Nutritive value of mixed proteins

2*. As determined by net protein utilization and protein efficiency ratio tests

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1. A fish meal, meat meal, soya-bean meal, groundnut meal and sunflower-seed meal of known amino acid composition were evaluated individually, and combined in all possible pairs, by the estimation of net protein utilization (NPU) and protein efficiency ratio (body-weight gain:crude protein intake; PER) using rats. Each pair provided a total of 100 g protein/kg diet made up so that the amounts of the constituents were (w/w) 100:0, 80:20, 60:40, 20:80 and 0:100.

2. Marked synergistic effects were noted only for mixtures of sunflower-seed meal with soya-bean, fish and meat meals.

3. Chemical score ([amount of limiting amino acid/the rat's requirement for the same amino acid] × 100; CS), but not essential amino acid index; geometric mean for the ratio, amount of essential amino acid: the rat's requirement for that amino acid, for all ten essential amino acids; EAAI), successfully predicted the rankings of all mixtures except groundnut meal–meat meal and groundnut meal–soya-bean meal, by both PER and NPU tests.

4. Although there is broad agreement linking results of PER and NPU tests with results obtained by a more practical feeding trial in which the mixtures were evaluated as supplements to cereals, neither of these two standard tests is capable of predicting in every instance the advantages to be gained by mixing protein concentrates.

An attempt has been made to relate the amino acid composition to the nutritive value for chicks of cereal-based diets containing pairs of protein concentrates combined in varying proportions (Woodham & Deans, 1977). It was shown that while the adequacy of the limiting amino acid, expressed as chemical score ([amount of limiting amino acid/the chick's requirement for the same amino acid] × 100; CS), frequently acted as a useful predictor of protein quality there were a number of instances in which it failed to do so, and this failure was attributed to short-comings in over-all amino acid balance in the diets concerned. The chick growth test used, total protein efficiency (g weight gain/g protein consumed; TPE), is a measure of the supplementary value of the protein concentrate, and further evaluations were carried out with similar mixtures to those used in the second part of the study described previously (Woodham & Deans, 1977), and using rat tests in which the mixed proteins were given alone, as the only protein sources. These standard tests were the protein efficiency ratio (body-weight gain:crude protein intake; PER) and the estimation of net protein utilization (NPU).

METHODS

PER. Male rats of the Rowett Hooded Lister strain, aged 19–21 d, were used. The technique was that described as the official method of the Association of Official Agricultural Chemists (Derse, 1960, 1962, 1965). Each diet was given to ten indivi-

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Table 1. *Mean intake of crude protein (nitrogen × 6·25) (g/rat per 28 d), mean body-weight gain (g/rat per 28 d), and protein efficiency ratio (body-weight gain: crude protein intake;* PER*) corrected to a casein value of 2·5 for rats given diets containing pairs of protein concentrates* (PC1, PC2) mixed in varying proportions to contribute* 100 g *protein/kg*

		Relative amounts of PC1–PC2 (w/w)					
	PC1–PC2	100:0	80:20	60:40	40:60	20:80	0:100
Protein intake	MM–GN	13·1	14·7	22·0	24·1	24·9	25·1
Body-wt gain		10	18	33	41	49	49
PER		0·55	0·90	1·13	1·27	1·48	1·46
Protein intake	FM–GN	34·2	34·2	34·2	31·2	29·4	21·3
Body-wt gain		90	101	90	75	63	36
PER		2·28	2·23	2·00	1·80	1·62	1·43
Protein intake	FM–MM	33·1	29·5	25·1	20·0	14·1	11·9
Body-wt gain		99	72	57	39	19	6
PER		2·24	1·84	1·70	1·45	1·04	0·36
Protein intake	SF–MM	18·3	21·6	23·6	20·4	13·5	10·2
Body-wt gain		37	42	47	39	20	6
PER		1·46	1·43	1·45	1·41	1·06	0·39
Protein intake	SB–MM	35·2	30·4	28·0	24·2	20·1	15·4
Body-wt gain		85	65	48	34	20	12
PER		1·90	1·70	1·35	1·13	0·81	0·60
Protein intake	SB–GN	19·6	19·5	20·4	20·1	17·7	16·0
Body-wt gain		45	51	47	45	36	26
PER		1·79	2·09	1·85	1·76	1·65	1·46
Protein intake	SB–SF	34·6	41·6	40·4	40·1	31·2	26·3
Body-wt gain		85	106	102	100	72	52
PER		2·05	2·13	2·09	2·07	1·92	1·64
Protein intake	SF–FM	26·7	33·2	37·8	38·9	34·1	30·5
Body-wt gain		55	83	109	118	107	93
PER		1·58	1·89	2·20	2·32	2·40	2·30
Protein intake	SF–GN	21·6	23·4	24·8	22·7	18·4	18·5
Body-wt gain		41	46	47	43	34	32
PER		1·50	1·54	1·52	1·50	1·48	1·39
Protein intake	SB–FM	29·3	35·2	37·5	34·3	30·0	32·7
Body-wt gain		79	100	110	103	94	105
PER		1·90	1·98	2·06	2·11	2·19	2·24

