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MUTUAL AND AMINO ACID SUPPLEMENTATION OF PROTEINS III. THE NUTRITIVE VALUE OF BLENDS OF SOYABEAN, SESAME AND MILK PROTEINS FORTIFIED WITH LIMITING AMINO ACIDS

V. A. DANIEL, R. LEELA, K. HARIHARAN, S. VENKAT RAO, D. RAJALAKSHMI, M. SWAMINATHAN AND H. A. B. PARPIA
Central Food Technological Research Institute, Mysore

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In view of the acute shortage in milk production in India and other developing countries,[1] investigations have been carried out by several workers on the preparation of dried milk substitutes suitable for feeding infants.[2-7] Infant foods based on oilseeds or blends of oilseeds and nuts should contain proteins of high nutritive value, approaching that of milk proteins. Available evidence would indicate[8] that the protein efficiency ratio of cow's milk proteins is about 3.1 while those of 1 : 1 blend of egg proteins and cow's milk proteins (similar in amino acid composition to that of human milk proteins) and of whole egg proteins about 4.3 and 4.7 respectively. Since groundnut proteins are limiting in three essential amino acids, viz., lysine, methionine and threonine and possess a medium PER (1.7) they are not suitable for use as the main protein source in infant foods.[9] Soya bean proteins are limiting only in methionine and the PER's of soya proteins and soya proteins fortified with methionine have been found to be 2.0 and 2.9 respectively.[10] It is evident that soya bean fortified with methionine would be a suitable raw material for the preparation of infant foods. Sesame proteins are rich in sulphur amino acids and a 2 : 1 blend of soya and sesame proteins has been reported to possess a higher PER than soya or sesame proteins.[11] Moreover it may be possible to effect further improvements in the nutritive value of infant foods, based on soya and sesame by incorporating a small percentage of skim milk powder as a source of lactose, proteins of high nutritive value and other nutrients and also by fortification with l-lysine or dl-methionine. It was therefore considered of importance to determine the nutritive value of the proteins of blends of soya, sesame and skim milk powder fortified with certain limiting essential amino acids as such blends will be suitable for feeding infants in developing countries.

Experimental

Materials: Processed soya bean meal and sesame flour were prepared according to Narayana Rao *et al.*[12] and Subrahmanyan *et al.*[13]

Protein blends: Three protein blends were prepared consisting of: (1) soya and sesame (ratio of proteins, 2 : 1), (2) soya, sesame and skim milk powder (ratio of proteins, 1 : 1 : 1) and (3) soya and skim milk powder (ratio of proteins, 2 : 1). The composition of the different protein blends is given in Table I.

Fortification with amino acids: L-lysine monohydrochloride (E. Merck), dl-methionine (E. Merck) and dl-threonine (AEC, France) were used for the fortification of the proteins, The fortified protein blends contained 6.6g/16gN of lysine, 5.4g/16gN of the sulphur amino acids and 5.0/16gN of threonine. Twice the quantity of dl-threonine was added as this has only half the biological activity of l-threonine.

Methods: *Essential amino acid composition of protein blends*: Lysine, methionine, cystine and threonine contents of the oilseed meals and skim milk powder were determined by microbiological methods.[14] The contents of these amino acids in the protein blends, calculated from the above data, are given in Table I.

Protein efficiency ratio: The protein efficiency ratio (PER) of the different protein

blends were determined by the method of Osborne *et al.*[15] Freshly weaned male albino rats (21 days old) from the laboratory stock colony (Wistar strain) were allotted to different groups (8 per group) in a randomised block design. The composition of the experimental diets and the procedure followed for the preparation of the diets and the feeding of the experimental animals were similar to those used by Panemangalore *et al.*[16] The rats were weighed weekly and records of individual food intake of rats were maintained. The protein efficiency ratios calculated from the above data, are given in Table II.

Results

PER of protein blends: The mean PER's of protein blends I to III were 2.70, 3.34 and 2.66 and were significantly higher than that of soya proteins. The PER of blend II (3.34) was significantly higher than that of milk proteins (3.08)

PER of protein blends fortified with limiting amino acids: PER of protein blends I and II fortified with lysine and methionine were 3 30 and 3.49 and that of blend III fortified with methionine was 3.78. These values were significantly greater than that obtained for milk proteins (3.08).

