J. Nutr. Dietet. (1964), **1**, 153

THE SUPPLEMENTARY VALUE OF LOW-FAT GROUNDNUT FLOUR AND GROUNDNUT PROTEIN ISOLATE FORTIFIED WITH CERTAIN VITAMINS AND CALCIUM TO POOR VEGETARIAN RICE DIET

J. V. PRABHAKAR, KANTHA JOSEPH, M. NARAYANA RAO AND M. SWAMINATHAN
Central Food Technological Research Institute, Mysore

(*Received 10 May 1964*)

The diets consumed by the low-income groups of the population in India and several Asian countries, Africa and Latin America, consist predominantly of cereals, roots and tubers and contain negligible amounts of protective and protein-rich foods.[1] Such diets are deficient in protein, certain vitamins and minerals. Consequently there is wide spread incidence of protein malnutrition and vitamin deficiency diseases particularly among children.[2-4] In view of the scarcity of milk and other protein-rich foods of animal origin in the countries mentioned above, the possibility of utilising protein-rich plant foods viz., oilseed meals and legumes as supplements to the diets of children and for the treatment of protein malnutrition has been investigated by several workers.[5-10] Aykroyd and Krishnan[11] reported that the addition of milk powder (full fat or skimmed) at 4-6% level to a poor rice diet, produced a remarkable improvement in the nutritive value of the diet, while addition of soya bean, produced only a slight improvement, as judged by the growth of albino rats and also of children. They concluded after an elaborate study[12] that poor Indian rice diet is deficient in calcium, vitamin A, certain vitamins of the B-complex and protein and the high supplementary value of milk is due to the fact that it is a rich source of all the above nutrients. Kuppuswamy *et al.*,[13] reported that supplementation of poor rice diet with groundnut flour did not cause any increase in the growth promoting value of the diet.

The need for the fortification of protein-rich foods so as to make them more balanced and suitable for making up the common deficiencies in the dietaries of low-income groups of the population has also been pointed out by some other workers.[9, 10] The present paper describes the results of investigations on the supplementary value of low-fat groundnut flour and groundnut protein isolate (with or without fortification with certain vitamins and calcium) to poor vegetarian rice diets.

Experimental

Materials: Groundnut flour was prepared according to Subrahmanyan *et al.*[9] Groundnut protein isolate was prepared from screw pressed groundnut cake according to Anantharaman *et al.*[14]

Animal Experiments: The experiments were conducted in two series. In the first series the supplementary value of groundnut flour (with or without fortification with calcium and vitamins) at 12.5% level to poor vegetarian rice diet was determined by growth experiments on albino rats. In the second series, the supplementary value of groundnut protein isolate (with or without fortification with calcium and vitamins) at 7.3% level to poor vegetarian rice diet was studied. The groundnut flour and groundnut protein isolate supplied about 6.3g of extra protein per 100 g of the diet. The composition of the diets is given in Table I.

Groups of freshly weaned albino rats weighing between 40-45g (ten in each group and distributed according to randomized block design ignoring litters) received the diets *ad lib.* A similar group of weanling rats was sacrificed at the start of the experiment for determining the initial protein content of the carcass. The methods of preparation of the diets and of feeding the experimental animals were the same as those described in an earlier paper.[15] The animals were weighed weekly.

Table I. *Percentage composition of experimental diets*

	Poor rice diet (control)	Control + groundnut flour	Control + groundnut flour + calcium	Control + groundnut flour + calcium and vitamins	Control + groundnut protein isolate	Control + groundnut protein isolate + calcium	Control + groundnut protein + calcium and vitamins
Rice flour	78.5	66.0	66.0	66.0	71.2	71.2	71.2
Turdhal (*cajanus cajan*)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Leafy vegetables (*Amaranthus gangeticus*)	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Non-leafy vegetables (potato and brinjal) (*Solanum melongena*)	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Skim milk powder	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Common salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Groundnut oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Groundnut flour*	—	12.5	12.5	12.5	—	—	—
Groundnut protein isolate*	—	—	—	—	7.3	7.3	7.3
Calcium carbonate	—	—	0.3	0.3	—	0.3	0.3
Vitamin A (I. U.)	—	—	—	375	—	—	375
Vitamin D (I. U.)	—	—	—	38	—	—	38
Thiamine (mg)	—	—	—	0.19	—	—	0.19
Riboflavin (mg)	—	—	—	0.38	—	—	0.38

