Br. J. Nutr. (1978), 39, 271

The utilization of proteins and amino acids in diets based on cassava (*Manihot utilissima*), rice or sorghum (*Sorghum sativa*) by young Nigerian men of low income

BY B. M. NICOL* AND THE LATE P. G. PHILLIPS

Nutrition Unit, Federal Ministry of Health, Kaduna, Nigeria

1. The net protein utilization (NPU, the percentage of dietary nitrogen retained in the body, allowance being made for endogenous urinary and faecal N) of diets composed of Nigerian foodstuffs, based on rice, sorghum (*Sorghum sativa*) or cassava (*Manihot utilissima*), was compared to that of a minimal protein diet used to determine endogenous N excretion, supplemented with whole egg. The addition of DL-methionine and L-tryptophan to the rice diet produced a small but non-significant increase in NPU, whereas the addition of DL-methionine to the cassava diet produced a very significant increase in NPU. The NPU of a diet based on home-pounded, winnowed, sorghum flour was higher than that of a diet based on milled whole-meal sorghum due to the low digestibility of the latter diet.

2. The digestibility of the rice and cassava diets were the same, although the total crude fibre content of the rice diets was lower than that of the cassava diets.

3. Nigerian men used the proteins of the egg diet and of mixed diets based on rice, sorghum and cassava more efficiently than predicted by applying methods recommended by the FAO/WHO *ad hoc* Expert Committee on Energy and Protein Requirements (FAO/WHO, 1973).

4. The recommendations of that Committee (FAO/WHO, 1973) to reduce the amounts of sulphur amino acids and tryptophan, contained in the 'provisional pattern of amino acids' proposed by the FAO Committee on Protein Requirements (FAO, 1957), are supported, but the increases in lysine and threonine are not supported, by the present results.

The similarity of endogenous urinary and faecal nitrogen excretion by young men of different ethnic, socio-economic and nutritional backgrounds, and the more efficient use of whole-egg N by young Nigerian men than by North American university students, have been reported (Nicol & Phillips, 1976*a*). Inter-individual variation of urinary and faecal N excretion was found to be significant, but intra-individual variation not significant, when Nigerian men were given a rice diet on two separate occasions. The physiological processes of adaptation to an efficient use of low-protein rice diets could be reversed within 2 or 3 weeks when they were given a diet containing amounts of high-quality protein greater than their customary intake (Nicol & Phillips, 1976*b*).

Three of the important staple foods consumed in Nigeria are cassava (*Manihot utilissima*), rice and sorghum (*Sorghum sativa*) (Nicol, 1959*a*, *b*). The first objective of this paper was to re-examine findings, obtained some years ago, on the utilization of proteins in diets of Nigerian pattern, based on these three staple foods, given at levels which maintained N balance close to equilibrium, while supplying sufficient energy for maintenance of body-weight of young Nigerian men. The second objective was to consider the effect of dietary crude fibre on the true digestibility (TD, the percentage of dietary N absorbed, allowance being made for endogenous faecal N), net protein utilization (NPU) and biological value (BV, the percentage of dietary N retained in the body, allowance being made for endogenous urinary N) of these diets. The third objective was to compare the amino acid composition of the diets with the 'provisional amino acid pattern' proposed by the FAO Committee on Protein Requirements (FAO, 1957) and with the 'provisional amino acid scoring pattern' recommended by the Joint FAO/WHO *ad hoc* Expert Committee on Energy and Protein Requirements (FAO/WHO, 1973).

This re-examination of our previous findings, submitted in detail as research reports to

* Present address: Trackway, 52 Golf Links Road, Ferndown, Wimborne, Dorset BH22 8BZ, UK.

the Committee on Protein Malnutrition of the US National Research Council's Food and Nutrition Board, has been prompted by statements made in the report of a Joint FAO/WHO Informal Gathering of Experts on Energy and Protein Requirements (FAO, 1975) that: 'NPU, as conventionally measured, tends to overestimate the ability of diets of intermediate and poor quality to meet human needs' and that 'amino acid scores of diets based on coarse whole-grained cereals and vegetables may have a correction for digestibility in the range of 85 per cent and that diets based on refined cereals may have applied a digestibility figure of about 90 per cent'.

The FAO Committee on Protein Requirements (FAO, 1957) and the Joint FAO/WHO *ad hoc* Expert Committee on Energy and Protein Requirements (1973) both applied the method used by Block & Mitchell (1946) to determine chemical scores of proteins or dietary protein mixtures. Block & Mitchell used the amino acid pattern of hen's egg protein as a reference pattern. Both Committees applied more recent information derived from experiments on animals and feeding trials on man in developing their 'provisional amino acid scoring patterns'. The resulting essential amino acid scoring patterns differed considerably from that of hen's egg protein, and one pattern from the other pattern. The FAO/WHO (1973) report states: 'In comparison with the 1957 pattern, significant changes included a lowering of tryptophan and methionine. Threonine levels were raised; lysine levels were raised in accordance with the suggestion that scores for lysine-limited foods overestimated the actual nutritive value of these proteins when fed to young children; estimation of lysine requirements is complicated by the fact that all chemically determined lysine in a food is not necessarily biologically available.'

The amounts and nature of dietary fibre provided by the diets considered in this paper were less than those reported by Southgate & Durnin (1970) which prompted the Joint FAO/WHO Informal Gathering of Experts on Energy and Protein Requirements (FAO, 1975) to suggest the correction of dietary amino acid scores for digestibility quoted above.

METHODS

Subjects. Nineteen young Nigerian men of the low-income class, whose socio-economic and nutritional backgrounds have been described (Nicol & Phillips, 1976*a*), participated in thirteen different feeding trials, each trial comprising a group of six men. They were ambulant in the metabolic compound of the Federal Nigerian Nutrition Unit laboratories, their only exercise being walking, mat-weaving, playing cards or other sedentary games.

