The Supplementary Value of a Low-Cost Protein Food Based on a Blend of Peanut, Coconut, and Chickpea (Cicer arietinum) Flours to a Maize-Tapioca Diet

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The supplementary value of a low-cost protein food—consisting of a blend of low fat peanut meal (50 parts), low-fat coconut meal (25 parts), and chickpea flour (25 parts) and fortified with thiamine, riboflavin, vitamins A and D, and calcium salts—when added to a maize-tapioca diet has been studied. When incorporated at 30% level in the diet (providing about 9% of extra protein in the diet), the protein food was as effective in promoting growth of rats as an equivalent amount of skim milk powder. There was no significant difference in protein content of the carcass or liver of rats receiving protein food, or skim milk powder. Comparison of livers from animals fed on maize-tapioca, protein food or skim milk powder diets, respectively, showed that both supplements were equally effective in correcting protein deficiency in the diet and in preventing liver damage. In many of the criteria employed, supplementation with skim milk powder produced significantly better results than the low-cost protein food mixture.

Diets consumed by the majority of children in India and other Asian countries, Africa, and Latin America are based largely on cereals, roots, and tubers and contain negligible amounts of protein-rich protective foods. Such diets are deficient in proteins, certain vitamins, and minerals (*2, 4, 8, 27*). Consequently, malnutrition and undernutrition are widely prevalent among vulnerable sections of the population. Incidence of protein malnutrition is particularly high among young children subsisting on maize-tapioca diets (*26*).

The problem of providing low cost nutritious food supplements to the diets of children has been engaging the attention of nutrition workers in several countries and also of certain international organizations such as the Food and Agricultural Organization (FAO), World Health Organization (WHO), and United Nations International Childrens' Emergency Fund (UNICEF) (*3, 27*). The most important protein-rich foods available in different underdeveloped countries include various oilseed meals and legumes. Investigations carried out by several workers have shown that protein foods based on blends of different oilseed meals and legumes can be used in treatment and prevention of protein malnutrition in children (*18, 24*). A low-cost protein food based on a blend of low fat peanut flour (50 parts), chickpea flour (25 parts), and coconut flour (25 parts) has been found to contain proteins of fairly high biological value (*15*). Subrahmanyan and coworkers (*23*) reported that supplementation of the diet of malnourished school children with 2 ounces of the protein food, daily over a period of 8 months, brought about a marked improvement in their growth and nutritional status. Tasker and coworkers (*25*) observed a significant increase in retention of nitrogen, calcium, and phosphorus in children receiving 2 ounces of protein food daily as compared with the control group. It was, at the same time, interesting to study the supplementary value of protein food fortified with calcium and certain vitamins to a low-protein diet based on maize and tapioca, since there is a high incidence of kwashiorkor in children subsisting on such diets (*6*).

This paper deals with studies on the effect of supplementing a maize-tapioca diet with protein food on the growth and composition of blood, liver, and carcass of albino rats.

Experimental

Materials and Methods. Maize flour (100% extraction) used in experiments

Table I. Composition of Low-Cost Protein Food and Skim Milk Powder

Nutrient and Amino Acid,[a]	Low-Cost Protein Food,[b] Grams	Skim Milk Powder,[c] Grams	FAO Reference Protein Pattern, Grams
Moisture	9.6[d]	4.1[d]	
Protein (N × 6.25)	36.5[d]	35.0[d]	
Ether extract	7.6[d]	0.1[d]	
Carbohydrate (by difference)	39.4[d]	54.0[d]	
Mineral matter	6.9[d]	6.8[d]	
Calcium	0.88[d]	1.37[d]	
Phosphorus	0.65[d]	1.00[d]	
Thiamine	1.82[e]	0.35[e]	
Riboflavin	3.11[e]	1.39[e]	
Vitamin A	3000[f]	3000[f]	
Vitamin D	300[f]	300[f]	
Amino acids			
Arginine	10.4	4.3	...
Histidine	2.1	2.4	...
Lysine	3.6	8.2	4.2
Tryptophan	1.0	1.6	1.4
Phenylalanine	4.9	5.6	2.8
Methionine	1.3 } 2.7	2.9 } 3.9	4.2
Cystine	1.4	1.0	
Threonine	2.8	4.8	2.8
Leucine	7.1	10.2	4.8
Isoleucine	4.9	7.0	4.2
Valine	4.8	7.0	4.2

