Amino Acid Supplementation of Grain[1]

JIRO KATO and NOBUTOSHI MURAMATSU,
Ajinomoto Co., Inc., Tokyo, Japan

ABSTRACT

Statistics indicate a rapid increase in world population, especially in the developing countries. Increasing demand for foods has come into existence in parallel with the growth of population. Amino acid supplementation of oil seed protein and grain is both practical and economical and can play a very important role in the campaign to improve the quality of world protein supplies. This paper reviews not only the nutritional significance but also the practical aspects of amino acid supplementation of foods and feedstuffs. Production of important amino acids is also reviewed.

INTRODUCTION

It is obvious that an abundant supply of food is necessary in order to maintain the health of the people of the world, and it is particularly important to have food which contains good quality proteins. According to United Nations statistics, the 1970 world population of 3.7 billion will increase to 6 billion by the year 2000, with explosive growth in Southeast Asia the most conspicuous factor of the upward trend. The problem of maintaining protein quality becomes increasingly important as the population expands.

Oilseed proteins assume a vital role in two ways, either to supply protein directly for human foods or to supply proteins for animals which eventually become a protein source for human beings.

It has been demonstrated repeatedly that vegetable proteins are inferior nutritionally to animal proteins because of an unbalance in the constituent amino acids. With advancing knowledge of animal nutrition it has been shown that marked improvement in the nutritive value of vegetable proteins can be achieved by adding some kind of amino acid which is in short supply. This balancing of the protein quality can take the form of addition of a protein source which contains a surplus of the missing amino acids or amino acids can be added directly. Amino acid supplementation can have a significant effect in solving nutritional problems in developing countries where grain is the major food staple. Large scale field tests of amino acid supplementation of grains are in progress in several areas and practical supplementation of foods is already being carried out in other areas.

The addition of amino acids to feeds for animals, which eventually become a protein source for human use, is being practiced on a large scale and economical basis, and prospects for future growth are bright.

GRAIN SUPPLEMENTATION

In 1960 the Economic and Social Council of the United Nations considered in detail the relationship between population expansion and food supplies with special emphasis on the critical protein component of foods (1). The policy directions for closing the protein gap utilizing protein from conventional sources include the following: promotion of increased quantity and quality; marine and fresh water fisheries; and prevention of loss of proteinaceous food. New sources of protein which could be utilized are: oil seed and oil seed protein; fish protein concentrates; amino acid fortification; and single cell protein (petroleum protein). The policy suggestions for closing the protein gap should be evaluated from the following viewpoints which encompass ideal criteria for rapid acceptance: (a) the protein sources should be readily available; (b) changes in traditional "food habits" and "cooking habits" should be avoided; (c) the protein sources must be acceptable to the populace (no religious or cultural prohibitions); (d) application of the proteins should not require any significant consumer education; (e) implementation time should be reasonably short; (f) the products must be harmless for use in foods. Toxicity must be checked thoroughly; (g) price should be reasonable and preferably as low or lower than familiar food items. The development of high lysine corn containing the Opaque-2 gene (2) is an example of promotion of increased quantity and quality. This discovery rates as a major agronomic achievement. The special corn contains a high level of natural L-lysine, but yield is low. On this basis hybridization with stronger types of corn has been carried out in an effort which promises to bring about a commercially acceptable and highly nutritious crop. The improved hybridization techniques which have been so important for corn have not been applied on a commercial scale to other grains such as rice and wheat, but experimental studies now in progress give hope for future success.

The studies by Scrimshaw and his coworkers (3) on supplementation of corn by oilseed proteins in the development of the very nutritive food, INCAPARINA, represents another method for improving the quality of cereal diets. Although there have been some problems in gaining widespread acceptance as a standard item of diet, the product is gradually gaining ground. Single cell protein (SCP) produced from petroleum-based food stocks (4) offers another solution to cope with the acute shortages of foods which are expected in the future. Its application to human foods remains to be proved, and utilization in animal feeds represent a more likely use of SCP.

In contrast to the above, amino acid supplementation of grain (5) appears to be the most practical method for immediate fortification of cereal proteins.

ESSENTIAL AMINO ACIDS AND LIMITING AMINO ACIDS IN GRAIN

All proteins are condensation polymers of something like two dozen amino acids. During digestion of food proteins the component amino acids are liberated and absorbed to build body protein. There are eight different amino acids, essential amino acids, which must be supplied by the protein food; whereas the remaining amino acids, the non-essential amino acids, can be synthesized in the body if they are not available in the food source. Essential amino acids must be supplied in an optimal ratio, and human milk and whole egg are considered as the best sources of protein having essential amino acids in good balance.

