

# Bioenergetic Cost of Altering the Amino Acid Composition of Cereal Grains<sup>1</sup>

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## ABSTRACT

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The bioenergetic costs (glucose requirement to provide carbon skeletons and energy production) for the synthesis of 18 amino acids and two amides common to the endosperm proteins of cereal grains were calculated. The amount of glucose required for synthesis of histidine, arginine, lysine, and tryptophan was nearly double that required by glutamic acid and about 30–40% more than that required by glutamine; these are the predominant amino acid and amide in cereal proteins. The energy necessary for the

synthesis of the eight essential amino acids for the nutrition of humans and monogastric animals was also higher compared with glutamic acid. Prolamins needed less assimilate for synthesis on a weight for weight basis than did the glutelins, albumins, and globulins of wheat, maize, rice, and oats. The implications for changing the amino acid composition of grain protein by plant breeding are discussed.

Concern for future world food needs inherently involves not only a question of quantity of food but also its quality (International Food Policy Research Institute 1977). One integral aspect is the requirement for protein supplies. This need will be met predominantly by production of plant proteins, particularly cereal proteins. Thus, plant breeders and nutritionists have shown substantial interest in improving the protein quantity and quality in grain (Scrimshaw et al 1975).

Recently we (Bhatia and Rabson 1976) showed that the energetic requirements for obtaining endosperm proteins with increased lysine content in maize and barley genotypes are slightly higher than in normal lysine stocks. We extended these studies to examine the bioenergetic costs for the synthesis of individual amino acids and amides, major protein fractions, and total proteins in different cereal grains.

## MATERIALS AND METHODS

Our calculations, as in the previous report, are based on the analysis of Penning de Vries et al (1974). The derivation of the values, the assumptions made, and the limitations were discussed earlier (Bhatia and Rabson 1976, Penning de Vries et al 1974). The values for heats of combustion ( $\Delta H_c^\circ$ ) of the amino acids and amides are expressed in kilocalorie per mole, as described by Domalski (1972).  $\Delta H_c^\circ$  is defined as the enthalpy change for the reaction of combusting the amino acid at constant pressure at 298° K to yield CO<sub>2</sub>, H<sub>2</sub>O, N<sub>2</sub> (gas), and sulfur as H<sub>2</sub>SO<sub>4</sub> (where applicable).

The data on the amino acid composition of grain proteins and the protein fractions of the various cereals derive from a number of sources. Values for amino acid composition for wheat, barley, rye, and oats are from Ewart (1967); sorghum, Singh and Axtell (1973); maize, Mertz et al (1966); rice, Eggum and Juliano (1973); pearl millet, Rabson et al (1978). Tryptophan and cysteine values are taken from the amino acid composition of RSJ variety of pearl millet, IARI Research Bulletin (1971); and for Table IV, Aykroyd et al (1954). Data for amino acid recovery are made 100% from the original data in all tables.

The sources used for data on the amino acid composition of protein fractions were: wheat, Mattern et al (1968); rice, IRRI Annual Report (1964); maize, Mertz et al (1966); and oats, Draper (1973).

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## RESULTS

### Cost of Production of Individual Amino Acids

The production value (PV), oxygen requirement factor, energy requirement factor (ERF), and hydrogen requirement factor for the 18 amino acids and the two amides normally found in cereal grain proteins are given in Table I. In the present context, PV is the most important factor, which is defined as the weight of the end product (either an amino acid or protein[s] of known amino acid composition) divided by the weight of the substrate required for carbon skeletons and energy production for the reactions. The reciprocal of PV thus gives the glucose requirement for the production of 1 g of the specific end product. The amino acids are listed (Table I) in the order of decreasing PVs. It is apparent that histidine, arginine, lysine, and tryptophan required nearly double the amount of glucose for their production, compared with glutamic acid. The adenosine triphosphate requirements, as reflected by the ERF of the basic amino acids, tryptophan, and sulfur-containing amino acids, were also relatively higher compared with the other amino acids. Using these values, the PVs of any protein(s) of known amino acid composition can be calculated by adding the glucose requirement for the synthesis of the specified amount of amino acid present in 100 g protein and the glucose requirement for other costs such as polymerization, maintenance of biosynthetic machinery, and uptake of minerals and glucose.

