

The metabolism of nitrogen, calcium and phosphorus in undernourished children

8.* The metabolism of nitrogen, calcium and phosphorus, and the digestibility coefficient and biological value of the proteins and the net protein utilization on poor Indian diets based on rice, maize or a mixture of rice and maize

BY P. K. TASKER, T. R. DORAISWAMY, M. NARAYANARAO,
M. SWAMINATHAN, A. SREENIVASAN AND V. SUBRAHMANYAN

Central Food Technological Research Institute, Mysore, India

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Maize is consumed widely as a staple food by the low-income groups in Africa, Latin America and other parts of the world (FAO, 1953). In India maize is consumed to a limited extent along with millets in some States (Patwardhan, 1952). The main nutritional defects of maize as compared with other cereals are (1) its low content of nicotinic acid and (2) the deficiencies of its proteins in tryptophan and lysine, and the imbalance between isoleucine and leucine (FAO, 1953). The incidence of pellagra and kwashiorkor is high among maize-eating populations (FAO, 1953; Autret & Béhar, 1954). Scrimshaw, Bressani, Béhar & Viteri (1958) reported that the retention of nitrogen in children aged 3–4 years was low on a maize diet providing about 3 g protein/kg body-weight. Similar observations on young children aged 1–3 years have recently been reported by Hansen, Schendel, Wilkins & Brock (1960). Truswell & Brock (1959) found that four out of six adults fed on a maize diet providing 1 g protein/kg body-weight maintained a slight positive N balance and two were in negative balance. No information is available on the metabolism of nitrogen, calcium and phosphorus in older children on poor maize diets.

In view of the shortage of rice in India, the use of maize and other grains as a partial substitute for rice needs to be studied. This paper describes the results of investigations on the effect of replacing rice in a poor Indian diet partly or completely by maize on the metabolism of N, Ca and P, and on the digestibility coefficient, biological value of the proteins and net protein utilization in children.

EXPERIMENTAL

The plan of the experiment and the analytical methods used were similar to those described by Kurien, Narayanarao, Swaminathan & Subrahmanyam (1960) in their studies with kaffir corn (*Sorghum vulgare*) diets. The ages, heights and weights of the eight boys studied are given in Table 1. They belonged to low-income groups and were inmates of a boarding home in Mysore city. They were accustomed to consuming diets based on a mixture of different cereals such as rice, wheat, ragi (*Eleusine coracana*)

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and kaffir corn. In this study, the diets were given in the same order as in earlier studies (Joseph, Kurien, Swaminathan & Subrahmanyam, 1959; Kurien *et al.* 1960). Since the experimental periods were of short duration (15 days, the first 10 days being treated as a preliminary period of adjustment), the design adopted was not likely to affect the interpretation of results. A similar procedure has been followed by Scrimshaw *et al.* (1958), in their investigation of the effect of amino acid supplementation of a maize diet on N retention in children.

Table 1. *Ages, heights and weights of the children at the beginning of the test*

Boy no.	Age (years)	Height (cm)	Weight (kg)
1	10	123	23.8
2	10	124	22.5
3	9	120	21.2
4	9	121	22.8
5	9	122	23.2
6	10	124	22.9
7	9	122	23.0
8	10	126	23.4

Table 2. *Percentage composition of the raw milled rice and maize*

	Raw milled rice	Maize (white)
Moisture	10.9	12.1
Protein (N × 6.25)	6.7	11.8
Fat (ether extractives)	0.6	3.8
Ash	0.6	1.7
Starch	80.1	60.2
Total sugars	0.4	0.9
Crude fibre	0.2	2.7
Pentosans and other hemicelluloses (by difference)	0.5	6.8
Calcium	0.01	0.01
Phosphorus (total)	0.150	0.330
Phytate phosphorus	0.088	0.210
Calories (kcal/100 g)	354	326

Table 3. *Mean daily intake (g) of foodstuffs by the children on the different diets**

Foodstuff	Rice diet	25 % maize diet	50 % maize diet	Maize diet
Rice, raw milled	360	270	180	—
Maize, white	—	90	180	360

* All the diets supplied in addition (g/day): red gram dhal (*Cajanus indicus* Spreng.) 26; groundnut oil 9; potato 29; brinjal (*Solanum Melongana* L.) 15; amaranth leaves (*Amaranthus gangeticus* L.) 15; radish 15; onions 14; tamarind fruit pulp (*Tamarindus indica* L.) 13; coconut kernel 10; meat 30; skim-milk powder 7; common salt 15; cane sugar 12.5; condiments (garlic, coriander seeds, mustard, red chillies and turmeric) 9; tea leaves 2.