MM, meat meal; GN, groundnut meal; FM, fish meal; SF, sunflower-seed meal; SB, soya-bean meal.

* For details, see Woodham & Deans (1977).

dually-caged rats. The levels of *myo*-inositol and riboflavin used in the vitamin mixture were increased to 250 and 10 mg/kg diet respectively (Campbell, 1963).

NPU. Male and female rats of the Rowett Hooded Lister strain were weaned at 19 d of age and given a stock diet for 10 d before commencing the experimental feeding. The method used was that described by Miller (1963) each diet being given to a group of four rats. NPU was determined both by body-water estimation and by carcass analysis.

Table 2. *Carcass nitrogen (g), N intake (g/d) and net protein utilization* (NPU) *for rats given diets containing pairs of protein concentrates* (PC1, PC2) mixed in varying proportions to contribute* 100 *g protein/kg*

	PC1–PC2	Bk	Ik	Relative amounts of PC1–PC2 (w/w)						PC1–PC2	Bk	Ik	Relative amounts of PC1–PC2 (w/w)					
				100:0	80:20	60:40	40:60	20:80	0:100				100:0	80:20	60:40	40:60	20:80	0:100
Carcass N	MM–GN	5·18	0·19	5·82	6·10	6·40	6·78	6·89	6·96	SB–GN	5·95	0·15	10·73	10·10	9·29	8·89	8·31	7·73
N intake				2·85	3·34	3·73	4·17	4·34	4·22				6·76	6·84	6·00	6·00	5·68	4·94
NPU				29	33	38	43	44	47				73	63	58	51	44	39
Carcass N	FM–GN	6·85	0·34	11·28	11·14	10·64	10·56	10·16	9·92	SB–SF	7·13	0·19	10·30	11·01	11·77	11·01	11·14	9·99
N intake				6·93	7·22	6·74	7·16	6·84	6·70				5·61	6·44	6·68	6·30	7·14	5·71
NPU				69	64	61	57	53	51				60	63	72	64	59	53
Carcass N	FM–MM	6·44	0·13	10·83	10·16	9·26	8·51	8·02	7·46	SF–FM	6·51	0·16	9·68	10·67	11·29	11·29	11·43	10·52
N intake				5·91	5·53	5·01	4·26	3·74	3·29				6·24	7·06	7·36	7·12	6·70	5·34
NPU				77	70	59	52	46	35				52	60	66	68	74	76
Carcass N	SF–MM	6·73	0·13	9·93	10·09	9·90	8·97	7·88	7·36	SF–GN	7·50	0·17	11·31	11·64	11·09	11·05	10·06	9·91
N intake				6·43	7·07	6·25	5·30	3·93	3·53				7·03	7·31	7·36	7·33	5·97	5·80
NPU				52	49	53	45	33	22				57	59	51	51	46	45
Carcass N	SB–MM	7·47	0·20	12·70	10·88	10·57	9·37	8·82	8·13	SB–FM	6·32	0·10	10·42	10·89	10·73	10·47	10·60	10·34
N intake				8·31	7·05	7·58	5·22	4·88	3·75				7·14	8·02	6·91	6·69	6·77	6·22
NPU				65	51	44	40	32	23				59	58	65	64	65	66

MM, meat meal; GN, groundnut meal; FM, fish meal; SF, sunflower-seed meal; SB, soya-bean meal; Bk, body N; Ik, N intake on the N-free diet of the NPU test.
* For details, see Woodham & Deans (1977).