Diets. The ingredients of the diets based on rice, cassava or sorghum, with their proximate composition (determined in duplicate by methods described by McCance & Walsham (1948)), are given in Table 1. The methods used to prepare and supervise the consumption of the diets have been described (Nicol & Phillips, 1976*a*). Cassava diets were given as three equal meals served at 08.00, 13.30 and 19.00 hours. The rice and sorghum diets were served as cassava and sauce at the morning and evening meals and as rice or sorghum and sauce at the mid-day meal, equal amounts of sauce being given with each meal. Cassava was provided as 'gari', a granular product prepared from the fermented rhizone by methods described by Phillips & Ladell (1959). Rice was milled and parboiled. Sorghum was given either as hammer-milled whole meal or as flour prepared in the home by the traditional method of pounding and winnowing. Water was freely available, consumption varying from 1000 to 1600 ml/d.

Riboflavin and thiamine 2 mg each, nicotinic acid 17 mg, calcium citrate 500 mg and 30 mg capsulated ferrous sulphate were added to all diets. When synthetic DL-methionine and L-lysine were given the amounts were equally divided between the three meals. (Vitamins, minerals and amino acids were all provided by British Drug Houses Ltd, Poole, Dorset, UK.)

Table 1. *The composition of egg and rice diets, 'low-protein score' and 'high-protein score' cassava* (Manihot utilissima) *diets, and of the whole-meal sorghum* (Sorghum sativa) *diet and the home-pounded sorghum diet, when each was given to groups of six young Nigerian men to determine their utilization of the dietary protein mixtures*

Diet / Ingredients* (g/d)	Egg	Rice	Cassava low-protein score	Cassava high-protein score	Sorghum milled whole-grain	Sorghum home-pounded
Cassava 'gari'	440	436	620	620	480	480
Maize starch	36	—	—	—	—	—
Refined cane sugar	30	—	—	—	—	—
Peppers						
Capsicum annum, fresh	4	8	8	8	8	8
Capsicum frutescens, dried	2	3	3	3	3	3
Tomato, fresh	20	22	22	22	22	22
Onion, fresh	10	10	10	10	10	10
Melon (*Citrullus vulgaris*) seed	—	12	—	17	12	12
Red palm oil (*Elaeis guineensis*)	45	21	15	14	22	22
Groundnut flour, defatted	—	—	30	—	—	—
Parboiled rice	—	174	—	—	—	—
Sorghum, milled whole-grain	—	—	—	—	140	—
Sorghum, home pounded	—	—	—	—	—	140
Dried fish	—	7	28	7	7	7
Whole guinea-fowl (*Numida* spp.) egg†	154	—	—	—	—	—
Common salt (NaCl), iodized	10	10	10	10	10	10
Vitamin and mineral mixture‡	0·551	0·551	0·551	0·551	0·551	0·551
Chemical analysis						
Energy						
MJ	10·08	10·06	10·25	9·87	10·38	10·42
kcal	2410	2405	2450	2360	2480	2490
Nitrogen	3·92	4·00	3·95	3·85	3·90	3·96
Protein (N × 6·25)	24·5	25·0	24·7	24·1	24·4	24·8
Energy (g/kg) from:						
Protein	40	40	40	40	40	40
Fat	250	110	110	120	140	140

* Edible portion.

† Homogenized whole egg, lightly cooked.

‡ Contained (mg): riboflavin 2, thiamine 2, nicotinic acid 17, calcium citrate 500, ferrous sulphate (capsulated) 30.

Table 2. *Age, body-weight, urinary creatinine, energy and nitrogen intake and N excretion, compared to apparent N balance, biological value* (BV),* *true digestibility (TD)*† *and net protein utilization* (NPU)‡ *of a rice diet when given to a group of six young Nigerian men whose endogenous urinary and faecal N excretion had been measured (subjects O, P, Q, R, S, T) and to two groups whose endogenous N excretion was not known (subjects A, B, C, D, E, F and G, I, J, H, E, K)*

Subjects	Age (years)	Body-wt§ (kg)	Urinary creatinine (g/d)	Energy intake (/d)		N intake (g/d)	N excretion (g/d)		Apparent N balance‖ (g/d)	BV	TD	NPU
				MJ	kcal		Urinary	Faecal				
O	23	51·3	1·17	10·04	2400	3·86	2·17	1·39	+0·30	0·86	0·89	0·77
P	21	58·7	1·33				2·13	1·41	+0·32	0·91	0·88	0·80
Q	28	55·6	1·34				2·55	1·35	−0·04	0·95	0·84	0·80
R	26	55·9	1·41				2·37	1·94	−0·45	0·70	0·88	0·62
S	25	50·2	1·29				1·87	1·80	+0·19	0·86	0·84	0·72
T	30	51·3	1·29				2·59	1·41	−0·14	0·67	0·97	0·65
Mean	25·5	53·83	1·31				2·28	1·55	+0·03	0·83	0·88	0·73
SE	1·3	1·38	0·03				0·11	0·10	0·21	0·06	0·02	0·04
A	20	58·1	1·12	10·21	2440	4·00	2·49	1·30	+0·21			
B	30	56·4	1·09				2·27	1·63	+0·10			
C	28	54·9	1·30				2·15	1·70	+0·15			
D	30	54·6	1·33				2·41	1·75	−0·16			
E	31	55·2	1·24				2·11	1·83	+0·06			
F	25	58·5	1·32				1·94	1·98	+0·08			
Mean	27·5	56·29	1·23				2·23	1·70	+0·07	0·84	0·86	0·72
SE	1·8	0·66	0·04				0·08	0·09	0·06			
G	26	58·3	1·21	10·21	2440	3·97	2·75	1·24	−0·02			
I	25	58·7	1·33				2·92	1·08	−0·03			
J	30	59·6	1·18				2·20	1·54	+0·23			
H	25	56·7	1·18				2·07	1·57	+0·33			
E	31	55·0	1·25				2·55	1·37	+0·05			
K	28	60·0	1·30				2·99	1·56	−0·58			
Mean	27·5	58·04	1·24				2·58	1·39	0	0·72	0·94	0·68
SE	1·0	0·75	0·03				0·15	0·08	—			

MPD, minimal protein diet used to determine endogenous urinary and faecal N excretion (Nicol & Phillips, 1976*a*).

* BV = absorbed N − (urinary $N_{\text{trial diet}}$ − urinary N_{MPD}) ÷ absorbed N.

† TD = dietary N − (faecal $N_{\text{trial diet}}$ − faecal N_{MPD}) ÷ dietary N.

‡ NPU = BV × TD.