It is relevant also to relate PVs to a thermodynamic property of the amino acids. The intrinsic energy content of a molecule is represented by the heat of combustion ( $\Delta H_c^\circ$ ). These values for all amino acids except histidine are also listed in Table I. Clearly, the heats of combustion for the amino acids arginine, tryptophan, and cystine were greater than those of glutamic acid and aspartic acid and their respective amides glutamine and asparagine. The heats of combustion of each of the aromatic amino acids was very large, whereas the PVs for these amino acids was correspondingly low. Further, 1/PV, which is the construction cost in terms of glucose equivalent, showed a significant correlation of 0.79\*\* with  $\Delta H_c^\circ$ . This indicates that the glucose equivalent needed for the production of any amino acid is closely correlated to the intrinsic energy content of the compound.

The data in Table II show that the eight amino acids considered essential for the nutrition of humans and monogastric animals cumulatively account for 26–35% of the total amino acids of the grain protein in the different cereals examined. In cereal grain, however, glutamic acid or its amide glutamine in combination may comprise 18–36% of the amino acid complement. A recent evaluation of available information by Mifflin and Shewry (1979) suggests that glutamic and aspartic acids exist almost exclusively as their respective amides in the prolamins and may represent a major proportion of those dicarboxylic acids in other fractions as well. The average PV of the eight essential amino acids was 0.4447

compared with 0.7054 for glutamic acid (glutamine, 0.5054). Moreover, for the first limiting amino acid of most cereals, lysine, the PV is 0.3952. In maize, the first limiting amino acid is lysine and the next limiting amino acid is tryptophan, which also has a low PV (0.3880).

#### Cost of Production of Major Protein Fractions in Cereals

The PVs for albumins, globulins, prolamins, and glutelins of wheat, rice, oats, and maize were calculated on the basis of reported amino acid compositions (Table III). In the four cereals, the prolamins had the highest PV and the albumin and globulin fractions in wheat, rice, and maize showed the lowest values. In oats, the PV of the glutelins was lowest. The data further show that the percent increase in glucose requirement for the production of 1 g of protein of the lowest PV relative to that of prolamins was in the range of 3–5%. For rice protein fractions (Table III) and proteins (Table IV), PVs were calculated on the basis of two nitrogen sources,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , since flooded rice utilizes N in the form of ammonium ion. When  $\text{NH}_4^+$  was considered as the N source, the

PVs of amino acids and proteins were much higher because the energy cost of nitrate reduction was excluded. The PV for rice prolamins was lowest when  $\text{NO}_3^-$  was considered as the N source.

#### Cost of Production of the Total Seed Proteins in Different Cereals

The above calculations suggested that the overall PVs for the grain proteins of different cereals would thus depend on their amino acid composition. The PVs were calculated for several cereals (Table IV). The crops are listed in the order of decreasing PVs, and a typical grain protein concentration also is indicated. Wheat proteins had the highest PV and the wheat grain also contained a high percentage of protein, whereas rice with the lowest PV had the lowest concentration of protein.

### DISCUSSION

#### Energetic Aspects of Protein Composition

Glucose was used as the basis of these calculations because most biochemical pathways have been studied using that sugar as a carbon source. Nevertheless, in plants, the first products of

TABLE I  
Production Costs and Energy Values of the Individual Amino Acids and Amides Found in Proteins<sup>a</sup>

	PV	ORF	ERF	HRF	Heat of Combustion ( $\Delta \text{H}^\circ$ ) (kcal/mol)	% Glucose Requirement to Synthesize 1 g of Amino Acid Instead of 1 g of Glutamic Acid
Aspartic acid	0.8024	0.1014	0.0300	0.0300	382.8	88
Glutamic acid	0.7054	0.0687	0.0241	0.0068	536.2	100
Serine	0.6928	0.1166	0.0381	0.0286	347.7	102
Glycine	0.6091	0.1571	0.0533	0.0133	232.7	116
Threonine	0.5711	0.1454	0.0588	0.0504	502.2	124
Alanine	0.5527	0.0732	0.0337	0.0449	387.3	128
Asparagine	0.5259	0.1744	0.0758	0.0606	461.0	134
Glutamine	0.5054	0.1251	0.0548	0.0342	614.5	140
Cystine	0.5018	0.2305	0.1000	0.0541	1,016.3	141
Cysteine	0.4913	0.2222	0.0992	0.0579	540.5	144
Valine	0.4750	0.0273	0.0171	0.0342	697.8	148
Proline	0.4669	0.0922	0.0434	0.0261	652.0	151
Leucine	0.4534	0.0146	0.0077	0.0077	856.4	156
Tyrosine	0.4443	0.1656	0.0387	0.0055	1,058.8	159
Methionine	0.4309	0.1875	0.0973	0.0671	809.5	164
Phenylalanine	0.4248	0.0780	0.0363	0.0000	1,110.3	166
Isoleucine	0.4190	0.0933	0.0534	0.0534	856.5	168
Lysine	0.3952	0.1139	0.0685	0.0685	880.3	178
Tryptophan	0.3880	0.1232	0.0637	0.0050	1,344.6	182
Arginine	0.3601	0.1724	0.1092	0.0862	893.9	196
Histidine	0.3499	0.1815	0.1097	0.0419	...	202

