland. Even though the levels of PAH in ordinary cereal products were low, the high level of consumption of these products (67.7 kg/person annually) makes them a significant source of PAH. The level of PAH in bolted flours and milled oats and wheat ranged from 25 to 38  $\mu\text{g}/\text{kg}$  and in rolled oats was 64  $\mu\text{g}/\text{kg}$ . The average concentration of PAH in bran was 220  $\mu\text{g}/\text{kg}$ . One specialty smoked cereal product contained PAH in concentrations as high as 4500  $\mu\text{g}/\text{kg}$  and benzo[a]pyrene in concentrations 100 times higher than in other cereal products. The average annual intake of the 25 PAH compounds from ordinary cereal products was estimated to be 2000  $\mu\text{g}$  per capita.

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants formed by the incomplete combustion of organic materials. By 1976 more than 30 PAH and several hundred derivatives of PAH were reported to have carcinogenic effects (Dipple, 1976). In the atmosphere PAH are present both as particles and in the vapor phase, and they can be transported long distances. Numerous studies have shown that the atmospheric deposit of PAH onto grains, leafy vegetables, fruits, and oil plants forms an important source of PAH in the diet (Grimmer and Duvel, 1970). Another important source is the smoking and grilling of foods (Santodonato et al., 1983). However, the low rate of consumption of smoked and grilled foods reduces their importance as a PAH source. Tobacco smoking is a third contributor to PAH exposure (Wynder and Hoffman, 1967), and the direct heating of food with air-containing

combustion gases, a fourth. Indeed some foods such as copra are traditionally dried without a heat transformer, and the smoke is directly in contact with large surface areas of the food. The use of natural gas and heating oil as a heat source without a heat transformer is a source of risk in many countries (Hutt et al., 1978).

We studied cereal products representative of consumption and of the most important farming areas in Finland. Among the samples were flours dried without a heat transformer and a local delicacy "talkkuna", a mixture of flours made from smoked oats, barley, and peas, which is eaten with dairy products.

#### EXPERIMENTAL SECTION

**Samples.** Commercial product samples were obtained from local retail outlets and pooled from five central government storages in the spring and autumn of 1985 (Figure 1). The sampling was representative of the major cereal products available in Finland and of the main farming areas (>75%).

\* Food Research Laboratory, Technical Research Centre of Finland, Biologinkuja 1, SF-02150 Espoo, Finland.