Generally speaking, grain proteins are inferior to animal proteins because they are dificient in essential amino acids. As shown in Table I, L-lysine is the first limiting and L-threonine or L-tryptophan are the second limiting essential amino acids of cereal products.

[1]One of 16 papers being published from the Symposium, "Oilseed Processors Challenged by World Protein Need," presented at the ISF-AOCS World Congress, Chicago, September 1970.

TABLE I

Limiting Amino Acids of Cereal Grains

Cereals	1st Limiting	2nd Limiting
Wheat	Lysine	Threonine
Rice	Lysine	Threonine
Corn	Lysine	Tryptophan
Millet	Lysine	Threonine
Sorghum	Lysine	Threonine
Barley	Lysine	Threonine

Figure 1 illustrates the concept of amino acid requirements in a model system. In the figure, water barrel A shows the amino acid pattern of a protein of reasonable quality for man. Barrel B corresponds to the amino acid pattern of wheat protein. The availability of wheat protein is limited by the L-lysine content, and other amino acids are worthless for body building in excess of the L-lysine level in much the same way as the volume of water in the barrel is limited to the low level of the L-lysine stave. By addition of L-lysine to this pattern, the capacity of the barrel, that is to say the availability of its protein, can be increased markedly as indicated by barrel C.

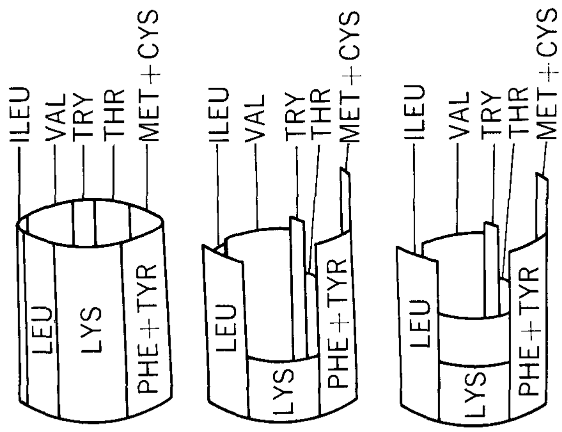

FIG. 1. Description of amino acid fortification.

THE EFFECTIVENESS OF GRAIN SUPPLEMENTATION BY AMINO ACID ADDITION

The significance of amino acid fortification lies in the fact that fortification of grains with relatively small levels of essential amino acids greatly increases the nutritional value of the protein. Animal feeding tests clearly prove this point. E.E. Howe (6) measured the protein efficiency ratio (PER) of various grains before and after addition of limiting amino acids (Table II). Rats fed with wheat flour containing 8% protein had an average 28 day weight gain of 8 g, and the PER was only 0.65. Supplementation of the wheat flour with 0.2% of L-lysine hydrochloride brought the weight gain to 35 g and the PER to 1.56, whereas supplementation with both L-lysine and L-threonine resulted in a weight gain of 78 g and a PER of 2.67, values comparable to those obtained for the casein controls. Analogous results were obtained in rice- and corn-based diets. Hegsted (7) measured the effectiveness of amino acid supplementation of wheat by determination of PER and biological value (BV) of the protein. Yang et al. (8) and Daniel (9) also studied the effect of wheat supplementation by L-lysine and L-threonine. Rosenberg (10,11) and Pecora (12) established supplementation levels of L-lysine and L-threonine for rice.

Understandably most of the supplementation studies are based on rat feeding, but there are many reports of the effect of supplementation in human feeding, particularly with children. Graham (13) recommended supplementation of wheat with 0.2% of L-lysine hydrochloride for diets of children.

Jansen (14,15) investigated supplementation of bread and showed that 0.2% of L-lysine hydrochloride was superior to 6% of fish flour from a nutrition standpoint. Furthermore, the lysine-fortified bread had no foreign odor or objectionable color to limit consumer acceptability.