<sup>a</sup>Production value (PV) = weight of end product/weight of substrate required for carbon skeletons and energy production. Oxygen requirement factor (ORF) = weight of oxygen consumed/weight of substrate required for carbon skeleton and energy production. Energy requirement factor (ERF) = gram moles ATP required/weight of end product. Hydrogen requirement factor (HRF) = gram moles  $\text{NADH}_2$  required/weight of end product. The nitrogen and sulfur sources are assumed to be  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ , respectively. No correction is made for the energy expenditure in the uptake of glucose and minerals across the cell membrane. The P/O ratio is considered as 3.00. The yields of ATP and  $\text{NADH}_2$  from 1 mol of glucose oxidation are assumed to be 38 and 12 mol, respectively.

TABLE II  
Essential Amino Acid Content of Representative Genotypes of Various Cereal Grains (Expressed as g/100 g of Protein)

	Pearl Millet	Wheat Wichita	Rye	Barley	Oats	Maize	Sorghum	Rice IR-8
Lysine	2.86	1.98	3.26	3.08	4.45	1.97	1.21	3.55
Methionine	3.77	1.34	1.70	1.70	1.87	2.37	1.96	2.35
Threonine	4.16	2.74	3.37	3.27	3.67	3.03	2.56	3.65
Tryptophan	1.85	1.12	1.77	1.99	1.96	...	0.86	1.01
Isoleucine	4.53	3.61	3.63	3.64	3.83	3.76	3.76	4.62
Leucine	9.86	6.74	6.72	7.16	7.69	14.60	14.81	7.92
Phenylalanine	5.10	5.07	4.93	5.48	5.22	4.99	5.13	5.47
Valine	5.76	3.68	4.36	4.58	4.97	3.63	4.38	6.29
% of Total	37.89	26.20	29.74	30.90	33.66	34.35	34.67	34.86
Glutamic acid								
(% of total)	19.23	36.33	29.36	29.27	22.42	20.94	26.02	18.06

**TABLE III**  
**Production Values (PV) and Energy Requirement Factors (ERF) of Major Seed Protein Fractions in Various Representative Cereal Genotypes**

	Wheat		Rice (NO <sub>3</sub> )		Rice (NH <sub>3</sub> )		Maize		Oats	
	PV	ERF	PV	ERF	PV	PV	ERF	PV	ERF	
Albumins	0.467	0.0916	0.441	0.0990	0.642	0.446	0.0873	0.458	0.0943	
Globulins	0.463	0.0919						0.457	0.0953	
Prolamins	0.477	0.0860	0.470	0.0858	0.613	0.467	0.0819	0.473	0.0872	
Glutelins	0.472	0.0893	0.462	0.0920	0.634	0.451	0.0931	0.453	0.0943	
% Increase in glucose required to synthesize 1 g of protein of the lowest PV relative to prolamins	3.02		6.58		...	4.71		4.42		

photosynthesis are phosphorylated sugars and other intermediates with sucrose as the principal product of translocation. In the interest of consistency, a common precursor, glucose, was used.

Glutamic acid and its amide are the most abundant amino compounds in cereal grain proteins. Glutamic acid also has a high PV (0.7054), which means that 1.42 g of glucose would yield 1 g of glutamic acid (1.98 g of glucose for glutamine). In contrast, 1 g of lysine would require consumption of 2.53 g of glucose. From energy considerations, it is therefore advantageous for the plant to store the available N in the seed endosperm for the nutrition of its progeny (the seedling developing on germination) in the form of glutamic acid and its amide, the most abundant amino constituents of the prolamins, particularly. The grain also has the enzymatic competence at germination to make the conversions from endosperm storage protein to nitrogen precursors for growth. For example, the data of Dalby and Tsai (1976), suggest a correlation between the amount of lysine produced in the germinating seedling and the concentration of prolamins in the endosperm. The storage of higher amounts of N with lower expenditure of photosynthates would have been a great selective advantage during the early evolution of the presently cultivated cereals from their wild ancestors if energy were limiting. The other amino acids with higher PVs than that of glutamine contain smaller amounts of N. Perhaps this is one reason for the abundance of glutamic acid and its amide in all cereal grains.