§ Mean value obtained during balance period of 6 d.

‖ N balance excluding cutaneous and other minor N losses.

The amino acid composition of the diets was calculated using tables (Orr & Watt, 1957), confirmatory and supplementary information being supplied by the Government Chemist, London (personal communication), from direct analysis of Nigerian foods, including batches of rice, cassava and sorghum.

Calculation of BV, *TD and* NPU *of the different diets.* The individual endogenous urinary and faecal N excretion had been measured for subjects O, P, Q, R, S, T and the BV, TD and NPU of the rice diet had been calculated for this group of subjects using the formulae given as footnotes to Table 2. BV, TD and NPU of the egg and rice diets were not significantly different when given to these subjects (Nicol & Phillips, 1976*b*). The endogenous N excretion of subjects A, B, C, D, E, F and G, I, J, H, E, K, was not known, but their urinary and faecal N excretions were not significantly different from those of subjects O, P, Q, R, S, T when the three groups were given the rice diet (Table 2). Application of the mean values for endogenous urinary (1·86 g/d) and faecal (1·14 g/d) N of subjects O, P, Q, R, S, T to the mean urinary and faecal N excretion of the other two groups of subjects indicated that the BV, TD and NPU of the rice diet did not vary significantly between the three groups of subjects (Table 2). Therefore the mean endogenous levels of N excretion of subjects O, P, Q, R, S, T were used when calculating the BV, TD and NPU of other diets. These three groups of subjects comprised seventeen of the nineteen men participating in the thirteen feeding trials reported in this paper.

Design of feeding trials. The groups, each of six men, who were given the egg, rice, cassava and sorghum diets are shown in Table 3. Each of the feeding trials comprised a 6 d prebalance period and a 6 d balance period, with the exception of the series of trials in which DL-methionine and L-tryptophan were added to the rice diet given to subjects G, I, J, H, E, K. In that instance the subjects were given the rice diet for a 6 d prebalance period and a 6 d balance period, followed by consecutive 4 d balance periods during which the amino acids were added (see Table 3), the trial concluding with a 6 d balance period on the rice diet. All trials, and the series of trials in which subjects G, I, J, H, E, K participated, were separated by periods of 3 or 4 weeks, during which the men ate a mixed Nigerian diet.

The amounts of DL-methionine and L-tryptophan added to the rice and cassava diets were equal to the calculated difference between the total S amino acids and tryptophan supplied (/g N) by the diet and the amounts proposed (/g N) in the FAO (1957) provisional amino acid pattern. In view of doubts about its biological activity (Camien, Malin & Dunn, 1951; Rose, Coon, Lockhart & Lambert, 1955) the amount of DL-methionine added to the rice diet was doubled for a period of 4 d. The ingredients of the 'low-protein score' cassava diets (Table 1) were determined by their S amino acid score relative to the FAO (1957) provisional amino acid pattern.

Analytical methods. The methods employed to collect samples of food, urine and faeces, and the analytical procedures used, have been described (Nicol & Phillips, 1976*a*). Crude fibre was determined by the method of the Association of Official Analytical Chemists (1955).

Statistical analysis. Significance of differences between mean values was determined by paired *t* tests, $P < 0.05$ being considered significant. Mean values are given with their standard error.

The term 'N balance' throughout the rest of this paper refers to 'apparent N balance', calculated as N intake – (urinary N + faecal N), excluding cutaneous and other minor N losses.

RESULTS

Utilization of egg, rice, cassava and sorghum proteins by young Nigerian men

The changes in body-weight recorded during the balance periods when different groups of men took part in thirteen feeding trials were not significantly related to energy or N intake, N balance, BV, TD or NPU.

N balance and the BV, TD and NPU of the egg diet and the rice diet did not differ significantly when given to subjects O, P, Q, R, S, T. When subjects G, I, J, H, E, K consumed the rice diet the addition of DL-methionine and L-tryptophan increased N balance significantly ($P < 0.02$), BV increasing from 0·72 to 0·81 and NPU from 0·68 to 0·75.

The addition of DL-methionine to the 'low-protein score' cassava diet (subjects G, B, H, I, E, F) produced an increase in N balance of 0·57 g/d, BV increasing from 0·66 to 0·82 and NPU from 0·59 to 0·76.

The difference in N balance was not significantly different when subjects L, I, M, H, E, K were given the milled whole-meal sorghum or the home-pounded sorghum diets, variances around mean values being high. The difference in BV was small, the considerable difference in NPU resulting from the low TD of the milled whole-meal sorghum.

The mean findings for the thirteen feeding trials (Table 3) indicated that young Nigerian men maintained constant body-weight and were in N equilibrium when energy intake was approximately 180 kJ (43 kcal/kg body-weight per d), protein intake 25 g/d (0·44 g/kg body-weight per d), BV, TD and NPU of dietary protein being 0·78, 0·90 and 0·70 respectively. NPU was correlated more closely to N balance ($r + 0.97$; $P < 0.001$) than were BV ($r + 0.77$; $P < 0.01$) or TD ($r + 0.73$; $P < 0.01$).

Dietary crude fibre and digestibility of the egg, rice, cassava and sorghum diets

The total crude fibre content of the thirteen diets is given in Table 3. Most of the dietary fibre was derived from cassava 'gari', and less from the cereal and sauce ingredients, as follows: egg diet: 85% from cassava, 15% from sauce; rice diets: 76% from cassava (range 72–79%), 9% from rice (range 8–12%), 14% from sauce (range 13–17%); cassava diets: 81% from cassava (range 78–86%), 19% from sauce (range 14–22%); milled whole-meal sorghum diet: 67% from cassava, 19% from sorghum, 14% from sauce; home-pounded sorghum diet: 72% from cassava, 13% from sorghum, 15% from sauce.

The mean crude fibre content of the four basal rice diets was 9.4 ± 0.5 g/d and that of the three cassava diets was 12.8 ± 0.7 g/d, but TD was approximately the same for each group, 0.90 ± 0.02 and 0.92 ± 0.02 respectively.

Fibre derived from milled whole-meal sorghum (% of total fibre) was only 6% higher than that derived from home-pounded sorghum. Yet this difference had a considerable effect on the TD and NPU of the two diets. TD and NPU of the home-pounded sorghum diet were the same as those of the rice diets, whereas the values for the milled whole-meal sorghum diet were inferior to those of the 'low-protein score' cassava diet.