Currently in Japan, 3.7 million school children, about 40% of the total, are fed daily with a wheat flour bread fortified with 0.2% of L-lysine hydrochloride. This program, which is promoted by the Ministry of Education, involved L-lysine fortification of wheat flour in the bakeries which produce bread for distribution under the school lunch system. By 1972 the distribution of L-lysine-fortified bread should embrace the entire school population of nine million children. When thisis accomplished L-lysine will be mixed with wheat flour in the milling process rather than as an ingredient of the dough at the bakery level. L-lysine-fortified bread is now being considered for general use in addition to the current distribution in the school lunch program.

In India the Modern Bread program involves distribution of 10,000 tons of L-lysine-fortified bread per year (16). An AID-sponsored large scale supplementation program will get under way in Tunisia by 1971 (17).

H.N. Parthasarathy (18) has performed nutritional tests on human subjects using fortified rice. H.R. Rosenberg (11) demonstrated in animal feeding studies that fortification of rice with 0.2% of L-lysine hydrochloride and 0.1% of L- threonine led to PER values which were comparable to casein. The practice of amino acid supplementation of rice is not as easily performed as in wheat, because in contrast to wheat which is used in a flour form, rice is consumed as a whole grain which is washed and boiled in water. Since amino acids are water soluble, losses are high during food preparation. In order to overcome this defect, the Ajino-

TABLE II

Effect of Grain Supplementation

Supplementation	28 day weight gain, g	Protein efficiency ratio
Wheat flour (8% protein)	8	0.65
Wheat flour + 0.2% L-lysine HCl	35	1.56
Wheat flour + 0.4% L-lysine HCl	36	1.63
Wheat flour + 0.4% L-lysine HCL+ 0.15% L-thrrconim	78	2.67
Casein	72	2.50
Rice (7.8% Protein)	35	1.50
Rice + 0.2% L-lysine HCl + 0.1% L-threanine	89	2.61
Casein (ca. 7.8% protein in diet)	68	2.50
Corn (8.55% protein)	50	1.41
Corn + 0.4% L-lysine HCl + 0.07% DL-tryptophan	98	2.33
Casein (CA. 8.55% protein in diet)	93	2.50

FIG. 2. Amino acid fortification of rice: left, rice fortification granules (RFG); right, rice containing RFG.

moto Company of Tokyo (19) has developed a Rice Fortification Granula (RFG). This RFG contains 20% of L-lysine hydrochloride, 10% of L-threonine and other nutritional ingredients in a rice-shaped granule (Fig. 2) which resists ingredient loss during conventional cooking of rice. Field tests of RFG are in process in Thailand. When this RFG is mixed with natural rice at a 1% level complete supplementation will be attained. All studies to date indicate that fortification with such granules does not influence traditional cooking processes nor does it change in any measurable way the acceptability of the final cooked rice.

ECONOMICS OF AMINO ACID SUPPLEMENTATION

Supplementation by amino acids is very effective in developing countries where the staple food is grain. U. Kracht (20) made a nutritional and economic evaluation of supplementation of various protein sources with L-lysine using the chemical score as a basis for comparison. His data has been recalculated (Table III) using L-lysine hydrochloride, soybean flour, fish protein concentrate and skim milk powder as sources of L-lysine, and using bulk, October 1970, prices in the United States. Of the various lysine sources providing equivalent utilizable protein, soyflour is somewhat more economical than L-lysine for fortification, and both milk and FPC are considerably more expensive. In many areas of the world where soy flour is available only by importation L-lysine provides lowest cost fortification of cereal grains. From the nutritional standpoint addition of one gram of L-lysine hydrochloride to cereals is equivalent to an increase of about 10 g of utilizable protein. Similar results have been calculated in animal testing using Protein Retention Efficiency (21) and Relative Nutritive Value (22).

SUPPLEMENTATION OF PROTEIN CONCENTRATES

There are several hundred million people in the world who economically cannot procure grains as food, but must survive on vegetables and fruits such as cassava, potatoes and bananas which contain negligible amounts of protein. Protein concentrates fortified with amino acids have been suggested to compensate for the protein shortage. In Table IV taken from results of WHO/FAO/UNICEF (23) the effectiveness of fortification with L-lysine and DL-methionine is quite apparent with peanut flour, sunflower meal, cottonseed flour, sesame flour and soybean flour.