[a] Calculated to 16.0 grams of nitrogen.
[b] Blend of low-fat peanut flour (50 parts), low-fat coconut meal (25 parts), and chickpea flour (25 parts) fortified with calcium phosphate (1 part), calcium carbonate (1 part), thiamine (1.5 mg./100 grams), riboflavin (3.0 mg./100 grams), vitamin A (3000 I.U./100 grams) and vitamin D (300 I.U./100 grams).
[c] Figures for added vitamins A and D included.
[d] Expressed as per cent. [e] Expressed as mg./100 grams. [f] Expressed as I.U./100 grams.

was prepared by powdering yellow maize (local variety), free from impurities, in a flour mill to pass through a 50-mesh sieve. Tapioca flour was prepared from fresh roots according to the method of Subrahmanyan and others (*22*). Low-cost protein food was prepared by blending 50 parts of low-fat peanut flour, 25 parts of low-fat cocoanut meal, and 25 parts of chickpea flour, and fortifying with vitamins A and D, thiamine, riboflavin, and calcium salts according to the method of Krishnamurthy and coworkers (*15*). A sample of imported, spray-dried skim milk powder (Maffco brand, Maffra Co-operative Milk Products Co., Ltd., Maffra, Victoria, Australia) was used. Table I shows chemical composition and essential amino acid composition of the protein food and skim milk powder. Chemical composition was determined according to the methods of the Association of Official Agricultural Chemists (*1*). Table II shows essential amino acid composition of the experimental diets calculated using amino acid values given in Table I for protein food and skim milk powder and amino acid composition determined in the laboratory for samples of maize and tapioca used in this investigation. Methionine was determined by the method of Horn, Jones, and Blums (*12*), tryptophan according to that of Spies (*20*), and histidine by the method of Macpherson (*16*). Other essential amino acids were determined according to the paper chromatographic method of Krishnamurthy and Swaminathan (*14*).

Animal Experiments. The supplementary value to a maize-tapioca diet of low-cost protein food and skim milk powder (at 30% levels) was determined by growth experiments on albino rats. Composition of experimental diets is shown in Table III. Groups of freshly weaned albino rats (Wistar strain) weighing about 40 to 45 grams (12 in each group and distributed equally according to sex, litter mates, and body weight) were fed *ad libitum* on different diets. Animals were allotted to different groups according to randomized block design, and were housed individually in raised wire screen-bottomed cages. One group of weanling rats weighing about 40 grams was used for determination of initial body composition. Diets were mixed with three times their weight of water, cooked in steam for 10 minutes, and fed to rats. Records of daily food intake were kept for all groups and rats were weighed weekly. After feeding for a period of 8 weeks, the hemoglobin and red blood cell count of the animals were determined in blood drawn from the tail of the rats. Hemoglobin was estimated by the acid hematin method using a Sahli-Hellige hemometer and red blood cell counts were made according to standard procedures using Neubauer's hemocytometer (*11*). The animals were then anesthetized with ethyl ether. Blood was drawn by heart puncture and the serum was separated. The total serum protein content was determined according to the method of King (*13*). The electrophoretic pattern of serum proteins was studied by the method of Durram (*7*), using a barbiturate buffer (pH 8.6; ionic strength 0.05). The rats were bled through the abdominal aorta to ensure minimum and uniform amount of residual blood in the livers. The whole liver was quickly excised, washed with normal saline to remove adhering blood, wiped between filter papers, and immediately weighed in glass dishes. Samples of liver from the left lobe were taken for histological examination. The moisture content of livers was determined by drying to constant weight at 90° to 95° C. in an air oven. The dry liver samples were powdered and aliquots taken for analysis of total nitrogen and fat. Total nitrogen was determined by the micro-Kjeldahl method and fat by the method of Hawk and Elvehjem (*10*). Contents of the gastrointestinal tract were removed by squeezing and the whole carcass was minced and weighed. Moisture, fat, and protein contents of carcass were determined according to the methods used for liver. The initial protein contents of the carcass of rats in different groups were calculated from values obtained for a group of weanling rats (6 males) weighing about 40 grams. Using these data, retention of protein in the carcass of rats receiving protein supplements over a period of 8 weeks was calculated. Results are given in Tables IV to VII.

Histological Examination of Liver. Samples of liver taken from the left lobe were fixed in 10% formalin. The tissues were processed through paraffin. Sections of 5-micron thickness were stained with hematoxylin and eosin. In addition, frozen sections were taken from each specimen and stained with Sudan IV in propylene glycol to study distribution of fat.