Dietary crude fibre for the thirteen diets was negatively, but not significantly, related to TD but was significantly correlated to NPU ($r - 0.62$; $P < 0.05$). NPU was significantly correlated to TD ($r - 0.67$; $P < 0.05$).

Essential amino acids, amino acid scores, N balance and NPU *of egg, rice, cassava and sorghum diets*

The essential amino acid content of each of the thirteen diets was compared with N balance, NPU and the first and second limiting amino acids calculated by applying the FAO 1957 'provisional amino acid pattern' (FAO, 1957) and the FAO/WHO 1973 'provisional amino acid scoring pattern' (FAO/WHO, 1973) (Table 4).

The amino acid score of the egg diet was 100 judged by both patterns, N balance being $+0.56 \pm 0.04$ g/d and NPU 0.84 ± 0.12. N balance was $+0.03 \pm 0.02$ g/d, NPU 0.71 ± 0.01 and the amino acid score 70 (S amino acids; FAO, 1957) or 81 (lysine; FAO/WHO, 1973) when the rice diet was given to three different groups each of six men. The relationship between N balance, NPU and amino acid scores of the cassava and sorghum diets is given in Table 4, together with the effects on amino acid patterns and scores observed by supplementing the rice and 'low-protein score' cassava diets with DL-methionine and L-tryptophan.

Inspection of Table 4 shows that in only one instance, the 'low-protein score' cassava diet, did both scoring patterns give the same first limiting amino acid (S amino acids), and that the second limiting amino acid was never the same for the thirteen diets. The S amino acids were limiting for all diets, whether based on rice, cassava or sorghum, the second being tryptophan, when compared against the FAO (1957) pattern. Addition of DL-methionine to the 'low-protein score' cassava diet in an amount sufficient to meet the amount proposed by FAO (1957), resulted in tryptophan being the first limiting, and isoleucine the second limiting, amino acid. When compared with the FAO/WHO (1973) scoring pattern, lysine was the first limiting amino acid for rice and sorghum diets, S amino acids being second for rice diets and threonine second for sorghum diets. When the FAO (1957) needs for S amino acids in rice diets were met, threonine was the second limiting amino acid, judged by the FAO/WHO (1973) pattern. In the instance of cassava based diets the picture presented by comparison with the FAO/WHO (1973) pattern became confused, valine being the first limiting amino acid of the 'high-protein score' cassava diet.

The amino acid scores of the thirteen diets were 79 ± 4 (FAO, 1957) and 80 ± 2 (FAO/WHO, 1973). N balance was $+0.06 \pm 0.11$ g/d and NPU 0.70 ± 0.02. Correlations between amino acid scores, N balance and NPU for all diets were: FAO 1957 score *v*. N balance, $r + 0.69$ ($P < 0.01$); FAO/WHO 1973 score *v*. N balance, $r + 0.70$ ($P < 0.01$); FAO 1957 score *v*. NPU, $r + 0.69$ ($P < 0.01$); FAO/WHO 1973 score *v*. NPU, $r + 0.74$ ($P < 0.01$). Correlations between BV and amino acid scores were: BV *v*. FAO 1957 score, $r + 0.58$ ($P < 0.05$); BV *v*. FAO/WHO 1973 score, $r + 0.58$ ($P < 0.05$).

The amounts of essential amino acids provided by the thirteen diets were expressed as mg/g N and mg/g protein, assuming a daily intake of 4 g N and 25 g protein ($N \times 6.25$). The least amount of each essential amino acid provided by those diets which maintained the subjects in N equilibrium or positive N balance, expressed as mg/g N and mg/g protein per d, were rounded to the nearest multiple of ten and compared, in Table 4, with the levels of essential amino acids in the scoring patterns suggested by FAO (1957) and FAO/WHO (1973). The major differences between the present findings and those of FAO (1957) and FAO/WHO (1973) were:

leucine > FAO 1957, = FAO/WHO 1973;
lysine = FAO 1957, < FAO/WHO 1973;
S amino acids < FAO 1957, < FAO/WHO 1973;
threonine = FAO 1957, < FAO/WHO 1973;
tryptophan < FAO 1957, = FAO/WHO 1973.

The separation of the values found for the egg diet from those of the other twelve diets made no significant difference to mean values for energy or protein intake, change in body-weight during balance periods, N balance, BV, TD or NPU, or to the essential amino acid pattern of all thirteen diets considered together (Tables 3 and 4).

Table 3. *Body-weight, energy and nitrogen intake and apparent N balance related to the biological value* (BV),* *true digestibility (TD)*,† *net protein utilization* (NPU)‡ *and dietary crude fibre when nineteen young Nigerian men were given diets based on egg, rice, cassava* (Manihot utilissima) *or sorghum* (Sorghum sativa), *certain diets being supplemented by synthetic* DL-*methionine and* L-*tryptophan*

