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# AMINO ACID SUPPLEMENTATION AS A MEANS OF IMPROVING THE QUALITY AND OVERCOMING SHORTAGE OF PROTEIN IN DEVELOPING COUNTRIES

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## Introduction

The diets consumed by a great majority of the human population in the developing countries are deficient in proteins and protein malnutrition is widely prevalent among weaned infants and young children.[1-3] The problem of providing adequate quantities of protein for the rapidly increasing population has been engaging the attention of research workers and international agencies.[4-6] Since the land available for cultivation is limited and the world population continues to increase at a rapid rate, the protein shortage is likely to become increasingly acute in the years to come.[7] However, recent developments in our knowledge of the amino acid composition and nutritive value of proteins have indicated possibilities of different methods of augmenting the protein supplies.[8,9] Of these one of the most promising is the amino acid supplementation of dietary proteins for enhancing their nutritive value.[10] Experiments with laboratory animals have shown that the nutritive value of several common dietary proteins can be increased 2 to 3 fold by supplementation with limiting amino acids.[11] Extensive studies carried out by many workers have shown that the primary limiting amino acids of the diets consumed in the developing countries are lysine and methionine while the secondary deficiencies are threonine and tryptophan. Methods for the large scale production at reasonable cost of lysine and methionine have been developed.[10,11] Amino acid fortification of certain dietary proteins with these two amino acids is now feasible on a large scale. The objective of this review is to briefly survey the following aspects of the problem: (1) amino acid composition and nutritive value of common dietary proteins, (2) improvement in the nutritive value of proteins by amino acid fortification, (3) large scale availability of amino acids for fortification and (4) improvement of proteins in poor dietaries by amino acid fortification.

## Amino acid composition and nutritive value of proteins

The nutritive value of proteins depends on their amino acid composition. Out of 22 amino acids commonly occurring in dietary proteins 10 amino acids have been found to be essential for the growth and maintenance of animals and human beings and these must be present in the diet in the proper proportions. Since the remaining 12 non-essential amino acids can spare the essential amino acids they must also be present in the dietary protein in required amounts.[8,9]

The essential amino acid composition of common foods has been determined by a large number of workers. The results have been summarised by Block & Weiss,[12] Kuppuswamy *et al*,[13] Orr and Watt[14] and Patwardhan and Ramachandran.[15] The data given in Table I are taken from the publication of Orr and Watt[14] and Patwardhan and Ramachandran.[15]

*Cereals*: Cereals contribute more than 50 per cent of proteins in the diets of low-income groups in almost all developing countries and hence form the most important sources of dietary proteins.[7,16] They contain moderate amounts of proteins varying from about 6–14% depending on the cereal.[16] The amino acid contents of different cereal proteins are given in Table I. The limiting amino acids in different