* Protein content (N×6.25) of groundnut flour was 50.7% and of groundnut protein isolate 87.0%

At the end of 8 weeks feeding, the haemoglobin concentration and red-blood cell count were determined in blood drawn from the tail according to the methods used by Joseph *et al.*[16] The animals were then sacrificed and the livers were removed. A portion of the left lobe of the liver was taken for histological examination according to Joseph *et al.*[16] The livers and carcasses were analysed for moisture, protein and fat according to Joseph *et al.*[16] For determining the quantity of protein retained by the different groups of rats, the initial protein content of the carcass of different groups of rats was calculated using the mean data obtained for a similar group of weanling rats at the beginning of the experiment. The results are presented in Tables II-IV.

Results

Growth rate : Table II shows that supplementation of a poor rice diet with groundnut flour or groundnut protein isolate to provide about 6.3% extra protein, did not produce any significient increase in the growth of rats. On the other hand, incorporation of calcium carbonate or calcium carbonate and certain vitamins along with groundnut flour or groundnut protein isolate caused a highly significant increase in the growth of rats.

Composition of blood and liver : Table II shows that supplementation of the poor rice diet with groundnut flour or groundnut protein isolate (with or without fortification with calcium or vitamins) produced a significant increase in the haemoglobin or red blood cell count as compared with the control group. The results given in Table III show that there was no significant difference in the mean moisture, protein and fat contents of the livers of animals fed on the different diets.

Composition of carcass and retention of protein : Table III shows that there was no significant difference in the protein content per 100g body weight between the control and the experimental groups receiving the protein supplements. The results presented in Table IV show that incorporation of calcium carbonate or calcium carbonate and vitamins along with groundnut flour or groundnut protein isolate caused a significant increase in the retention of protein over that observed in rats receiving unfortified groundnut flour or groundnut protein isolate.

Histological structure of liver : The livers of animals fed on poor rice diet showed a mild degree of parenchymal damage of the protein deficiency type.[17] No appreciable fatty change or necrosis of the liver cells was observed. The livers of rats receiving a

Table II *Effect of supplementing rice diet with groundnut flour or groundnut protein isolate (with and without fortification with calcium and vitamins) on the growth and composition of blood of albino rats*
(Mean values for 5 males and 5 females in each group ; duration of experiment : 8 weeks)

Diet	Protein content of diet* (%)	Initial body weight (g)	Diet intake* (g/day)	Weekly gain in body wt (g)	Haemo-globin (%)	RBC Count (10^6/cu. mm)
Series I :						
Poor rice diet (control)	8.4	42.3	7.1	5.6	13.5	7.3
Control + groundnut flour	15.0	42.2	7.3	6.5 (24df)	14.3 (24df)	7.7 (24df)
Control + groundnut flour + calcium	15 0	41.9	7.9	8.4 ±0.40	14.9 ±0.21	8.0 ±0.15
Control + groundnut flour + calcium + vitamins	15.0	42.1	8.4	12.3	15.3	8.4
Critical difference at 5% level				1.18	0.62	0.44
,, 1% ,,				1.60	0.84	0.59
,, 0.1% ,,				2.14	1.12	0.79
Series II :						
Poor rice diet (control)	8.4	45.5	6.7	5.7	13.6	7.3
Control + groundnut protein	15.0	45.6	6.5	6.0 (24df)	14.4 (24df)	7.6 (24 df)
Control + groundnut protein + calcium	15.0	45.6	6.5	7.9 ±0.03	14.7 ±0.17	7.6 ±0.18
Control + groundnut protein + calcium + vitamins	15.0	45.6	6.9	10 9	15.7	8.2
Critical difference at 5% level				0.83	0.52	0.50
,, 1% ,,				1.12	0.70	0.67
,, 0.1% ,,				1.50	0.94	0.90