	Body-wt (kg)		Change in§ body-wt (kg)		Energy intake (/d)		N intake and excretion (g/d)				
								Urinary		Faecal	
Diet and subjects	Mean	SE	Mean	SE	MJ	kcal	Intake	Mean	SE	Mean	SE
Rice											
O, P, Q, R, S, T	53·83	1·38	+0·05	0·21	10·04	2400	3·86	2·28	0·11	1·55	0·10
A, B, C, D, E, F	56·29	0·66	+0·30	0·13	10·21	2440	4·00	2·23	0·08	1·70	0·09
G, I, J, H, E, K	58·04	0·75	−0·12	0·10	10·21	2440	3·97	2·58	0·15	1·39	0·08
+318 mg Met											
G, I, J, H, E, K	57·94	0·73	−0·11	0·11	10·21	2440	4·00	2·48	0·14	1·37	0·06
+318 mg Met+75 mg Try											
G, I, J, H, E, K	57·80	0·68	−0·06	0·09	10·21	2440	4·00	2·38	0·11	1·48	0·08
+636 mg Met+75 mg Try											
G, I, J, H, E, K	57·75	0·71	−0·05	0·09	10·21	2440	4·04	2·36	0·08	1·40	0·09
G, I, J, H, E, K	57·70	0·66	−0·05	0·06	10·04	2400	3·92	2·45	0·12	1·46	0·06
Cassava											
Low-protein score											
G, B, H, I, E, F	57·87	0·66	−0·03	0·15	10·25	2450	3·95	2·66	0·13	1·59	0·07
+510 mg Met											
G, B, H, I, E, F	57·77	0·63	−0·23	0·08	10·25	2450	4·00	2·32	0·11	1·40	0·08
High-protein score											
G, B, H, I, E, F	57·31	0·92	+0·05	0·10	9·87	2360	3·85	2·56	0·17	1·32	0·11
Sorghum											
Milled whole-meal											
L, I, M, H, E, K	56·76	0·98	−0·13	0·07	10·38	2480	3·90	2·37	0·14	1·94	0·04
Home-pounded flour											
L, I, M, H, E, K	56·89	1·00	−0·13	0·12	10·42	2490	3·96	2·39	0·12	1·57	0·09
Mean ± SE (*n* 12)	57·16	0·82	−0·04	0·11	10·19 ± 0·04		3·95 ± 0·02	2·42	0·12	1·51	0·08
Egg: O, P, Q, R, S, T	53·21	1·33	+0·41	0·07	10·08	2410	3·92	2·15	0·18	1·21	0·14
Mean ± SE (*n* 13)	56·86	0·86	−0·01	0·10	10·18 ± 0·04		3·95 ± 0·02	2·40	0·13	1·49	0·08

Table 3 (*cont.*)

Diet and subjects	Apparent N‖ balance (g/d) Mean	SE	BV Mean	SE	TD Mean	SE	NPU Mean	SE	Dietary crude fibre (g/d)
Rice									
O, P, Q, R, S, T	+0·03	0·21	0·83	0·06¶	0·88	0·01	0·73	0·04¶	9·3
A, B, C, D, E, F	+0·07	0·06	0·84		0·86		0·72		9·5
G, I, J, H, E, K	0		0·72		0·94		0·68		9·5
+318 mg Met									
G, I, J, H, E, K	+0·15	0·11	0·76		0·94		0·71		9·5
+318 mg Met+75 mg Try									
G, I, J, H, E, K	+0·13	0·10	0·79		0·92		0·73		9·4
+636 mg Met+75 mg Try									
G, I, J, H, E, K	+0·28	0·07	0·81		0·93		0·75		9·5
G, I, J, H, E, K	+0·01	0·11	0·76		0·92		0·70		9·4
Cassava									
Low-protein score									
G, B, H, I, E, F	−0·29	0·13	0·66		0·89		0·59		13·5
+510 mg Met									
G, B, H, I, E, F	+0·28	0·12	0·82		0·93		0·76		13·5
High-protein score									
G, B, H, I, E, F	−0·03	0·11	0·72		0·95		0·68		11·3
Sorghum									
Milled whole-meal									
L, I, M, H, E, K	−0·41	0·20	0·74		0·79		0·58		12·5
Home-pounded flour									
L, I, M, H, E, K	−0·01	0·14	0·78		0·89		0·69		11·6
Mean ± SE (*n* 12)	+0·02	0·11	0·77	0·02	0·90	0·01	0·69	0·02	10·7 ± 0·5
Egg: O, P, Q, R, S, T	+0·56	0·04	0·90	0·08¶	0·93	0·06	0·84	0·12¶	8·2
Mean ± SE (*n* 13)	+0·06	0·11	0·78	0·02	0·90	0·01	0·70	0·02	10·5 ± 0·5

Met, methionine; Try, tryptophan; MPD, minimal protein diet used to determine endogenous urinary and faecal N excretion (Nicol & Phillips, 1976*a*).

* BV = absorbed N − (urinary $N_{trial\ diet}$ − urinary N_{MPD}) ÷ absorbed N.

† TD = dietary N − (faecal $N_{trial\ diet}$ − faecal N_{MPD}) ÷ dietary N.

‡ NPU = BV × TD.

§ During the balance periods of the feeding trials.

‖ Excluding cutaneous and other minor N losses.

¶ Individual values for endogenous urinary and faecal N had been determined for these subjects so the standard error could be calculated.

Table 4. *Apparent nitrogen balance,* net protein utilization* (NPU),† *essential amino acid content and first and second limiting amino acids (mg/g protein and mg/g N), calculated by both the FAO* (1957) *and FAO/WHO* (1973) *patterns,‡ when young Nigerian men were given egg, rice, sorghum* (Sorghum sativa) *and cassava* (Manihot utilissima) *diets, certain diets being supplemented by synthetic* DL-*methionine and* L-*tryptophan*

	Apparent N balance (g/d)			Amino acid scores (First and second limiting amino acids)			
				FAO 1957		FAO/WHO 1973	
Diet and subjects	Mean	SE	NPU	First	Second	First	Second
Rice							
O, P, Q, R, S, T	+0·03	0·21	0·73	71 (Met+Cys)	81 (Try)	81 (Lys)	87 (Met+Cys)
A, B, C, D, E, F	+0·07	0·06	0·72	69 (Met+Cys)	81 (Try)	80 (Lys)	87 (Met+Cys)
G, I, J, H, E, K	0		0·68	70 (Met+Cys)	83 (Try)	80 (Lys)	88 (Met+Cys)
+318 mg Met							
G, I, J, H, E, K	+0·15	0·11	0·71	83 (Try)	100	80 (Lys)	88 (Thr)
+318 mg Met+75 mg Try							
G, I, J, H, E, K	+0·13	0·10	0·73	100	100	80 (Lys)	88 (Thr)
+636 mg Met+75 mg Try							
G, I, J, H, E, K	+0·28	0·07	0·75	100	100	80 (Lys)	88 (Thr)
G, I, J, H, E, K	+0·01	0·70	0·70	70 (Met+Cys)	80 (Try)	81 (Lys)	88 (Met+Cys)
Cassava							
Low-protein score							
G, B, H, I, E, F	−0·29	0·13	0·59	56 (Met+Cys)	78 (Try)	69 (Met+Cys)	76 (Thr)
+510 mg Met							
G, B, H, I, E, F	+0·28	0·12	0·76	78 (Try)	90 (Ile)	76 (Thr)	78 (Lys)
High-protein score							
G, B, H, I, E, F	−0·03	0·11	0·68	79 (Met+Cys)	84 (Try)	88 (Val)	92 (Thr)
Sorghum							
Milled whole-meal							
L, I, M, H, E, K	−0·41	0·20	0·58	74 (Met+Cys)	88 (Try)	73 (Lys)	85 (Thr)
Home-pounded flour							
L, I, M, H, E, K	−0·01	0·14	0·69	75 (Met+Cys)	86 (Try)	72 (Lys)	85 (Thr)
Mean ± SE (*n* 12)	+0·02	0·11	0·69 ± 0·02	77 ± 3	87 ± 2	78 ± 2	86 ± 2
mg/g protein (N × 6·25)							
mg/g N							