Table 3. *Limiting amino acid (LAA), essential amino acid index* (EAAI), and chemical score† (CS) calculated for pairs of protein concentrates‡ (PC1, PC2) mixed in varying proportions to contribute 100 g protein/kg diet*

		Relative amounts of PC1–PC2 (w/w)							Relative amounts of PC1–PC2 (w/w)					
	PC1–PC2	100:0	80:20	60:40	40:60	20:80	0:100	PC1–PC2	100:0	80:20	60:40	40:60	20:80	0:100
LAA	MM–GN	C+M	C+M	C+M	C+M	C+M	C+M	SB–GN	C+M	C+M	C+M	C+M	C+M	C+M
EAAI		57·2	58·3	59·2	60·0	60·6	61·1		82·3	78·7	74·5	70·2	65·7	61·1
CS		31·6	32·0	32·3	32·6	33·0	33·3		40·0	38·6	37·3	36·0	34·6	33·3
LAA	FM–GN	C+M	C+M	C+M	C+M	C+M	C+M	SB–SF	C+M	C+M	C+M	LYS	LYS	LYS
EAAI		76·1	73·5	70·7	67·7	64·5	61·1		82·3	82·0	80·9	79·5	77·8	75·9
CS		46·6	44·0	41·3	38·6	36·0	33·3		40·0	46·3	52·6	55·3	48·2	41·1
LAA	FM–MM	C+M	C+M	C+M	C+M	C+M	C+M	SF–FM	LYS	LYS	LYS	C+M	C+M	C+M
EAAI		76·1	72·4	68·7	64·9	61·1	57·2		75·9	76·5	76·7	76·8	76·6	76·1
CS		46·6	43·6	40·6	37·6	34·6	31·6		41·4	49·1	57·1	56·6	51·6	46·6
LAA	SF–MM	LYS	LYS	LYS	C+M	C+M	C+M	SF–GN	LYS	LYS	LYS	LYS	LYS	C+M
EAAI		75·9	72·7	69·3	65·6	61·6	57·2		75·9	73·2	70·5	67·5	64·4	61·1
CS		41·1	44·4	47·7	47·6	39·6	31·6		41·1	40·0	38·8	37·7	36·6	33·3
LAA	SB–MM	C+M	C+M	C+M	C+M	C+M	C+M	SB–FM	C+M	C+M	C+M	C+M	C+M	C+M
EAAI		82·3	78·0	73·0	67·9	62·6	57·2		82·3	81·7	80·4	79·0	77·6	76·1
CS		40·0	38·3	36·6	35·0	33·3	31·6		40·0	41·3	42·6	44·0	45·3	46·6

MM, meat meal; GN, groundnut meal; FM, fish meal; SF, sunflower-seed meal; SB, soya-bean meal; C+M, cystine+methionine; LYS, lysine.

* Geometric mean for the ratio, amount of essential amino acid: the rat's requirement for that amino acid, for all ten essential amino acids.

† (Amount of LAA/the rat's requirement for the same amino acid) × 100.

‡ For details, see Woodham & Deans (1977).