Table I. *Essential amino acid content of common food proteins (g/16g N)*

| Foodstuff | Botanical name | Arginine | Histidine | Isoleucine | Leucine | Lysine | Cystine | Methionine | Phenylalanine | Threonine | Tryptophan | Valine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| *Cereals and millets* | | | | | | | | | | | | |
| Rice | *Oryza sativa* | 10.3 | 1.6 | 6.0 | 8.0 | 3.9* | 1.8 | 2.2 | 4.6 | 3.3* | 1.2 | 6.1 |
| Wheat | *Triticum vulgare* | 5.2 | 1.5 | 3.5 | 6.5 | 2.2* | 2.2 | 1.2 | 5.3 | 2.6* | 1.1 | 4.0 |
| Kaffir corn | *Sorghum vulgare* | 6.8 | 1 6 | 6.1 | 12.9 | 3 4* | 1.8 | 1.6 | 5.1 | 3.9* | 1.2 | 5.9 |
| Oats | *Avena sterilis* | 6.1 | 1.7 | 4.8 | 7.0 | 3.4* | 2.0 | 1.4 | 5.0 | 3.1* | 1.2 | 5.6 |
| Pearl millet | *Pennisetum typhoideum* | 8.1 | 1.7 | 5.9 | 9.5 | 3.8* | 1.9 | 1.8 | 4.3 | 3 8* | 1.9 | 6.3 |
| Ragi | *Eleucine coracana* | 5.2 | 1 3 | 6.4 | 9.5 | 3.4* | 2.2 | 3.0 | 4.4 | 3.5* | 1.6 | 6.7 |
| Barley | *Hordeum vulgare* | 4.8 | 1.7 | 4.0 | 6.5 | 3.2* | 1.9 | 1.3 | 4.8 | 3.2* | 1.2 | 4.7 |
| Italian millet | *Setaria italica* | 3.6 | 2.1 | 7.6 | 16.7 | 2.2* | 1.8 | 2.8 | 6.7 | 3.1* | 1 0 | 6.9 |
| Samai | *Panicum miliare* | 4.7 | 1.9 | 6.7 | 10.9 | 1 8* | 1.7 | 2.3 | 4.8 | 3.4* | 0.6 | 6.1 |
| Corn(Maize) | *Zea mays* | 3.5 | 2.1 | 4.6 | 13.0 | 2 9* | 1.3 | 1.9 | 4.5 | 4.0* | 0.6 | 5.1 |
| Varagu | *Paspalum scrobiculatum* | 4.0 | 1.8 | 5.3 | 8.5 | 2.1* | 2.2 | 2.8 | 6.9 | 3.8* | 0.6 | 5.6 |
| Rajkeera | *Amaranthus paniculatus* | 14.8 | 2.9 | 6.9 | 8.0 | 8.2 | 2.2 | 2.5 | 4.8 | 4.4* | 0.9 | 6.1 |
| *Legumes* | | | | | | | | | | | | |
| Red gram | *Cajanus cajan* | 5.4 | 3.4 | 5.7 | 7.0 | 6.4 | 0.8* | 0.9* | 9.1 | 3.4 | 0.2* | 5.1 |
| Bengal gram | *Cicer arietinum* | 6.9 | 2.3 | 6.0 | 8.0 | 6.4 | 0.8* | 1.7 | 5.0 | 4 8 | 0.6* | 5.4 |
| Green gram | *Phaseolus radiatus* | 6.3 | 2.7 | 6.3 | 7 7 | 7.0 | 0.6* | 1.0* | 5.9 | 3.5 | 0.4* | 6.4 |
| Lentil | *Lens esculenta* | 7.0 | 2.1 | 5.4 | 5.4 | 5.8 | 0.8* | 0.8* | 4 1 | 2.9 | 0.3* | 5.4 |
| Black gram | *Phaseolus mungo* | 5.7 | 2.7 | 5.5 | 7.2 | 6.0 | 0 7* | 1.1* | 5.4 | 4.3 | 0.5* | 6.4 |
| Cow pea | *Vigna catiang* | 6.9 | 3.1 | 4.9 | 7.5 | 6.2 | 0.7* | 1.0* | 5.2 | 3.2 | 0.6* | 6.3 |
| Horse gram | *Dolichos biflorus* | 5.4 | 3.0 | 6.7 | 7.9 | 8.3 | 1.3* | 0.8* | 8.5 | 3.8 | 0.6* | 5.4 |
| Khesari dhal | *Lathyrus sativus* | 7.8 | 2.5 | 6.6 | 6.6 | 6.9 | 1.4* | 0.4* | 4.1 | 2.3 | 0.4* | 4.7 |
| Field bean | *Dolichos lablab* | 9.2 | 2.8 | 6.0 | 8.9 | 8.1 | 1.0* | 0.7* | 5.3 | 3.3 | 0.5* | 5.6 |