Table 4 (*cont.*)

Diet and subjects	Apparent N balance (g/d)		NPU	Amino acid scores (First and second limiting amino acids)			
				FAO 1957		FAO/WHO 1973	
	Mean	SE		First	Second	First	Second
Egg: O, P, Q, R, S, T	+0·56	0·04	0·84±0·12	100	100	100	100
Mean±SE (n 13)	+0·06	0·11	0·70±0·02	79±4	89±2	80±2	87±2
mg/g protein (N×6·25)	—	—	—	—	—	—	—
mg/g N	—	—	—	—	—	—	—
FAO/WHO 1973 scoring pattern							
mg/g protein (N×6·25)	—	—	—	—	—	—	—
mg/g N	—	—	—	—	—	—	—
FAO 1957 scoring pattern							
mg/g protein (N×6·25)	—	—	—	—	—	—	—
mg/g N	—	—	—	—	—	—	—
Minimum requirement for N equilibrium (present results)							
mg/d	—	—	—	—	—	—	—
mg/g protein (N×6·25)	—	—	—	—	—	—	—

Table 4 (*cont.*)

Diet and subjects	Essential amino acids (mg/d)							
	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Try	Val
Rice								
O, P, Q, R, S, T	1040	1830	1090	740	1905	850	290	1320
A, B, C, D, E, F	1050	1860	1080	745	1915	885	290	1370
G, I, J, H, E, K	1040	1840	1080	740	1920	880	290	1345
+318 mg Met								
G, I, J, H, E, K	1040	1840	1080	1060	1920	880	290	1345
+318 mg Met+75 mg Try								
G, I, J, H, E, K	1040	1840	1080	1060	1920	880	365	1345
+636 mg Met+75 mg Try								
G, I, J, H, E, K	1040	1840	1080	1380	1920	880	365	1345
G, I, J, H, E, K	1020	1800	1080	760	1920	840	280	1300
Cassava								
Low-protein score								
G, B, H, I, E, F	950	1830	1040	600	1735	750	280	1075
+510 mg Met								
G, B, H, I, E, F	950	1830	1040	1110	1735	750	280	1075
High-protein score								
G, B, H, I, E, F	1040	1660	1520	820	1490	880	290	1060
Sorghum								
Milled whole-meal								
L, I, M, H, E, K	1140	2700	950	770	1680	810	295	1160
Home-pounded flour								
L, I, M, H, E, K	1180	2830	970	800	1740	840	305	1205
Mean ± SE (*n* 12)	1044	1975	1091	881	1817	844	302	1245
	42	79	44	35	73	34	12	50
mg/g protein (N × 6·25)								
mg/g N	261	494	273	220	454	211	76	311
Egg: O, P, Q, R, S, T	1450	1960	1460	1165	2230	1095	380	1600
Mean ± SE (*n* 13)	1075	1980	1119	903	1848	863	308	1273
	43	79	45	36	74	35	12	51
mg/g protein (N × 6·25)								
mg/g N	269	495	280	226	462	216	77	318

Table 4 (*cont.*)

Diet and subjects	Essential amino acids (mg/d)							
	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Try	Val
FAO/WHO 1973 scoring pattern								
mg/g protein (N × 6·25)	40	70	55	35	60	40	10	50
mg/g N	250	440	340	220	380	250	60	310
FAO 1957 scoring pattern								
mg/g protein (N × 6·25)	42	48	42	42	56	28	14	42
mg/g N	270	306	270	270	360	180	90	270
Minimum requirement for N equilibrium (present results)								
mg/d	950	1700	1050	750	1500	750	280	1300
mg/g protein (N × 6·25)	40	70	40	30	60	30	10	50

Met, methionine; Try, tryptophan; Ile, isoleucine; Leu, leucine; Lys, lysine; Cys, cystine; Phe, phenylalanine; Tyr, tyrosine; Thr, threonine; Val, valine.

* Excluding cutaneous and other minor N losses.

† NPU = BV × TD.

‡ For details, see pages 275 and 284.

DISCUSSION

The amino acid scores of diets are not always dependent on the first limiting amino acid of the staple foodstuff due to the amino acid composition of the supplementary foods which are added to make diets acceptable to consumers. Calculated amino acid scores of staple foodstuffs or diets are dependent also on the reference pattern of essential amino acids used as a basis for comparison. The amino acid scores and first limiting amino acids of rice proteins were calculated to be 54 (lysine), 72 (tryptophan) and 69 (lysine) when compared respectively with the amino acid pattern of hen's egg protein (Lunven, De St Marcq, Carnovale & Fratoni, 1973), the FAO 1957 'provisional pattern of amino acids' and the FAO/WHO 1973 'provisional amino acid scoring pattern'. Using the same three standards for comparison the amino acid scores of sorghum proteins were calculated to be 41, 66 and 52 respectively, lysine being limiting in each instance, and the score of cassava proteins were 17, 22 and 27 respectively, S amino acids being limiting in each instance. The amino acid scores of the rice diet proteins were 54 (S amino acids), 70 (S amino acids) and 80 (lysine); of the home-pounded sorghum diet 56 (S amino acids = lysine), 75 (S amino acids) and 72 (lysine); and of the 'low-protein score' cassava diet 43, 56 and 69 respectively, S amino acids being limiting in each instance. It was impossible to devise a cassava-based diet acceptable to our subjects having an amino acid score less than 56 (FAO, 1957) or 69 (FAO/WHO, 1973).