Results

Chemical and Amino Acid Composition of Protein Food. Chemical composition and amino acid composition of the low-cost protein food as compared

Table II. Essential Amino Acid Composition of Experimental Diets

Amino Acid[a]	*Control, Maize-Tapioca Diet*	*Control + Low Cost Protein Food*	*Control + Skim Milk Powder*
Arginine	4.3	8.7	4.3
Histidine	2.0	2.1	2.3
Lysine	3.0	3.4	6.8
Tryptophan	0.7	0.9	1.4
Phenylalanine	4.3	4.7	5.3
Methionine	1.7	1.4	2.6
Cystine	1.3	1.4	1.1
Threonine	3.9	3.1	4.5
Leucine	11.9	8.5	10.7
Isoleucine	4.4	4.8	6.3
Valine	5.0	4.8	6.4

[a] Calculated to 16.0 grams of nitrogen.

Table III. Composition of Experimental Diets

Constituents	*Maize-Tapioca Diet, %*	*Maize-Tapioca Diet + Low-Cost Protein Food, %*	*Maize-Tapioca Diet + Skim Milk Powder, %*
Maize flour (100% extraction)	47	32	32
Tapioca flour	47	32	32
Peanut oil	5	5	5
Sodium chloride	1	1	1
Low-cost protein food	..	30	..
Skim milk powder, fortified with vitamins A and D	..	..	30

Table IV. Gain in Body Weight of Rats Fed on Maize-Tapioca Diet and the Same Supplemented with Low-Cost Protein Food or Skim Milk Powder[a]

Diet	*Protein Content of Diet,[b] %*	*Av. Body Weight, Grams: Initial*	*Av. Body Weight, Grams: Final*	*Av. Daily Food Consumed,[b] Grams*	*Av. Weekly Gain in Body Weight, Grams*
Control, maize-tapioca diet[c]	6.50	48.8	87.0	8.7	4.77 ± 0.48[d] (19 d.f.)
+ low-cost protein food	15.70	48.6	187.2	11.9	17.32 ± 0.45
+ skim milk powder	16.17	48.9	192.7	11.8	17.97 ± 0.45

[a] Average of 6 males and 6 females per group, duration of experiment 8 weeks.
[b] Expressed on moisture-free basis.
[c] Gain in body weight of one rat on maize-tapioca diet was determined by missing plot technique, as animal died during the course of experiment. [d] Standard error.

Table V. Effect of Supplementation of Maize-Tapioca Diet with Low-Cost Protein Food and Skim Milk Powder on Composition of Blood of Rats[a]

Diet	Hemoglobin, %	Erythrocytes, Millions/Cu. Mm.	Total Serum Protein, %	Albumin, %	Globulins, %			
					Alpha$_1$	Alpha$_2$	Beta	Gamma
Control, maize-tapioca diet[b]	13.5 ± 0.21[c]	6.07 ± 0.22[c]	5.68 ± 0.030[c]	1.59 ± 0.016[c]	1.05[c] ± 0.010	0.73[c] ± 0.006	1.06[c] ± 0.008	1.26[c] ± 0.035
+ low-cost protein food	15.3 ± 0.19	8.35 ± 0.21	5.91 ± 0.028	1.84 ± 0.015	1.17 ± 0.009	0.76 ± 0.006	1.13 ± 0.007	1.01 ± 0.033
+ skim milk powder	14.8 ± 0.19	7.57 ± 0.21	6.10 ± 0.028	1.90 ± 0.015	1.21 ± 0.009	0.79 ± 0.006	1.28 ± 0.007	0.92 ± 0.033

[a] Averages of 6 males and 6 females per group, duration of experiment 8 weeks.
[b] Values for one rat estimated by missing plot technique, as rat died.
[c] Standard error based on 19 degrees of freedom.

to skim milk powder are shown in Table I. Table I shows that the protein content of the low-cost protein food (36.5%) was nearly of the same order as that of skim milk powder, but calcium content was somewhat less. The low-cost protein food contained lesser amounts of all essential amino acids (except arginine) as compared with the proteins of milk; but as compared with FAO reference protein pattern (*9*), it was deficient only in lysine, tryptophan, methionine, and cystine.

Growth and Composition of Blood. The average weekly increase in weight of rats fed on different diets is shown in Table IV. Results show that incorporation of protein foods at 30% level in a maize-tapioca diet resulted in a highly significant ($P < 0.001$) increase in growth rate of rats. There was no significant difference, however, in growth rate of rats receiving the low-cost protein food or skim milk powder. Values for serum protein, hemoglobin, and red blood cell counts in the blood of rats fed on different experimental diets are shown in Table V. The serum protein, hemoglobin, and red blood cell counts of rats receiving supplements of low-cost protein food or skim milk powder were significantly higher ($P < 0.001$) than those of the control maize-tapioca diet. On the other hand, no significant differences in these values were observed in different groups of rats receiving protein supplements. Values for albumin, alpha$_1$, alpha$_2$, beta, and gamma globulin fractions of the serum are given in Table V. The serum of rats fed on the maize-tapioca diet had a lower albumin content and a higher gamma globulin content than that of rats receiving supplements of low-cost protein food or skim milk powder.