Discussion

The object of the present investigation was to determine the PER of protein blends containing proteins of high nutritive value and suitable for the preparation of infant foods in developing countries. The results reported here have shown that protein foods based on blends of soya, sesame and skim milk powder possess high PER's ranging from 2 66-3 34. Fortification of these blends with l-lysine and/or dl-methionine brought about significant increase in the PER's (3.30 to 3.78). It may be concluded that blends of soya, sesame and skim milk powder (with or without fortification with l-lysine and dl-methionine) containing proteins of high nutritive value will be suitable for the preparation of infant food formulations for feeding infants in the developing countries.

Summary

1) The protein efficiency ratio of three protein blends (Protein blend I: 64 parts of soya flour and 36 parts of sesame flour; Protein blend II: 28 parts of soya flour, 32 parts of sesame flour and 40 parts of skim milk powder; and Protein blend III: 58 parts of soya flour and 42 parts of skim

Table I. *Protein, lysine, methionine, cystine and threonine contents of the unfortified and amino acid fortified protein blends as compared with those of cow's milk (g/16gN)*

Blend No.	Ingredients	Protein (Nx 6.25) %	Lysine	Methionine	Cystine	Total sulphur amino acids	Threonine
1	Soya flour	49.5	6.3	1.4	1.7	3.1	4.0
2	,, +dl-methionine (2.39g/16gN)	,,	6.3	3.7	1.7	5.4	3.2
3	Sesame flour	44.0	2.7	2.8	2.3	5 1	3.2
4	,, +l-lysine (4.0g/16gN)	,,	6.7	2.8	2.3	5.1	3.2
5	Soya flour 64% + sesame flour 36% (Blend I)	47.5	5.1	1.9	1 9	3.8	3.8
6	Blend I + l-lysine (1.5g/16gN)	,,	6.6	1.9	1.9	3.8	3.8
7	,, ,, +dl-methionine (1.7g/16gN)	,,	6.6	3.6	1.9	5.5	3.8
8	,, ,, +dl-threonine (2.8g/16gN)	,,	6.6	3.6	1.9	5.5	5.2
9	Soya flour 28%+sesame flour 32%+skim milk powder 40% (Blend II)	42.0	5.7	2.1	1.7	3.8	4.0
10	Blend II+lysine (1 0g/16gN)+dl-methionine (1.6g/16gN)	,,	6.7	3.7	1.7	5.4	4.0
11	,, dl-threonine (1.1g/16gN)	,,	6.7	3.7	1.7	5.4	5.1
12	Soya flour 58%+skim milk powder 42% (Blend III)	43.3	5.9	1.6	1.5	3.1	4.3
13	Blend III+dl-methionine (2.2g/16gN)	,,	6.9	3.8	1.5	5.3	4.3
14	Skim milk powder,	34.7	8.1	2.0	1.1	3.1	4.8
15	,, +dl-methionine (2.3g/16gN)	,,	8.1	4.3	1.1	5.4	4.8

Table II. *Protein efficiency ratios of unfortified and amino acid fortified protein blends as compared with skim milk powder*

(Level of protein in the diet, 10% ; 8 male rats per group ; duration of experiment 4 weeks)

Group No.	Source of protein in the diets	Initial body weight (g)	Gain in body weight (g)	Protein intake (g)	PER
1	Soya flour	37.0	51.4	24.0	2.14
2	,, +methionine	36.9	89.2	29.0	3.07
3	Sesame flour	36.9	39.5	21.9	1.80
4	,, +lysine	36.9	101.8	34.4	2.96
5	Soya flour 64+sesame flour 36 (Blend I)	36.9	82.9	30.7	2.70
6	Blend I+lysine	37.0	84.1	28.6	2.93
7	,, ,, +methionine	36.9	97.1	29.4	3.30
8	,, ,, +threonine	37.0	113.1	31.5	3.59
9	Soya flour 28%+Sesame flour 32%+skim milk powder 40% (Blend II)	37.0	105.0	31.4	3.34
10	Blend II+lysine+methionine	36.8	107.4	30.8	3.49
11	,, ,, +threonine	37.0	111.2	30.4	3.66
12	Soya flour 58%+skim milk powder 42% (Blend III)	36.9	75.5	28.3	2.66
13	Blend III+methionine	37.0	107.6	28.5	3.78
14	Skim milk powder	36.8	89.8	29.3	3.08
15	Skim milk powder+methionine	36.9	104.9	27.7	3.78
	Standard error of the mean (98 d.f.)				±0.06