| Peas | *Pisum sativum* | 17.7 | 2 7 | 8.4 | 10.9 | 11.4 | 1.2* | 1.3* | 5.8 | 4.9 | 0.7* | 7.8 |
| *Roots and tubers* | | | | | | | | | | | | |
| Sweet potato | *Ipomeoa batatas* | 5.2 | 1.5 | 6.2 | 6.8 | 5.0 | 0.7* | 1.4* | 5.0 | 5.3 | 0.8* | 9.5 |
| Potato | *Solanum tuberosum* | 5.2 | 1.2 | 5.2 | 5.4 | 6.7 | 1.2* | 1.0* | 4,1 | 3.9 | 0.7* | 5.0 |
| Elephant yam | *Amorphophallus campanulatus* | 11.2 | 1.6 | 5.0 | 5.9 | 4.4 | 2.1* | 1.0* | 6.2 | 4.5 | 0.6* | 5.0 |
| Tapioca | *Manihot utilissima* | 7.7 | 1.5 | 5.3 | 5·6 | 6.2 | 0.9* | 0.6* | 3.5 | 3.8 | 0.5* | 4.5 |
| Colocasia | *Colocasia antiquorum* | 6.2 | 1.8 | 5.2 | 8.9 | 6.2 | 2.7* | 1.0* | 5.2 | 4.7 | 1.4 | 6.0 |
| Green plantain | *Musa paradisiaca* | 4.1 | 4.5 | 5.1 | 5.4 | 5.6 | 0.4* | 0.6* | 4.5 | 2.7 | 0.7* | 4.4 |
| *Oilseeds and nuts* | | | | | | | | | | | | |
| Peanut | | 10.7 | 2.4 | 4.1 | 5.1 | 3.6* | 1.5* | 0.9* | 5.1 | 2.7* | 1.1 | 5.0 |
| Soyabean | | 7.2 | 2.4 | 5.4 | 7.7 | 6.3 | 1.8* | 1.3* | 4.9 | 3.9 | 1.4 | 5.3 |
| Coconut | | 12.1 | 1.7 | 4.5 | 6.7 | 3.8* | 1.6* | 1.8* | 4.3 | 3.2 | 0.8 | 5.3 |
| Cottonseed | | 11.2 | 2.7 | 3.8 | 5.9 | 4.3* | 1.6* | 1.4* | 5.2 | 3.5 | 1.2 | 4.9 |
| Sesame | | 8.3 | 1.9 | 4.2 | 7.4 | 2 6* | 2.2 | 2.8 | 6.4 | 3.1 | 1.5 | 3.9 |
| Sunflower | | 8.7 | 2.2 | 4.7 | 6.4 | 3.2 | 1.7 | 1.6 | 4.5 | 3.4 | 1.3 | 5.0 |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Milk and egg* | | | | | | | | | | | | |
| Milk, cow | | 3.7 | 2.7 | 6.5 | 10.0 | 7 9 | 0.9* | 2.5* | 4.9 | 4.7 | 1.4 | 7.0 |
| Milk, human | | 4.1 | 2.2 | 5.5 | 9.1 | 6.6 | 2.0 | 2.4 | 4 4 | 4.5 | 1.7 | 6.3 |
| Milk, buffalo | | 3.3 | 2.1 | 5.0 | 10.2 | 8.1 | 1.4* | 2.7* | 4.3 | 5.2 | 1 4 | 6.2 |
| Milk, goat | | 2.2 | 2.0 | 5.2 | 8.7 | 6.3 | 0.7* | 2.2* | 4.4 | 4.3 | 1.3 | 5.8 |
| Egg, hen | | 6.6 | 2.4 | 4.6 | 8.8 | 6.4 | 2.3 | 3.1 | 5.8 | 5.0 | 1.7 | 7.4 |
| *Meat and fish* | | | | | | | | | | | | |
| Beef | | 6.5 | 3.5 | 5.2 | 8.2 | 8.7 | 1.3 | 2 5 | 4.1 | 4.4 | 1.2 | 5 6 |
| Lamb | | 6.5 | 2.8 | 5.2 | 7.7 | 8.1 | 1.3 | 2.4 | 4.1 | 4.6 | 1.3 | 4.9 |
| Pork | | 6.1 | 3.5 | 5.1 | 7.4 | 8.2 | 1.2 | 2.5 | 3 9 | 4.6 | 1.3 | 5.2 |
| Poultry (chicken muscle) | | 6.3 | 2.9 | 5.3 | 7.2 | 8.8 | 1.3 | 2.6 | 3 9 | 4.3 | 1.2 | 4.9 |
| Fish | | 5.6 | ... | 5.1 | 7.6 | 8.8 | 1.3 | 2.9 | 3.7 | 4.3 | 1.0 | 5.3 |
| *Miscellaneous* | | | | | | | | | | | | |
| Food yeast | | 7.2 | 2.7 | 7.2 | 8.0 | 7.9 | 0.9* | 1.5* | 5.1 | 5.0 | 1.4 | 6.3 |
| Chlorella | | 7.0 | 3.5 | 5.3 | 9.0 | 7.6 | 0.3* | 2.0* | 4.1 | 3.7 | 2.1 | 7.9 |