The chemical composition of the rice and maize used is given in Table 2. The test diets (Table 3) contained different proportions of rice and maize with other foods normally consumed in small quantities in India by the low-income groups. The diets

designated: 'rice diet' (in which rice was the only cereal); '25% maize diet' (in which 25% of the rice was replaced by maize); '50% maize diet', containing equal amounts of rice and maize; and 'maize diet', in which all the rice was replaced by maize. The essential amino acid composition of the mixed proteins in the different diets, determined by the methods of Krishnamurthy, Tasker, Ramakrishnan, Rajagopalan & Swaminathan (1960), is given in Table 4, compared with the FAO reference protein pattern (FAO, 1957). The protein 'scores' for the mixed proteins of the different diets calculated by the FAO method are also given in Table 4.

The procedures for feeding the children and the collection of excreta were similar to those described by Kurien *et al.* (1960). Total N, Ca and P in food, urine and faeces were determined by the methods of Murthy, Swaminathan & Subrahmanyam (1954).

Table 4. Essential amino acid content* (g/16 g N) of the mixed proteins of the different diets

Amino acid	Rice diet	25 % maize diet	50 % maize diet	Maize diet	FAO (1957) reference protein pattern
Arginine	5.71	5.34	4.95	4.23	—
Histidine	2.02	2.14	2.15	2.32	—
Lysine	5.26	4.94	4.63	4.14	4.2
Tryptophan	1.10	1.03	0.91	0.72	1.4
Phenylalanine	5.34	5.26	5.15	4.92	2.8
Methionine	1.82	1.83	1.80	1.91	2.2
Cystine	1.30	1.31	1.33	1.34	—
Total sulphur amino acids	3.12	3.14	3.13	3.25	4.2
Threonine	3.91	3.92	4.05	4.14	2.8
Leucine	8.23	9.14	10.01	11.72	4.8
Isoleucine	5.04	4.92	4.91	4.84	4.2
Valine	6.23	5.95	5.66	5.14	4.2
Protein score (FAO, 1957)					
	74	72	65	51	—

* The limiting amino acids in bold-faced type.

Table 5. Mean daily intake (g) of foodstuffs by the children on a low-protein diet*

Maize starch	158.0
Tapioca flour (washed with dilute alkali)	60.0
Sago	20.0
Cane sugar	55.0
Groundnut oil	50.0
Vitaminized sugar†	5.0
Common salt, refined	12.5
Salt mixture‡	5.0
Onion	15.0
Tamarind fruit pulp	6.4
Condiments (garlic, coriander seeds, mustard, red chillies and turmeric)	10.0
Brinjal (<i>Solanum Melongana</i> L.)	22.0
Lemon juice	60 ml

* Nitrogen content of the diet, 0.15%; calorific value, 1670 kcal.

† Provided the daily requirements of the vitamins as recommended by the (U.S.A.) National Research Council: Food and Nutrition Board (1958).

‡ Osborne & Mendel (1913).

After the collection of urine and faeces had been completed, the children were fed on a low-protein diet (Table 5) for 10 days, and urine and faeces were collected during the last 5 days, for determination of endogenous urinary and faecal N. The low-protein diet consisted of savoury and sweet preparations made from a vermicelli-like product obtained from cooked maize starch and washed tapioca flour, sago pudding, lime juice and vegetable soup.