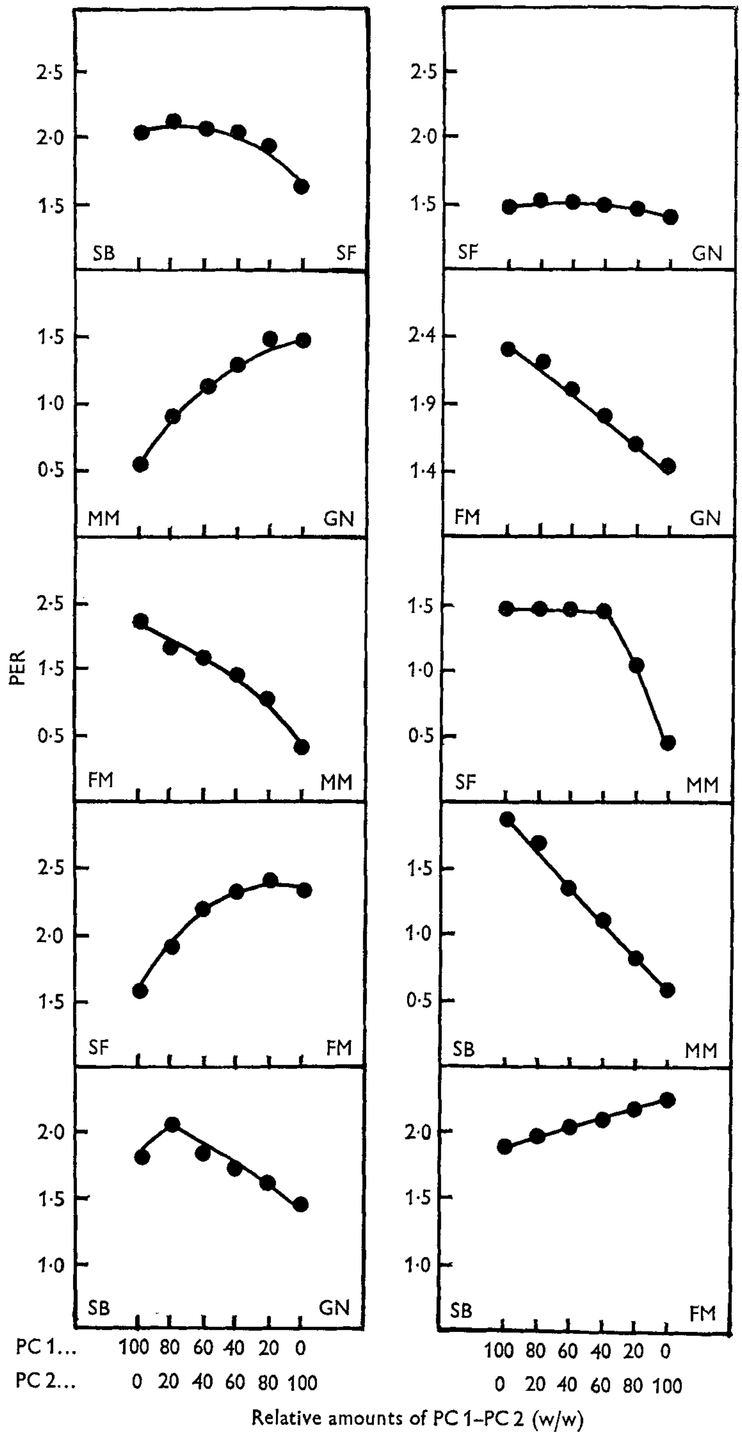

Fig. 1. Protein efficiency ratio (body-weight gain: crude protein intake; PER) values for rats of pairs of protein concentrates (PC1, PC2) mixed in varying proportions to give a total of 100 g protein/kg diet. SB, soya-bean meal; SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; FM, fish meal. For details of protein concentrates, see Woodham & Deans (1977).