* Limiting amino acids

cereals are as follows: rice, lysine and threonine; wheat, lysine and threonine; maize, lysine, tryptophan and threonine; and millets, lysine and threonine. Among cereals and millets the proteins of oats, rice and barley possess higher nutritive value than wheat and millet proteins. The protein efficiency ratios of cereals and millets range widely from 0.5 to 2.4 depending on the grain and are very much less than that (4.3) of egg proteins.

*Legumes*: Legumes are good sources of protein. About 15–30% of the total proteins in average diets consumed by the low-income groups in developing countries may be derived from legumes.[15,17,18] Legume proteins are good sources of lysine and threonine but are deficient in sulphur amino acids and tryptophan. The nutritive value of several legume proteins is seriously affected by the growth inhibitors present in them.[19] Optimal heat processing has been shown to inactivate these and bring about an increase in the nutritive value of the proteins. The protein efficiency ratios of optimally heat processed legume proteins (at 10% level) range from 1.5 to 2.0 depending on the legume.

*Nuts and oilseeds*: Nuts and oilseeds form important potential sources of proteins for overcoming protein malnutrition in developing countries.[15,20] The most important of these are soya bean, groundnut, cottonseed, sesame seed, coconut and sunflower seed. Soya bean proteins are deficient in sulphur amino acids, cottonseed and coconut proteins in lysine and sulphur amino acids, while sesame and sunflower seed proteins are deficient in lysine. Groundnut proteins are deficient in lysine, methionine and threonine.[13] The protein efficiency ratios of nuts and oilseeds range from 1.5 to 2.3 depending on the material.[13]

*Roots and tubers*: The important roots and tubers consumed in the different developing countries are potato, yam (*Amorphophallus camapanulatus*), colocasia (*Colocasia esculenta*), sweet potato (*Ipomeoa batatas*) and tapioca or cassava (*Manihot esculenta*).[3–6] Among these potato, yam and colocasia are fair sources of proteins while sweet potato and tapioca are poor sources. Potato proteins possess fairly high nutritive value Data regarding the nutritive value of the proteins of yam and colocasia are not available. Potato proteins are deficient in sulphur amino acids.

*Egg and milk*: Among the dietary proteins egg proteins possess the highest nutritive value.[13] Milk forms the main source of proteins in the diets of infants and young children and milk proteins possess high protein efficiency ratio (2.8–3.3) though lower than that (4.0–4.5) of egg proteins. Milk proteins are deficient in sulphur amino acids.

*Meat and fish*: The proteins of meat and fish possess high nutritive value comparable to that of milk proteins. The limiting amino acids in fish and meat proteins are sulphur amino acids and tryptophan.

*Yeast and algae*: During recent years

considerable amount of work has been carried out on the possible uses of dried yeast and algae (*chlorella*) as protein supplements to human diets.[13] Both dried yeast and chlorella contain about 35 to 50% protein. The protein efficiency ratios have been found to range from 1.5 to 2.0 depending on the strain and cultural conditions. They are rich sources of lysine but are deficient in sulphur amino acids.