AMINO ACIDS IN ANIMAL FEEDS

In order to produce large quantities of meat at lowest cost it is essential to have a well balanced feedstuff at a reasonable low price. Supplementation with lysine and methionine can be highly effective in balancing rations to

TABLE III

Nutritional and Economic Evaluation of Protein Enrichment of Wheat Flour

Wheat flour, g	Supplement	Grams	Total protein	Chemical Score	Utilizable protein g,	U.S. $[a]	Proportional cost (L-lysine HCl = 100)
542	---	---	49.9	26	13.0	---	---
542	L-Lysine-HCl	1.09	51.0	48	24.5	0.0801	100
507	Soybean flour	29.0	61.1	41	24.5	0.0763	95
506	Skim milk powder	36.1	59.6	42	25.0	0.0914	114
535	Fish protein conc	12.5	58.3	42	24.5	0.0889	111

[a]Ingredient prices in U.S. $/kg:wheatflour, 0.143; L-lysine HCl, 2.20; soy flour, 0.132; skim milk powder, 0.528; fish protein conc, 0.99 (nominal).

TABLE IV

Amino Acid Supplementation of Protein Concentrates

Supplementation	Protein efficiency ratio
Peanut flour	1.53
Peanut flour + 0.4% DL-thrconine	1.70
Peanut flour + 0.4% DL-threonine + 0.2% L-lysine • HCl + 0.2% DL-methionine	2.22
Sunflower meal	1.28
Sunflower meal + 0.43% L-lysine• HCl	1.69
Cottonseed flour	1.66
Cottonseed + 0.39% L-lysine•HCl	2.24
Sesame flour	0.81
Sesame + 0.35% L-lysine•HCl	1.97
Soybean flour (full fat)	1.99
Soybean flour (full fat) + 0.23 DL-methionine	2.62
Casein	2.50

TABLE V

Available Lysine in the Proteins of Fish and Soybean Meal

Total lysine, %	Available lysine, %	Availability, %
Fish meal, Peru		
6.3	4.1	65
5.3	4.2	79
Fish meal, Japan		
6.8	4.1	60
6.8	4.2	62
6.1	2.3	38
4.5	1.8	40
Soybean meal		
3.8	3.1	82
5.1	4.2	82
3.8	2.2	58

improve feed efficiency.

It is roughly estimated that the current world production of mixed feeds is in the range of 100 million tons per year. The major feed ingredients are cereal grains, oilseed meals, animal protein sources and mineral supplements. Fish meal is a preferred protein concentrate, but as a marine-derived resource, production, quality and price are rather unstable. There is a strong tendency among the feedstuff producers to rely more on agricultural protein concentrates such as soybean meal, peanut meal or cottonseed meal. However, the vegetable proteins contain less lysine and methionine than fish meal and supplementation with amino acids is desirable to secure a better balanced high protein feed.

Cereal grains such as corn or sorghum are the major energy sources in mixed feeds, but additionally they are significant contributors to protein. Here again the vegetable proteins are incomplete, lacking in L-lysine, DL-methionine and DL-tryptophan. Fortification with amino acids is a logical expedient to improve protein quality. Increasingly, the formulation of mixed feeds is calculated by computors to provide a mixture of ingredients having the highest feed efficiency at the lowest cost. In this computerized formulation both L-lysine and DL-methionine are playing a more important role in the thinking of the more progressive feed manufacturers.

Heat is applied during production of both fish meals and oilseed meals, and unstable amino acids in the proteins may be destroyed or converted to a nutritionally unavailable form. L-lysine is particularly susceptible to such heat damage. L-lysine availability data from the Central Research Laboratories of the Ajinomotor Company are supplied in Table V for fish meal and soybean meal. The more than twofold variation in L-lysine availability from 1.8% of the protein of one Japanese fish meal up to 4.2% for a Peruvian meal and a different Japanese meal is particularly noteworthy. Synthetic L-lysine supplementation can be extremely important in improving quality in such cases of heat damage.

PRODUCTION OF AMINO ACIDS

Table VI shows the production of four kinds of amino acids which are important for amino acids supplementation of grain. The manufacturing process, today, of L-lysine is by fermentation (24), and the total production in the entire world for 1970 is estimated to be about 7000 metric tons with projections to 10,000 tons in the near future. Of this quantity 90% is manufactured by Ajinomoto Co., Inc. and Kyowa Hakko Kogyo Co., both in Japan.

For the moment, as far as marketing is concerned, L-lysine is sold principally for the supplementation of feedstuffs. In the very near future, amino acid fortification for human beings will be brought forward more forcefully as a necessity throughout the entire world.