Composition of Liver and Carcass. Results of analysis of liver and carcass of rats fed on different diets are shown in Table VI. There was no significant difference in mean fat and protein contents of livers and carcasses of rats receiving protein food or skim milk powder. Mean fat content of livers of rats fed on maize-tapioca diet was significantly higher ($P < 0.001$) and the protein content significantly lower ($P < 0.001$) than that of rats receiving the supplements. Protein content of the carcass of rats fed on maize-tapioca diet was also significantly lower ($P < 0.001$) than that of animals receiving protein supplements.

Histological Structure of Liver. Livers of animals on maize-tapioca diet showed a mild to moderate degree of parenchymal damage of the protein deficiency type. Liver cells showed reduction in cytoplasmic protein. Varying degrees of cytoplasmic vacuolation of the protein deficiency type were observed. Frozen sections stained with Sudan IV showed abundant stainable fat around periportal areas. Fatty infiltration was not seen around the central vein. Necrosis of the liver cells

Table VII. Retention of Protein in Rats Fed on Maize-Tapioca Diet with and without Supplementation of Low-Cost Protein Food or Skim Milk Powder[a]

Diet	Body Weight Gain, Grams	Total Body Protein (x), Grams	Initial Body Protein (y),[b] Grams	Protein Retained (x − y), Grams	Protein Retained/ 100 Grams Increase in Body Weight, Grams
Control, maize-tapioca diet[c]	39.3	14.78	8.9	5.88	14.97 ± 0.59[d]
+ low cost protein food	166.0	39.12	8.8	30.32	18.26 ± 0.52[d]
+ skim milk powder	171.0	41.82	8.9	32.92	19.25 ± 0.52[d]

[a] Averages of 6 males per group, duration of experiment 8 weeks.
[b] Calculated from data obtained for a similar group of weanling rats.
[c] Value of one rat determined by missing plot technique.
[d] Standard error.

Table VI. Effect of Supplementation of Maize-Tapioca Diet with Low-Cost Protein Food and Skim Milk Powder on the Composition of Liver and Carcass of Rats[a]

Diet	Liver Weight, Grams	Liver Composition, %			Body Weight, Grams	Carcass Composition, %		
		Protein	Fat	Moisture		Protein	Fat	Moisture
Control, maize-tapioca diet	3.34	14.55[b]	4.72[c]	72.2[d]	88.3	16.44[e]	14.44[f]	64.5[g]
+ low-cost protein food	8.45	18.00	3.96	69.8	215.0	18.38	18.65	59.4
+ skim milk powder	8.78	18.49	3.92	70.0	220.0	18.97	21.86	57.0

Averages of 6 males per group, duration of experiment 8 weeks.
[a] Standard error with degrees of freedom.
[b] ±0.54 (10 d.f.).
[c] ±0.18 (10 d.f.).
[d] ±0.48 (10 d.f.).
[e] ±0.35 (10 d.f.).
[f] ±0.73 (10 d.f.).
[g] ±1.02 (10 d.f.).

was absent. Liver sections of rats receiving low-cost protein food or skim milk powder were normal indicating thereby that the low-cost protein food was as effective as skim milk powder in correcting deficiencies of protein and other dietary essentials in the diet and in preventing liver damage.

Retention of Protein. Retention of protein by rats fed on different diets is given in Table VII. No significant difference was observed in the amount of protein retained per 100-gram increase in body weight of rats receiving low-cost protein food or skim milk powder. On the other hand, the amount of protein retained per 100-gram increase in body weight on maize-tapioca diet was significantly lower than that observed in groups receiving protein food or skim milk powder.

Discussion

Results obtained in the present investigation have shown that a low-cost protein food based on a blend of peanut meal, coconut meal, chickpea flour, and fortified with calcium, vitamins A and D, thiamine, and riboflavin, when incorporated at 30% level in a maize-tapioca diet, could meet as effectively as an equivalent amount of skim milk powder the protein requirements of albino rats. Livers of animals receiving different protein supplements had a normal histological appearance. On the other hand, livers of rats fed on the control maize-tapioca diet showed peri-portal fatty infiltration and cytoplasmic vacuolation. This may be due to the low protein content of the diet and also to deficiencies of certain essential amino acids in the proteins of maize (*19*).