**Improvement in the nutritive value of proteins by amino acid supplementation**

Considerable amount of work has been carried out by various workers on the amino acid supplementation of dietary proteins.[21,22] The results of studies on amino acid supplementation of some common dietary proteins are summarised in Table II. A brief summary of the results as may be applicable to developing countries is presented here.

*Cereals*: Supplementation of the proteins of wheat and bread with lysine has been found to bring about a marked improvement in the protein efficiency ratio. Addition of threonine along with lysine brings about a further increase in the nutritive value of protein.[24] The protein efficiency ratio of rice proteins has been reported to improve when fortified with both lysine and threonine.[25,26] From the chemical analysis, one would not expect that threonine supplementation would be required. Since threonine is almost as limiting as lysine, it would appear that 40% of the threonine in the rice protein is not available for growth.[8] In the case of corn proteins a marked improvement in the protein efficiency ratio was observed when supplemented with both lysine and tryptophan.[27] Tang *et al*[28] reported that lysine supplementation improved the nutritive value of oats proteins and a further improvement was observed when methionine and threonine were added along with the lysine. Venkat Rao *et al*[29] found that lysine fortification improved markedly the protein efficiency ratio of sorghum, (*Sorghum vulgare*), pearl millet (*Pennisetum typhoideum*) and ragi (*Eleucine coracana*).

*Legumes*: When supplemented with methionine the nutritive value of legume proteins are known to improve to a marked extent.[30-36] For example the nutritive value of the proteins of Alaska field pea is increased almost to that of casein when supplemented with methionine. In the case of split pea, lentils and pigeon pea, addition of tryptophan and threonine along with methionine brought about a marked improvement in the nutritive value of the proteins.

*Oilseeds and nuts*: Supplementation with methionine increases markedly the protein efficiency ratio of both raw and processed soya bean.[37-39] The protein efficiency ratio of methionine fortified processed soya bean is nearly equal to that of milk proteins. Highly significant increases in the protein efficiency ratio of sesame proteins as a result of fortification with lysine have been reported.[40,40a] Lyman *et al*[41] reported that fortification of cottonseed meal with lysine increased the protein efficiency ratio to a significant extent. Supplementation of groundnut proteins with lysine, methionine and threonine increased the protein efficiency ratio to a marked extent.[42-44]

*Egg and milk*: Mitchell[45] reported that supplementation of egg proteins (at 9% level) with lysine brought about a slight but significant increase in its growth promoting value but Venkat Rao *et al*[46] did not observe any supplementary effect of lysine to egg proteins (at 10% level). Fortification of milk proteins with methionine produced a marked increase in the nutritive value of milk proteins.[47,48]

*Yeast and chlorella*: Fortification of food yeast and chlorella with methionine brings about a marked increase in the protein efficiency ratios.[48a, 48b]

*Blends of protein-rich foods*: Panemangalore *et al*[49] reported that fortification of a protein food based on a 1:1 blend of soya flour and groundnut flour with lysine and methionine increased the protein efficiency ratio to a highly significant extent. The protein efficiency ratio of protein foods based on 2:2:1 blend of groundnut flour, Bengal gram flour and sesame flour or 4:3:3 blend of groundnut flour, soya bean flour and seame flour increased to a significant extent when supplemented with lysine and methionine.[50]