The apparent and true digestibility coefficients and the biological value of the proteins and net protein utilization (operative) ($NPU_{(op)}$) of the diets were calculated according to the following formulas:

$$\text{Apparent digestibility coefficient} = 100 \times \frac{\text{N intake} - \text{faecal N}}{\text{N intake}},$$

$$\text{True digestibility coefficient} = 100 \times \frac{\text{N intake} - (\text{faecal N} - \text{endogenous faecal N})}{\text{N intake}},$$

$$\text{Biological value} = 100 \times \frac{\text{N intake} - (\text{faecal N} - \text{endogenous faecal N}) - (\text{urinary N} - \text{endogenous urinary N})}{\text{N intake} - (\text{faecal N} - \text{endogenous faecal N})},$$

$$NPU_{(op)} = \frac{\text{True digestibility coefficient} \times \text{biological value}}{100},$$

$$\text{Net dietary protein calories \% (ND-p Cals \%)} = \frac{NPU_{(op)} \times \text{percentage of protein calories}}{100}.$$

RESULTS

Essential amino acid composition of the diets and their protein score (Table 4). The protein 'scores' based on the essential amino acid composition and calculated by the method of FAO (1957) fell progressively from 74 for the rice diet to 51 for the maize diet.

Table 6. *Daily urinary and faecal excretion (g) of nitrogen by the children on the low-protein diet*

Boy no.	Urinary	Faecal	Total
1	1.72	0.67	2.39
2	1.64	0.58	2.22
3	1.26	0.65	1.91
4	1.52	0.75	2.27
5	1.42	0.66	2.08
6	1.11	0.92	2.03
7	1.02	0.86	1.88
8	1.46	0.77	2.23
Mean value with its standard error (7 df)	1.39 ± 0.087	0.73 ± 0.041	2.13 ± 0.064

N Balance of the children, digestibility coefficient and biological value of the proteins (Table 7). The mean daily intake of N from the different diets ranged from 6.6 to 8.7 g. The values for endogenous urinary and faecal N are given in Table 6. The mean

daily N retentions ranged from 2.04 g on the rice diet to 0.99 g on the maize diet. The apparent and true digestibility coefficients and the biological value of the proteins of the different diets progressively decreased as the proportion of maize in the diet increased.

Table 7. Mean daily intake, excretion and balance of nitrogen of the children on the different diets

Diet Description	Calorie value (kcal/ day)	Intake (g)	Excretion (g)			Appa- rent digesti- bility coeffi- cient (%)	Balance (g)	True digesti- bility coeffi- cient (%)	Bio- logical value
			Urinary	Faecal	Total				
Rice	1704	6.61	3.05	1.51	4.56	77.1	2.04	88.2	71.5
25 % maize	1700	7.19	3.45	1.86	5.32	74.1	1.87	84.2	66.2
50 % maize	1646	7.53	3.43	2.75	6.17	63.5	1.35	73.3	62.0
Maize	1615	8.66	3.69	3.98	7.67	54.0	0.99	62.5	57.6
Standard error of the mean (21 df)	—	—	—	—	—	± 1.05	± 0.15	± 1.04	± 2.42

$NPU_{(op)}$ and $ND-p$ Cals % (Table 8). $NPU_{(op)}$ values ranged from 63.1 for the rice diet to 35.9 for the maize diet, and the $ND-p$ Cals % values ranged from 6.12 for the rice diet to 4.81 for the maize diet.

Protein intake and net available protein (Table 8). The mean daily protein intake ranged from 41.3 g on the rice diet to 54.1 g on the maize diet. The net available protein provided by the different diets, calculated as total protein intake \times net protein utilization \div 100, ranged from 26.1 to 19.4 g.

Mean intake and absorption of essential amino acids. The amount of essential amino acids absorbed was calculated as amino acid intake \times digestibility coefficient of the protein \div 100, on the assumption that all amino acids were digested and absorbed to the same extent. Table 9 shows that the amounts of tryptophan absorbed from the maize diet and the 50% maize diet were lower than that required by the reference protein pattern suggested by FAO (1957).

Ca balance (Table 10). Ca intakes ranged from 362 to 382 mg and all the subjects were in positive balance. The mean daily retention decreased as the level of maize in the diet increased.