Subrahmanyan and coworkers (*24*), Bharucha and Edibam (*5*), and Purushothama Rao (*17*) reported blends of peanut flour, chickpea flour, and skim milk powder to be effective in treatment of protein malnutrition in children. In view of the wide spread occurrence of kwashiorkor in many underdeveloped countries, it may be concluded from results obtained in the present study and from those reported in earlier studies (*24, 25*) that processed protein foods based on peanut meal, coconut meal, chickpea flours, and fortified with calcium and certain essential vitamins could be used as safe protein supplements for prevention of protein malnutrition in children in underdeveloped countries.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 7th ed., 1945.
(2) Autret, M., Behar, M., *Food and Agr. Organization U. N., Nutrition Studies,* No. 13, FAO, Rome, 1951.
(3) Autret, M., van Veen, A. G., *Am. J. Clin. Nutrition* **3,** 234 (1955).
(4) Behar, M., Bressani, R., Scrimshaw, N. S., in "World Review of Nutrition and Dietetics," Vol. 1, G. H. Bourne, ed., Pitman Medical Pub. Co., Ltd., London, 1959.
(5) Bharucha, E. P., Edibam, B. C., *Indian J. Child Health* **261** (1960).
(6) Brock, J. F., Autret, M., *Food and Agr. Organization U. N., Nutrition Studies,* No. **8,** FAO, Rome, 1952.
(7) Durram, E. L., "A Microelectrophoretic and Microionophoretic Technique," Rept. Medical Dept. Field Research Lab., Fort Knox, Project No. **6-64-12-06,** 1949.
(8) Flores, M., Flores, Z., Meneses, B., *Arch. venezolanos nutric.* **8,** 57 (1957).
(9) Food and Agriculture Organization, *Food and Agr. Organization U. N., Nutrition Studies,* No. **16,** Rept. FAO Committee, 1951.
(10) Hawk, E. A., Elvehjem, C. A., *J. Nutrition* **49,** 495 (1953).
(11) Hepler, O. E., "Manual of Clinical Laboratory Methods," 7th ed., Charles C Thomas, Springfield, Ill., 1950.
(12) Horn, M. J., Jones, D. B., Blums, A. E., *J. Biol. Chem.* **166,** 313 (1946).
(13) King, E. J., "Micro Analysis in Medical Biochemistry," 2nd ed., Grune and Stratton, New York, 1951.
(14) Krishnamurthy, K., Swaminathan, M., *Anal. Chem.* **27,** 1936 (1955).
(15) Krishnamurthy, K., Tasker, P. K., Indira, K., Rajagopalan, R., Swaminathan, M., Subrahmanyan, V., *Ann. Biochem. and Exptl. Med. (Calcutta)* **18,** 175 (1958).
(16) Macpherson, H. T., *Biochem. J.* **36,** 59 (1942).
(17) Purushothama Rao, *Indian J. Child Health* **207** (1960).
(18) Scrimshaw, N. S., *J. Am. Dietet. Assoc.* **35,** 441 (1959).
(19) Shils, M. E., Friedland, I., Stewart, W. B., *Proc. Soc. Exptl. Biol. Med.* **87,** 483 (1954).
(20) Spies, J. R., *Anal. Chem.* **22,** 1447 (1950).
(21) Subrahmanyan, V., Bhagavan, R. K., Swaminathan, M., *Indian J. Pediat.* **25,** 216 (1958).
(22) Subrahmanyan, V., Bhatia, D. S., Bains, G. S., Swaminathan, M., Raghunatha Rao, Y. K., *Bull. Cent. Food Technol. Research Inst. Mysore* **3,** 180 (1954).
(23) Subrahmanyan, V., Doraiswamy, T. R., Bhagavan, R. K., Tasker, P. K., Sankaran, A. N., Rajagopalan, R., Swaminathan, M., *Ann. Biochem. and Exptl. Med. (Calcutta)* **19,** 147 (1959).
(24) Subrahmanyan, V., Doraiswamy, T. R., Joseph, K., Narayana Rao, M., Swaminathan, M., *Indian J. Pediatrics* **24,** 112 (1957).
(25) Tasker, P. K., Narayana Rao, M., Swaminathan, M., Subrahmanyan, V., *Ann. Biochem. and Exptl. Med. (Calcutta)* **19,** 153 (1959).
(26) Trowell, H. C., Davies, J. N. P., Dean, R. F. A., "Kwashiorkor," Edward Arnold, London, 1954.
(27) Venkatachalam, P. S., Srikantiah, S. G., Gopalan, C., *Indian J. Med. Research* **42,** 555 (1954).

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