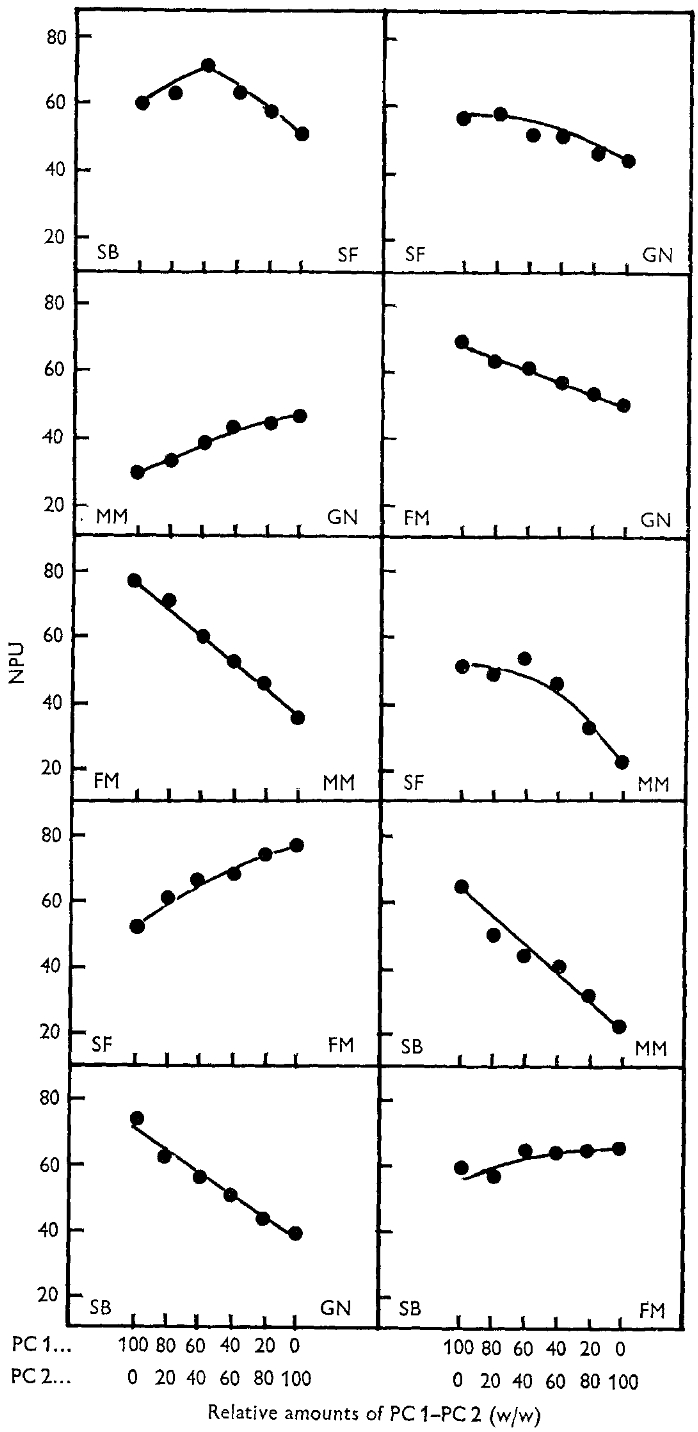

Fig. 2. Net protein utilization (NPU) values for rats of pairs of protein concentrates (PC1, PC2) mixed in varying proportions to give a total of 100 g protein/kg diet. SB, soya-bean meal; SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; FM, fish meal. For details of protein concentrates, see Woodham & Deans (1977).

Table 4. *The essential amino acid requirements of rats**

Amino acid	Requirement (g/kg diet)
Threonine	5·0
Valine	6·0
Cystine + methionine	6·0
Isoleucine	5·5
Leucine	7·5
Tyrosine + phenylalanine	8·0
Lysine	9·0
Histidine	3·0
Arginine	6·0
Tryptophan	1·5

* From (US) National Research Council (1972).

Diets

The five protein concentrates used in this study were those used for the study of mixed proteins in chick diets (Woodham & Deans, 1977) and were: meat meal (MM), fish meal (FM), soya-bean meal (SB), groundnut meal (GN) and sunflower-seed meal (SF). The protein concentrates referred to in this paper were series 2, and their amino acid compositions are given in Table 2 of Woodham & Deans (1977). The five protein concentrates were combined in the ten possible pairs to provide 100 g crude protein (nitrogen × 6·25)/kg diet, the proportions of the components of each pair being (w/w) 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100.

RESULTS

The protein intakes, mean body-weight gains and PER values obtained for the sixty mixtures are tabulated in Table 1, and the results accumulated for the calculation of NPU values are presented in Table 2. The PER and NPU values for all mixtures are shown in Figs 1 and 2. The limiting amino acids for each of the mixtures and the essential amino acid index (geometric mean for the ratio, the amount of essential amino acid: the rat's requirement for that amino acid, for all ten essential amino acids; EAAI) and CS, calculated as described by Woodham & Deans (1977), are presented in Table 3. The lower level of protein used in both the NPU and PER determinations (100 g/kg diet) resulted in the level of essential amino acids being less than 'requirement' levels ((US) National Research Council, 1972) much more frequently than with the diets containing 180 g protein/kg used in the chick TPE estimations (Tables 4 and 5). Consequently EAAI values were low and there were significant differences between mixtures. Accordingly EAAI values have been included in Fig. 3 for comparison with CS values.

DISCUSSION

None of the single concentrates or any of the mixtures provided an adequate level of essential amino acids (Table 5) and the extent to which they are deficient in lysine and the sulphur-containing amino acids is shown in Fig. 4. PER values provided some-