* On dry weight basis

Table III. *Effect of supplementing rice diet with groundnut flour or groundnut protein isolate (with and without fortification with calcium and vitamins) on the chemical composition of liver and the carcass of albino rats*
(Mean values for 5 males in each group)

Diet	Body weight (g)	Weight of liver (g)	Composition of liver			Composition of carcass		
			Mois-ture (%)	Protein (%)	Fat (%)	Mois-ture (%)	Protein (%)	Fat (%)
Series I :								
Poor rice diet (control)	87.4	3.8	72.4	17.3	3.75	60.7	17.8	16 4
Control + groundnut flour	93.9	4.9	71.5 (12 df)	18.0 (12 df)	3.67 (12 df)	58.6 (12 df)	17.8 (12 df)	17.6 (12 df)
Control + groundnut flour + calcium	109.2	4.8	71.7 ± 0.40	18.8 ± 0.22	3.73 ± 0.12	58.2 ± 0.38	17.9 ± 0.46	18.4 ± 0.45
Control + groundnut flour + calcium + vitamins	140.1	6.0	70.8	18.7	3.89	58.0	18.2	19.1
Critical difference at 5% level			1.23	0.66	0,36	1.2	1.4	1.5
,, 1% ,,			1.73	0,93	0.51	1.7	2.0	2.1
,, 0.1% ,,			2.44	1.31	0.72	2.3	2.8	2.7
Series II :								
Poor rice diet (control)	93.7	3.7	69.7	18.5	3.95	60.6	18-1	16.4
Control + groundnut protein	90.9	3.8	69.6 (12 df)	19.1 (12 df)	3.99 (12df)	58.8 (12 df)	18.3 (12	17.7 (12 df)
Control + groundnut protein + calcium	109.0	4.6	69.2 ± 0.49	19.8 ± 0.53	3.98 ± 0.70	58.3 ± 0.77	18.6 ± 0.30	18 3 ± 0.40
Control + groundnut protein + calcium + vitamins	132.4	5.2	69.2	19 9	4.07	56.9	18.8	19 0
Critical difference at 5% level			1.50	1.64	0.36	2.00	0.83	1.07
,, 1% ,,			2 11	2.30	0.50	2.81	1.16	1 50
,, 0.1% ,,			2.98	3.61	0.71	3,97	1.64	2.12

Table IV. *Intake and retention of protein in the different groups of rats receiving supplements of groundnut flour or groundnut protein isolate (with or without fortification with calcium and vitamins)*
(Mean values for 5 males in each group; duration of experiment : 8 weeks)

Diet	Total protein intake (g)	Initial body protein (g)	Final body protein (g)	Protein retained (g)	Protein retained (as % of intake)	
Series I :						
Control + groundnut flour	65.9	7.38	16.71	9.33	14.2	±0.13 (8df)
Control + groundnut flour + calcium	70.6	7.33	19.54	12.21	17.3	
Control + groundnut flour + calcium + vitamins	76.4	7.37	25.50	18.13	23.7	
		Critical difference at 5% level			0.43	
		,, 1% ,,			0.62	
		,, 0.1% ,,			0.93	
Series II .						
Control + groundnut protein	58.6	7.98	17.84	9.86	16.8	±0.27 (8df)
Control + groundnut protein + calcium	58 9	7.98	22.46	14.48	24.6	
Control + groundnut protein + calcium + vitamins	62.5	8.02	25.80	17.78	28.5	
		Critical difference at 5% level			0.87	
		,, 1% ,,			1.47	
		,, 0.1% ,,			1.90	

supplement of groundnut flour or groundnut protein isolate (with or without fortification with calcium and vitamins) were quite normal, indicating thereby that addition of 6.3% extra groundnut protein was effective in correcting the protein deficiency in the poor rice diet.

Discussion

The results obtained in the present investigation have shown that groundnut flour or groundnut protein isolate fortified with calcium carbonate and vitamins A,D, thiamine and riboflavin form an excellent supplement to a poor vegetarian rice diet. The livers of animals receiving the groundnut flour or groundnut protein isolate had normal appearance, while those of the animals fed on the poor rice diet showed mild parenchymal damage.