The feeding trials reported in this paper were carried out before the FAO/WHO (1973) 'provisional amino acid scoring pattern' had been published. Therefore DL-methionine and L-tryptophan were added to the rice and cassava diets in amounts calculated to cover the deficits between dietary levels and the amounts of these amino acids proposed in the FAO (1957) 'provisional pattern of amino acids' (Table 4).

Allison, Anderson & Seeley (1947) and Allison (1955) reported a protein-sparing effect of DL-methionine when added to a protein-free diet given to protein-depleted dogs, evidenced by reduction of urinary N excretion. When casein was added to the protein-free diet urinary urea-N increased, but fell after the addition of DL-methionine to the casein. S amino acids are limiting in casein, thus this fall in urinary N could be attributed to increased BV of the dietary protein mixture. Our subjects were not depleted of protein but the addition of DL-methionine to the rice diet led to a fall in urinary N excretion, which increased when L-tryptophan was added, resulting in higher values for BV and NPU (Table 3). These higher values were reflected by an increase in amino acid scores calculated by comparison with the FAO (1957) scoring pattern, but were not reflected by comparison with the FAO/WHO (1973) scoring pattern (Table 4).

The present results indicate that healthy young ambulant Nigerian men lost or gained only small amounts of body-weight when given mixed diets based on rice, cassava or sorghum when energy intake was approximately 180 kJ (43 kcal)/kg body-weight per d and protein intake 0·41–0·44 g/kg body-weight per d.

The amount of protein ($N \times 6{\cdot}25$) provided by the twelve mixed diets was $24{\cdot}7 \pm 0{\cdot}1$ g/d, or 0·43 g/kg body-weight per d. NPU was $0{\cdot}69 \pm 0{\cdot}02$ and N balance approximately equilibrium. Applying methods recommended by the Joint FAO/WHO *ad hoc* Expert Committee on Energy and Protein Requirements (FAO/WHO, 1973) the safe level of intake of egg protein to maintain N equilibrium in adult men was calculated to be 0·44 g/kg body-weight per d. Adjusted by the NPU of the egg diet (0·84) the amount of protein provided by the twelve mixed sorghum, rice and cassava diets for maintenance of equilibrium would be 0·54 g/kg body-weight per d. The NPU of eight mixed diets not supplemented by amino acids was $0{\cdot}67 \pm 0{\cdot}02$, and the amount of protein required for maintenance of N balance in this instance would be 0·55 g/kg body-weight per d.

These findings indicate that Nigerian men use ingested protein more efficiently than would be expected by applying the recommendations of the FAO/WHO Committee (FAO/WHO, 1973). The present results are in agreement with our earlier findings for the utilization of egg and rice diets by Nigerian men (Nicol & Phillips, 1976*a*, *b*). The physiological processes involved in adaptation to traditionally low protein intakes have been described by Stephen & Waterlow (1968) and Waterlow (1968).

The results obtained by adding DL-methionine and L-tryoptophan to the rice diet (Tables 3 and 4) in amounts which bridged the gap between dietary intake and the levels suggested by the FAO Committee (FAO, 1957), suggest that S amino acids were limiting in this diet and that the level of tryptophan in the FAO (1957) provisional amino acid pattern may have been too high. The effects on N balance and NPU produced by adding twice the calculated requirement of methionine as the DL-isomer are in agreement with the view that DL-methionine has only half the biological activity of the L-isomer (Irwin & Hegsted, 1971). Such conclusions must be qualified by recognition of the short duration of each balance period (4 d) and the fact that they were consecutive, possibly resulting in a cumulative effect on N balance and NPU for a 12 d period. The effect of adding DL-methionine to the 'low-protein score' cassava diet cannot be qualified by the duration of the feeding trial, which comprised 6 d prebalance and balance periods. The increase in N balance and NPU resulting from the addition of 510 mg DL-methionine to this diet resulted in values equal to those recorded for the rice diet. This amount of S amino acids is provided by the amounts of pumpkin and melon seeds, food legumes, fish and meat included in diets based on starchy roots eaten in many areas of Southern Nigeria (Nicol, 1953, 1959*b*). The effect of amino acid supplementation, or the addition of small amounts of high quality protein, to diets having NPU values of approximately 0·60 or less, is well known to be more effective than that of similar additions to diets with NPU 0·70 or above (FAO, 1957).

TD of the rice and cassava diets was not significantly different, $0{\cdot}91 \pm 0{\cdot}01$ and $0{\cdot}92 \pm 0{\cdot}02$ respectively, although the amounts of dietary crude fibre were $9{\cdot}4 \pm 0{\cdot}03$ g/d for rice diets and $12{\cdot}8 \pm 0{\cdot}7$ g/d for cassava diets, indicating the high digestibility of cassava 'gari' fibre (Phillips & Ladell, 1959). The crude dietary fibre of the sorghum diets was lower than that of the cassava diets yet N balance, TD and NPU of the milled whole-meal sorghum diet were lower, and faecal N and dry faecal weight were higher, than the values recorded for any other diet in this series of feeding trials. The NPU and TD of home-pounded winnowed sorghum flour were similar to those of rice. These findings are in line with those of Subrahmanyan, Narayanarao, Ramarao & Swaminathan (1955), who compared the protein digestibilities of whole-meal 'ragi' (*Eleucine coracana*) and rice.

The NPU of dietary protein by definition includes its digestibility. Present results show that NPU is very closely related to N balance for subjects in approximate N equilibrium (Allison, 1955), this correlation being the same for all thirteen diets, and for the eight diets unsupplemented by synthetic amino acids, included in this feeding trial. The correlation between amino acid scores and NPU for the eight diets was the same ($P < 0{\cdot}05$) whether calculated by the FAO (1957) or the FAO/WHO (1973) patterns, but the levels of score were different: FAO 1957, 71 ± 2; FAO/WHO 1973, 78 ± 2. Present findings (Table 4) show that the FAO 1957 scores were in good agreement with NPU determined by feeding trials participated in by Nigerian men, exceptions being the sorghum-based diets. The FAO/WHO 1973 amino acid scores for rice and cassava diets, unsupplemented by amino acids, were approximately 14% higher than those calculated using the FAO 1957 pattern, except in the instance of sorghum-based diets when the scores were the same, although the first and second limiting amino acids changed from S amino acids and tryptophan to lysine and threonine. NPU of the whole-meal sorghum diet was 78% of the calculated amino acid scores, and that of the home-pounded sorghum diet 92% of the calculated amino acid scores.