P balance (Table 10). The mean intake of P on the different diets ranged from 697 to 1389 mg and all the subjects were in positive balance. The mean daily retention of P ranged from 153 to 341 mg. The mean daily retention increased as the level of maize in the diet increased.

DISCUSSION

Maize, like kaffir corn, contains fairly large amounts (9.5%) of roughage, i.e. cellulose and hemicelluloses. The mean daily faecal bulk increased with the amount of maize in the diet. Similar observations have been made earlier with diets containing ragi (Joseph *et al.* 1959) and kaffir corn (Kurien *et al.* 1960). It should be noted that the replacement of rice by maize to the extent of 50% affected adversely the retention of N and Ca. The results indicate that about 25% of rice in the diet could be replaced

Table 8. Mean values for net protein utilization, net dietary protein value and net dietary protein calories on the different diets eaten by the children

Diet	Protein intake		Protein in diet (%)	Protein calories (%)	NPU ^(pp)	Net available protein* (g)	ND-pv (%)	ND-p Cals %
	g	g/kg body-weight						
Rice	41.3	2.0	10.09	9.70	63.12	26.1	6.36	6.12
25% Maize	44.9	2.2	10.86	10.56	55.63	25.0	6.04	5.87
50% Maize	47.1	2.3	11.57	11.45	45.33	21.4	5.24	5.19
Maize	54.1	2.6	13.14	13.41	35.90	19.4	4.72	4.81
Standard error of the mean (21 df)	—	—	—	—	± 1.97	—	—	± 0.21

* The reference protein requirement of the children calculated according to FAO (1957) amounts to 25.1 g.

Table 9. Mean daily intake (g) and absorption* by the children of essential amino acids from the different diets

Amino acid	Rice diet		25% maize diet		50% maize diet		Maize diet		FAO (1957) reference protein requirement†
	Intake	Absorption	Intake	Absorption	Intake	Absorption	Intake	Absorption	
Arginine	2.36	2.08	2.40	2.03	2.33	1.71	2.29	1.43	—
Histidine	0.83	0.73	0.96	0.81	1.02	0.75	1.26	0.79	—
Lysine	2.17	1.91	2.22	1.87	2.18	1.60	2.24	1.40	1.06
Tryptophan	0.45	0.40	0.46	0.39	0.43	0.32	0.39	0.24	0.35
Phenylalanine	2.20	1.94	2.36	1.99	2.43	1.78	2.66	1.66	0.70
Methionine	0.75	0.66	0.82	0.69	0.85	0.62	1.03	0.64	0.55
Cystine	0.54	0.48	0.59	0.50	0.63	0.46	0.73	0.46	—
Total sulphur amino acids	1.29	1.14	1.41	1.19	1.48	1.08	1.76	1.10	1.05
Threonine	1.62	1.43	1.76	1.48	1.91	1.40	2.24	1.40	0.70
Leucine	3.40	3.00	4.10	3.45	4.71	3.45	6.34	3.96	1.21
Isoleucine	2.08	1.84	2.21	1.86	2.31	1.69	2.62	1.64	1.05
Valine	2.58	2.28	2.67	2.25	2.67	1.96	2.78	1.74	1.05

The limiting amino acids are in bold faced type.

* Calculated as $\frac{\text{intake} \times \text{digestibility coefficient of the protein}}{100}$.

† The reference protein requirement of the children calculated according to FAO (1957) amounts to 25.1 g.