It is also of interest to note that incorporation of unfortified groundnut flour or groundnut protein isolate in poor rice diet did not produce any significant increase in the growth of rats. These results confirm the earlier observations of Kuppuswamy *et al.*[13] Aykroyd and Krishnan[11] made the interesting observation that the addition of a pure protein like casein to poor rice diet was only slightly effective while addition of skim milk powder was highly effective in increasing the growth of rats. It may be concluded from the results obtained in the present investigation that for obtaining the maximum supplementary value, it is necessary to fortify protein rich plant foods and protein isolates with essential vitamins and minerals in which the diets consumed by the low-income groups of the population in the different developing countries are deficient.

Summary

1. The supplementary value of low-fat groundnut flour and groundnut protein isolate (with or without fortification with calcium, vitamins A and D, thiamine and riboflavin) to poor vegetarian rice diet has been studied using albino rats.

2. Unfortified groundnut flour or groundnut protein isolate, when incorporated at 12.5% and 7.3% levels respectively in the poor rice diet (providing about 6.3% extra protein) did not cause any significant increase in the growth of rats. On the other hand, incorporation of groundnut flour or groundnut protein isolate fortified with calcium and vitamins produced a highly significant ($P<0.001$) increase in

the growth of rats and also in the percentage retention of the ingested dietary protein.

3. The livers of animals fed on the poor rice diet showed parenchymal damage of a mild protein deficiency type; the livers of animals receiving the groundnut flour or groundnut protein isolate had, however, a normal histological appearance indicating thereby that the two supplements were effective in correcting the protein deficiency in the poor rice diet.

REFERENCES

1. Food and Agriculture Organisation of the United Nations. (1962). *Year Book of Food & Agricultural Statistics*, Vol. 16, FAO, Rome.
2. Trowell, H. C., Davies J. N. P. and Dean, R. F. A. (1954), *Kwashiorkor*, Edward Arnold Publishers, London.
3. Brock, J. F. and Autret, M. (1952), *Kwashiorkor in Africa*, FAO Nutrition Studies No. 8, FAO, Rome.
4. Waterlow, J. C. and Vergara, A. (1956), *Protein malnutrition in Brazil*, FAO Nutrition Studies No. 14, FAO, Rome.
5. Dean, R.F.A. (1952), *Brit. Med. J.*, **2**, 791.
6. Dean, R. F. A. (1953), *Plant Proteins in child feeding*, Spec. Rep. Ser. Med. Res. Coun. No. 279, London.
7. Autret, M. and Van Veen, A. G. (1955), *Amer. J. Clin. Nutr.*, **3**, 234.
8. Venkatachalam, P. S., Srikantiah, S. G. and Gopalan, C. (1954), *Indian J. Med. Res.*, **42**, 555.
9. Subrahmanyan, V., Rama Rao, G., Kuppuswamy, S., Narayana Rao, M. and Swaminathan, M. (1957), *Food Science*, **6**, 76.
10. Scrimshaw, N. S. (1959), *J. Amer. Dietet. Assoc.*, **35**, 441.
11. Aykroyd, W. R. and Krishnan, B. G. (1936-37), *Indian J. Med. Res.*, **24**, 1093.
12. Aykroyd, W. R. and Krishnan, B. G. (1937-38), *Indian J. Med. Res.*, **25**, 367.
13. Kuppuswamy, S., Giri, K. V. and Subrahmanyan, V. (1949), *J. Amer. Dietet. Assoc.*, **35**, 441.
14. Anantharaman, K., Subramanian. N., Bhatia, D. S. and Subrahmanyan, V. (1959), *Indian Oilseeds J.*, **3**, 85.
15. Subrahmanyan, V., Murthy, H. B. N. and Swaminathan, M. (1954), *Brit. J. Nutr.*, **8**, 1.
16. Joseph, K., Narayana Rao, M., Swaminathan, M., Sankaran, A. N., Paul Jayaraj, A. and Subrahmanyan, V. (1962), *Brit. J. Nutr.*, **16**, 49.
17. Sriramachari, S., Ramalingaswamy, V. and Patwardhan, V. N. (1958), *Brit. J. Nutr.*, **12**, 8.