*Results of test of significance (at 5% level) by Duncan's multiple range test**

Group No.	III	I	XII	V	VI	IV	II	XIV	VII	IX	X	VIII	XI	XIII	XV
Means	1.80	2.14	2.60	2.70	2 93	2.96	3.07	3.08	3.30	3.34	3.49	3.59	3.66	3.78	3.78

Note : Any two means not underscored by the same line are significantly different
Any two means underscored by the same line are not significantly different.

* D. B. Duncan, *Biometrics*, Vol. 2, No 1. 1955.

milk powder), determined at 10% level of protein over a period of 4 weeks were 2.70, 3.34 and 2.66 respectively, as compared with 2.14 for soya proteins and 3.08 for milk proteins.

2) Fortification of protein blends I and II with l-lysine and dl-methionine and of of protein blend III with dl-methionine brought about an increase in the PERs to 3.30, 3.49 and 3.78 as compared with values of 3.07 and 3.78 respectively obtained for soya flour and skim milk powder fortified with dl-methionine.

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REFERENCES

1. FAO *Production Year Book* (1958) Vol. 12 Food and Agricultural Organisation, Rome.
2. ICMR Spl. Report No. 31 '*Milk substitutes of Vegetable origin*' (1955) Indian Council of Medical Research, New Delhi.
3. Dean, R. F. A. (1953) *Plant Protein in child feeding, Spl. Rep. Ser. No. 279* Medical Research Council, London.
4. Meyer, H. F. (1960) *Infant Foods and Feeding Practices* C. C. Thomas, Publisher, Illinois, U. S. A.
5. Shurpalekar, S. R., Chandrasekhara, M. R., Lahiry, N. L., Swaminathan, M., Indiramma, K. and Subrahmanyan, V. (1960), *Ann. Biochem. Exp. Med.* **20**, 145.
6. Shurpalekar, S. R., Chandrasekhara, M. R., Korula, S., Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V. (1964). *Food Tech.*, **18**, 898.
7. Teply, L. J. and Gyorgy, P. (1962), *J. Pediat* **61**, 925.
8. Venkat Rao, S., Daniel, V. A., Joseph A. A. Sankaran, A. N. and Swaminathan, M., (1964), *J. Nutr , Dietet.* **1**. 103.
9. Tasker. P. K., Joseph, K., Rajagopalan, R., Narayana Rao, M., Indiramma, K., Swaminathan, M., Sreenivasan, A. and Subrahmanyan V (1963). *Ann. Biochem. Exp. Med.* **23**, 279.

10. Parthasarathy, H. N., Joseph K., Narayana Rao, M., Swaminathan, M., Sankaran, A. N., Sreenivasan, A. and Subrahmanyan, V. (1964), J. *Nutr. Dietet.* **1**, 14.

11. Shurpalekar, S. R., Chandrasekhar, M. R., Lahiry, N.L., Swaminathan, M., Indiramma, K., and Subrahmanyan, V. (1961), *Ann. Biochem. Exp. Med.*, **21**, 143.

12. Narayana Rao, M., Ananthachar, T. K., Kurup, K. R., Rajagopalan, R, Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V. (1964), J. *Nutr. Dietet.* **1**, 1.

13. Subrahmanyan, V., Rama Rao, G., Kuppuswamy, S., Narayana Rao, M. and Swaminathan, M. (1957), *Food Science*, **6**, 84.

14. Barton-Wright, E. C. (1952), *Microbiological Assay of vitamin B-complex and amino acids*, Sir Issac Pitman & Sons, Ltd., London.

15. Osborne, T. B., Mendel, L.B. and Ferry, E. L. (1919), J. *Biol. Chem.*, **37**, 223.

16. Panemangalore, M., Balaji Rao, M., Narayana Rao, M., Rajagopalan, R., Chandrasekhara, B S., Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V., (1964), J. *Nutr. Dietet.* **1**, 4.