Table 10. *Mean daily intake, excretion and balance of calcium and phosphorus by the children on the different diets*

Description	Calorie value (kcal/day)	Intake (mg)	Excretion (mg)			Balance (mg)
			Urinary	Faecal	Total	
Calcium						
Rice	1704	362	56	157	213	+149
25% maize	1700	366	62	194	266	+110
50% maize	1646	373	59	225	284	+89
Maize	1615	382	61	254	315	+67
Standard error of the mean (21 df)	—	—	—	—	—	±4.8
Phosphorus						
Rice	1704	697	249	295	544	+153
25% maize	1700	860	292	376	668	+192
50% maize	1646	1093	297	546	843	+250
Maize	1615	1389	235	813	1048	+341
Standard error of the mean (21 df)	—	—	—	—	—	±12.2

by maize without influencing the retention of N or Ca. The net amounts of available proteins supplied daily by the rice diet (26.1 g) and the 25% maize diet (25.0 g) met the daily requirement of 25.1 g of reference protein calculated according to FAO (1957); on the other hand, the net amounts supplied by the 50% maize diet (21.4 g) and the maize diet (19.4 g) did not meet this requirement. These results are in line with the quantities of essential amino acids absorbed from the different diets (Table 9). Table 9 shows that the proteins absorbed after digestion from the rice and the 25% maize diets met fully the essential amino acid requirements defined by the FAO reference amino acid pattern (FAO, 1957) whereas the proteins absorbed after digestion from the 50% maize diet and the maize diet did not provide an adequate amount of tryptophan. According to Platt, Miller & Payne (1961), the protein requirements of boys of the age group 9–10 years recommended by the FAO Committee (FAO, 1957) will be about 6.0 when expressed as ND-p Cals%. Judged on this basis, the rice diet and the 25% maize diet are just adequate, but the 50% maize diet and the 100% maize diet are inadequate to meet the protein needs of children.

SUMMARY

1. The effect on nitrogen, calcium and phosphorus metabolism of replacing 25%, 50% or all of the rice in a poor Indian diet by maize was studied in eight boys aged 9–10 years.

2. The daily intake of N increased slightly with the increase in the level of maize in the diet. All the subjects were in positive balance. The apparent and true digestibility coefficient and biological value of the proteins and the mean daily retention of N decreased as the proportion of maize increased.

3. The net protein utilization (operative) values and the net dietary protein calories % of the different diets decreased steadily with the increase in the level of maize in the diet.

4. The mean daily intake of Ca was nearly the same on the different diets and the mean retention of Ca decreased as the proportion of maize increased. All the subjects were in positive balance.

5. Both the intake and retention of P increased as the proportion of maize in the diet increased.

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REFERENCES

- Autret, M. & Béhar, M. (1954). *F.A.O. nutr. Stud.* no. 13.
FAO (1953). *F.A.O. nutr. Stud.* no. 9.
FAO (1957). *F.A.O. nutr. Stud.* no. 16.
Hansen, J. D. L., Schendel, H. E., Wilkins, J. A. & Brock, J. F. (1960). *Pediatrics, Springfield*, **25**, 258.
Joseph, K., Kurien, P. P., Swaminathan, M. & Subrahmanyam, V. (1959). *Brit. J. Nutr.* **13**, 213.
Krishnamurthy, K., Tasker, P. K., Ramakrishnan, T. N., Rajagopalan, R. & Swaminathan, M. (1960). *Ann. Biochem.* **20**, 73.
Kurien, P. P., Narayanarao, M., Swaminathan, M. & Subrahmanyam, V. (1960). *Brit. J. Nutr.* **14**, 339.
Murthy, H. B. N., Swaminathan, M. & Subrahmanyam, V. (1954). *Brit. J. Nutr.* **8**, 11.
National Research Council: Food and Nutrition Board (1958). *Publ. nat. Res. Coun., Wash.*, no. 589.
Osborne, T. B. & Mendel, L. B. (1913). *J. biol. Chem.* **15**, 317.
Patwardhan, V. N. (1952). *Nutrition in India*. Bombay, India: Indian Journal of Medical Sciences.
Platt, B. S., Miller, D. S. & Payne, P. R. (1961). In *Recent Advances in Human Nutrition with Special Reference to Clinical Medicine*, p. 351. [J. F. Brock, editor.] London: J. and A. Churchill Ltd.
Scrimshaw, N. S., Bressani, R., Béhar, M. & Viteri, F. (1958). *J. Nutr.* **66**, 485.
Truswell, A. S. & Brock, J. F. (1959). *S. Afr. med. J.* **33**, 98.