There are two methods for manufacturing L-threonine, by fermentation (25) and by synthesis (26). L-threonine is generally used in the pharmaceutical industry, and the price is relatively high. Production is very small, but once it starts being used in amino acid supplementation and produced on a far larger scale, the price could be lowered greatly.

L-tryptophan, the second limiting amino acid of many cereals is indispensable for increasing the effectiveness of corn protein. It is produced by a synthetic method (27), but at present L- or DL-tryptophan is used only in the field of pharmaceuticals, and the usage for amino acids supplementation is left for future.

DL-methionine is an important adjunct to fish and soybean meals for feeds. It is manufactured by synthetic methods (28) from acrolein as a raw material. Manufactured in Germany, France, U.S.A. and Japan, the production totals roughly 35,000 tons per year.

REFERENCES

1. Report to the Economic and Social Council of the Advisory Committee on the Application of Science and Technology, United Nations, "*International action to avert the impending crisis.*" New York, 1968.
2. Bell, E., T. Humphreys, H.S. Slayter and C.E. Hall, Science 145:280 (1964).

TABLE VI

Production of Amino Acids

Amino acids	Method	Production, ton year	Supplier
L-lysine•HCl	F[a]	7,000 ~ 10,000	Japan, France
DL-methionine	S	30,000 ~ 35,000	Japan, USA, France, Germany
L-threonine	S F	30 ~ 50	Japan
L-tryptophan	S	15 ~ 20	Japan

[a]F, fermentation; S, synthesis.

3. Bressani, R., L.G. Elices, Arch. Venez. Nutr. 12(2):245-257 (1963).
4. "Production and Utilization of BP Protein Concentrate," presented at the Second International Conference on Global Impacts of Applied Microbiology, Addis Ababa, Ethiopia, November, 1967.
5. PAG. Statement No. 9, "Amino Acid Fortification," August 24, 1970.
6. Howe, E.E., G.R. Jansen and E.W. Gilfillan, Amer. J. Clin. Nutr. 16:315 (1965).
7. Hegsted, D.M., Ibid. 21:668 (1968).
8. Yang, S.P., Helen E. Clark and G.E. Vall, J. Nutr. 75:241 (1961).
9. Daniel, V.A., T.R. Doraiswamy, S.V. Pao, M. Swaminathan and H.A.B. Parpia, J. Nutr. Diet. 5:134 (1968).
10. Rosenberg, H.R., and R. Culick, J. Nutr. 63:477 (1957).
11. Rosenberg, H.R., R. Culik and R.E. Eckert, Ibid. 69:217 (1959).
12. Pecora, L.J., and J.M. Hundley, Ibid. 44:101 (1951).
13. Graham, G.G., R.P. Placko, G. Acewedo and E. Morales, Amer. J. Clin. Nutr. 22:1459 (1969).
14. Jansen, G.R., C.F. Hutchison and M.E. Zanetti, Food Technol. 20:323-326 (1966).
15. Jansen, G.R., and S.R. Ehle, Ibid. 19:1435 (1965).
16. Iwan, J.L., Cereal Sci. Today 13:202 (1968).
17. Rosenfield, D., and F.J. Stare, Mod. Gov. 11(5):47 (1970).
18. Parthasarathy, H.N., K. Joseph, V.A. Daniel et al., Can. J. Biochem. 14:385 (1964).
19. Ajinomoto Co., Inc. Japanese patent pending.
20. Kracht, U., presented at Ad Hoc Group on Amino Acid Fortification, Rome, May 1969.
21. Jansen, G.R., Amer. J. Clin. Nutr. 22:38 (1969).
22. Hegsted, D.M., "Protein Enriched Cereal Foods for World Needs," AACS, Minneapolis, 1969.
23. Howe, E.E., E.W. Gilfillan and M. Milner, Amer. J. Clin. Nutr. 16:321 (1965).
24. Ajinomoto Co., Inc., Japanese Patents 41-51266 and 41-57005; French Patent 1,533,688.
25. Ajinomoto Co., Inc., Japanese Patent 40-28815; French Patent 1,484,846; British Patent 1,143,218.
26. Akabori, S., K. Okawa and M. Sato, Bull. Chem. Soc. Japan 30:937 (1957).
27. Ajinomoto Co., Inc., U.S. Patent 3,019,232.
28. U.S. Ind. Chem. Inc., British Patent 605,311.

[Received December 7, 1970]