here. Bressani *et al*[54] have shown that lysine supplementation caused a marked increase in nitrogen retention in all of 6 preschool children who had recovered from severe protein malnutrition and were receiving daily 2g protein/kg body weight in the form of wheat flour. In some of the children, the retention approximated that obtained with milk protein. Rice *et al*[55] have carried out nitrogen balance experiments with college students receiving 95% of their protein from white bread containing 4% milk solids. These authors found statistically significant increases in nitrogen retention with lysine-supplementation on intakes of 0.7g and 1.0g protein/kg/day. This experiment proves that lysine supplementation of the diet based entirely on wheat flour will improve nitrogen retention even in the case of young adults. Scrimshaw *et al*[56] and Bressani *et al*[57] studied the effects of supplementation of corn masa diet fed to young children with lysine and tryptophan and found a marked increase in N retention on protein intakes of 1.5 to 3.0 g per kilogram per day. Truswell and Brock[58] have reported that supplementation of maize diets consumed by adults on protein intakes of 0.5 to 1.0g/kg/day with lysine, tryptophan and isoleucine resulted in a significant increase in nitrogen retention. Gomez and associates[59] reported that supplementation of corn-bean diet consumed by preschool children with lysine and tryptophan caused considerable increase in nitrogen retention. King *et al*[60] and Laughlin *et al*[60] have reported that lysine supplementation of the diets of growth-retarded Haitian children 6 to 16 years of age increased their growth rate. Parthasarathy *et al*[61] found that supplementation of a poor Indian rice diet with lysine, methionine and threonine resulted in a highly significant increase in the net protein utilisation in children aged 8-9 years. When a similar rice diet was supplemented with methionine fortified soya flour, the nitrogen retention increased almost to the same extent as that observed with an equivalent amount of milk proteins[62] Parathasarathy *et al*[63] reported that nitrogen retention in children aged 8–9 years receiving daily 1g. soya bean protein per kilogram body weight markedly increased on supplementation with methionine. Doraiswamy *et al*[64]and Parthasarathy *et al*[65] found that supplementation of a poor Indian rice diet consumed by school children (8–9 years) with a protein food based on 1:1 blend of groundnut flour and soya flour fortified with lysine and methionine brought about considerable improvement in their growth and nitrogen retention.

In many developing countries, the supplies of protein-rich foods of high nutritive value for supplementing the low protein basal diets of the population are highly inadequate.[7] In such cases improvement in the amino acid balance in the dietary proteins can be achieved by fortification with the limiting amino acids.[8] A number of typical examples are given in Table III based on studies with children. An increase in nitrogen retention means better utilisation of dietary proteins and an increase in the amount of body tissue. In the case of children better growth has also been observed as a result of such supplementation. The results so far obtained have indicated that amino acid fortification provides an effective means of improving the nutritive value of available protein supplies in the developing countries. In view of the widespread occurrence of protein malnutrition in these regions,there is urgent need to fortify protein blends based on locally available protein-rich foods such as oilseed meals and legumes with lysine and methionine for enhancing their nutritive value and distribute these foods for use as supplements to the diets of weaned infants, young children and expectant and nursing mothers.

Table III. *Effect of amino acid supplementation of certain dietary proteins on the retention of nitrogen by children*

| Diet | Age and sex | Nitrogen intake mg/kg/day | Nitrogen retention mg/kg/day | Reference no. |
|---|---|---|---|---|
| Wheat diet | Boy, 2 years 2 months | 295 | 3.0 | 54 |
| ,, + lysine (2.4g/16gN) | Boy, 2 years 2 months | 323 | 39.5 | 54 |
| Corn-masa diet | Boy, 3 years 7 months | 449 | —3.0 | 56 |
| ,, + lysine (3.9g/16gN) and tryptophan (1.2g/16gN) | Boy, 3 years 7 months | 450 | 69.0 | 56 |
| Corn-masa diet | Boy, 4 years 9 months | 241 | —17.5 | 57 |
| ,, + lysine (3.9g/16gN) and tryptophan (1.2g/16gN) | Boy, 4 years 9 months | 239 | 29.5 | 57 |
| Rice diet | Girls, 8-9 years | 207 | 17.6 | 61 |
| ,, + lysine (3.5g/16gN) methionine (1.2g/16gN) and threonine (0.9g/16gN) | Girls, 8-9 years | 217 | 50.1 | 61 |
| Processed soya flour | Girls, 8-9 years | 190 | 15.1 | 63 |
| ,, + methionine (1.2g/16gN) | Girls, 8-9 years | 200 | 43.5 | 63 |

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