The major sources of variation in the glucose requirement for the synthesis of proteins are due to differences in their amino acid composition. At the level of protein fractions, again the prolamins require a smaller expenditure of photosynthates for biosynthesis compared with the other protein fractions. In most cereals, except rice and oats, prolamins constitute a major component of the total seed proteins. An increase in the prolamins fractions relative to other seed protein fractions in wheat and barley was reported when plants were grown at high soil N levels or following N fertilizer applications during the grain filling period (Mitra and Bhatia 1973, Zoschke 1970). This could be the result of a mechanism designed to store a maximal amount of N with the minimum expenditure of assimilates.

#### Implications of Changing the Amino Acid Composition

Mutants that show enhanced levels of lysine in their endosperm proteins have been discovered in maize, barley, and sorghum. These are being extensively used in plant breeding programs to develop cultivars with improved lysine content. In most of these mutants, the increase in lysine is brought about by reduced synthesis of the prolamins with a concomitant increase in the other protein fractions (Axtell 1976). Glutamic acid and glutamine levels in these high-lysine genotypes are lower than in the normal lysine types. Nelson (1970) suggested that the nutritional value of wheat, maize, sorghum, and barley can be improved by reducing the prolamins content of the endosperm proteins. Our calculations show that the prolamins are the least energy expensive of the major endosperm protein fractions. Plant breeders should therefore keep in mind that replacing prolamins in the endosperm proteins has an

**TABLE IV**  
**Production Values (PV) and Energy Requirement Factors (ERF) of Grain Proteins of Representative Cereals**

	PV	ERF	% Grain Protein
Wheat	0.478	0.0903	14
Rye	0.469	0.0925	14
Sorghum	0.468	0.0874	12
Barley	0.466	0.0922	9
Maize	0.460	0.0873	10
Pearl Millet	0.459	0.0921	13
Oats	0.459	0.0975	13
Rice N as NO <sub>3</sub>	0.454	0.0972	8
Rice N as NH <sub>3</sub>	0.623	0.0514	

energy cost that must be met by the plant. From the point of view of human nutrition, more desirable amino acid composition of the grain protein and the production cost appear to be negatively correlated, i.e., more energy expenditure is required for synthesis of the more nutritious proteins. It would seem, however, that the enhanced nutritional value of such proteins would result in increased efficiency of utilization and could well more than offset the greater energy requirement for synthesis.

Concern has been expressed that the values used by Penning de Vries may be in error and reflect greater efficiency by the plant than is the case. Considering the incompleteness of the data used, this may be so. For instance, there is considerable uncertainty about the site and cost in photosynthate of nitrate reduction in the plant (Mifflin 1979). However, it is unlikely that any future corrections will alter the *relative* figures presented in the calculations. This is also suggested by the close correspondence between the construction cost (1/PV) and the heat of combustion data.

From our previous article (Bhatia and Rabson 1976), it was erroneously inferred by some that genetic improvement in both yield and protein concentrations (and/or protein quality) are mutually exclusive on bioenergetic grounds. This would be true only when the total energy available for grain production remains the same. Because the enhancement of grain yield and grain protein concentration involves an extra expenditure of energy, the improvement of one will make less energy available for the expression of the other. Nevertheless, we believe that a simultaneous increase in grain yield and grain protein concentration (and/or quality) can be and was achieved (Johnson et al 1979) through plant breeding efforts. This improvement, however, will be at a measured pace with no large increments to be expected in the short term. Although they take advantage of energy gains offered by new genetic combinations, breeders will still be confronted with a choice of aiming at higher and still higher grain yields or use of a portion of these gains in improving protein. Perhaps it will be necessary to strike a balance between yield and protein concentration and/or quality. The demand for more nutritious cereals and consumer willingness to pay for them, the cost of external energy inputs (in fertilizers), and other such economic factors will ultimately decide the acceptance of new cultivars with improved protein content.

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