These results for sorghum-based diets lend some credence to the proposal of the Joint FAO/WHO Informal Gathering of Experts on Protein and Energy Requirements (1975) that amino acid scores calculated by using the FAO/WHO (1973) pattern of amino acids be corrected for digestibility by 85% and 90% respectively in the instance of diets based on whole-meal or refined cereal flours. Regarding the diets based on rice and cassava, with vegetables, any adjustment of the FAO/WHO (1973) amino acid score on the grounds of digestibility seems to be unreasonable. In these instances a reduction of the FAO/WHO (1973) amino acid score in the range of 85–90% would be reasonable on the grounds that the score is 10–15% higher than the NPU as measured by feeding trials.

It is not possible, from present results, to assess the effects of the complete change in first and second limiting amino acids which results by applying the FAO/WHO (1973) pattern rather than the FAO (1957) pattern. The effects of variation of the essential amino acid scoring pattern when applied to national food balance sheets have been discussed by Lunven *et al.* (1973). The essential amino acid pattern, calculated from the least amount of each provided by the mixed rice, cassava and sorghum diets, which maintained N equilibrium or slightly positive N balance, when given to young Nigerian men (Table 4), suggests that the increase of lysine proposed by FAO/WHO (1973) in excess of the amount suggested by FAO (1957) was unnecessary. Traditional methods of food preparation and cooking in Nigeria may be of importance in this regard. The reductions in levels of S amino acids and tryptophan was justified by our findings. The requirements of the Nigerian men for S amino acids were approximately the same, on a body-weight basis, as those of North American students (Clark, Howe, Shannon, Carlson & Kolski, 1970; Zezulka & Calloway, 1976). The increase in the amount of threonine was not in line with the needs of Nigerian men.

The low amounts of leucine included in the FAO (1957) pattern was prompted by the concept of isoleucine:leucine imbalance (Harper, Benton, Winje & Elvehjem, 1954). Present findings agree with those of FAO/WHO (1973). The question of the low NPU of sorghum protein has been attributed to isoleucine:leucine imbalance. The addition of 1 g L-leucine to a rice-based diet increased leucine:isoleucine to that of a sorghum-based diet, given at similar levels of energy and N intake. It was concluded that the low NPU of sorghum was due to poor digestibility rather than amino acid imbalance (Nicol & Phillips, 1961).

Amino acid requirements of man, and essential amino acid patterns, are produced by one expert committee or group after another. The only common factor is that their findings are always carefully labelled 'provisional'.

The authors are indebted to the late Sir Samuel Manuwa, Chief Medical Adviser to the Federal Government of Nigeria, who originally gave them permission to publish the research results and the material included in this paper. They wish to thank the entire staff of the Federal Nutrition Unit who made it possible to carry out this study. The work was supported by a grant (RF-NRC-8) from the Committee on Protein Malnutrition of the US National Research Council's Food and Nutrition Board.

REFERENCES

Allison, J. B. (1955). *Physiol. Rev.* **35**, 664.
Allison, J. B., Anderson, J. A. & Seeley, R. D. (1947). *J. Nutr.* **33**, 361.
Association of Official Analytical Chemists (1955). *Official Methods of Analysis*, 8th ed. Washington, DC: Association of Official Analytical Chemists.
Block, R. J. & Mitchell, H. H. (1946). *Nutr. Abstr. Rev.* **16**, 249.
Camien, M. N., Malin, R. B. & Dunn, M. S. (1951). *Archs Biochem.* **30**, 62.
Clark, H. E., Howe, J. M., Shannon, B. M., Carlson, K. & Kolski, K. (1970). *Am. J. clin. Nutr.* **23**, 731.
FAO (1957). *Nutr. Stud.* no. 16.
FAO/WHO (1973). *Tech. Rep. Ser. Wld Hlth Org.* no 522.
FAO/WHO (1975). *Fd Nutr.* (FAO, Rome) **1**, no. 2.

Harper, A. E., Benton, D. A., Winje, M. E. & Elvehjem, C. A. (1954). *Archs Biochem. Biophys.* **51**, 523.
Irwin, M. I. & Hegsted, D. M. (1971). *J. Nutr.* **101**, 539.
Lunven, P., C. Le Clement De St Marcq, Carnovale, E. & Fratoni, A. (1973). *Br. J. Nutr.* **30**, 189.
McCance, R. A. & Walsham, C. M. (1948). *Br. J. Nutr.* **2**, 26.
Nicol, B. M. (1953). *Proc. Nutr. Soc.* **12**, 66.
Nicol, B. M. (1959*a*). *Br. J. Nutr.* **13**, 293.
Nicol, B. M. (1959*b*). *Br. J. Nutr.* **13**, 307.
Nicol, B. M. & Phillips, P. G. (1961). *Publs natn. Res. Coun., Wash.* no. 843.
Nicol, B. M. & Phillips, P. G. (1976*a*). *Br. J. Nutr.* **35**, 181.
Nicol, B. M. & Phillips, P. G. (1976*b*). *Br. J. Nutr.* **36**, 337.
Orr, M. L. & Watt, B. K. (1957). *Home Econ. Res. Rep. US Dept. Agric.* no. 4.
Phillips, P. G. & Ladell, W. S. S. (1959). *J. trop. Med. Hyg.* **63**, 3.
Rose, W. C., Coon, J. M., Lockhart, H. B. & Lambert, G. F. (1955). *J. biol. Chem.* **215**, 101.
Southgate, D. A. T. & Durnin, J. V. G. A. (1970). *Br. J. Nutr.* **24**, 517.
Stephen, J. M. L. & Waterlow, J. C. (1968). *Lancet* i, 118.
Subrahmanyan, V., Narayanarao, M., Ramarao, G. & Swaminathan, M. (1955). *Br. J. Nutr.* **9**, 550.
Waterlow, J. C. (1968). *Lancet* ii, 1091.
Zezulka, A. Y. & Calloway, D. H. (1976). *J. Nutr.* **106**, 212.

Printed in Great Britain