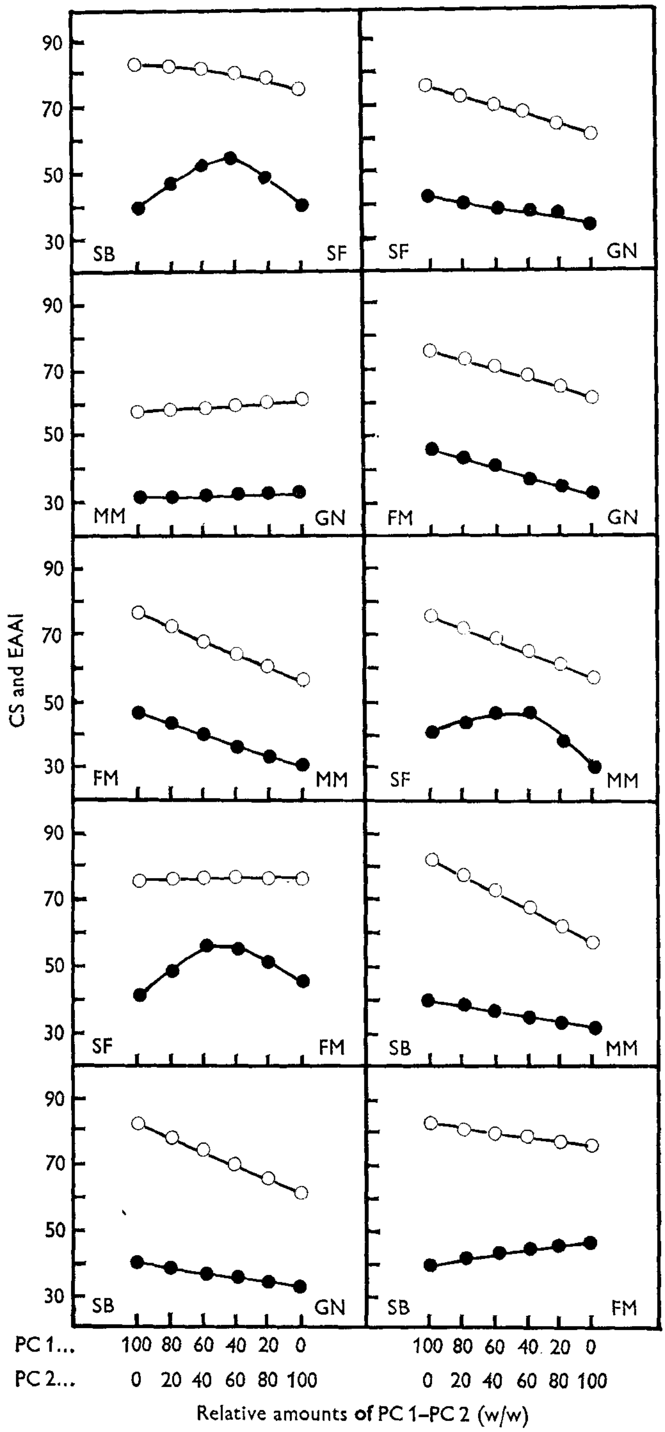

Fig. 3. For legend see facing page.

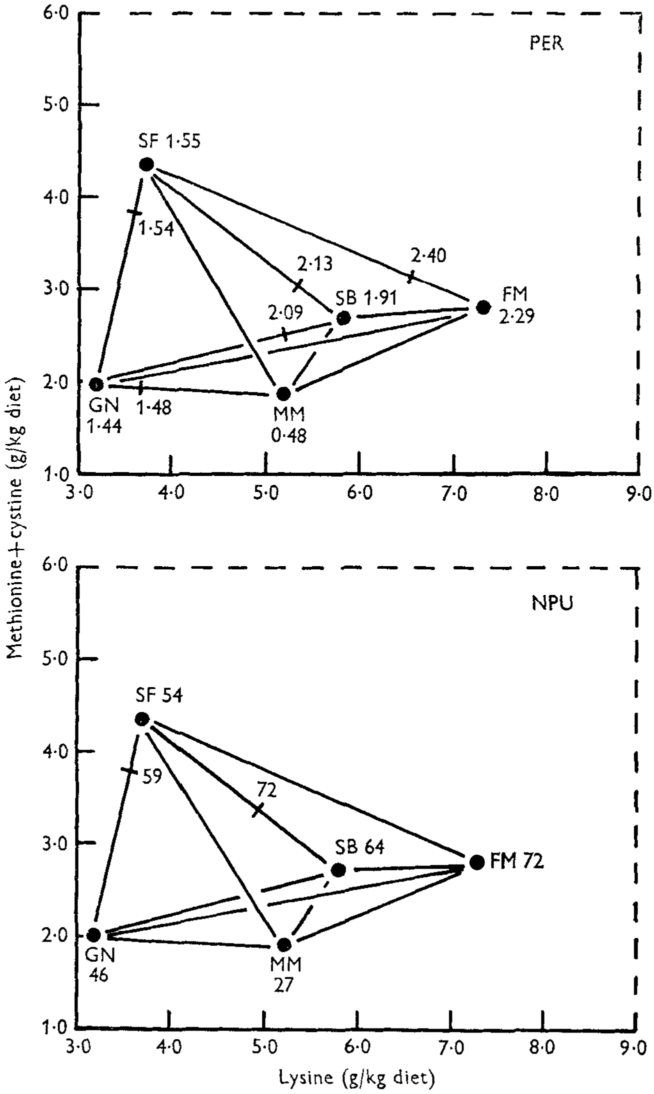

Fig. 4. The content of lysine and of sulphur-containing amino acids (methionine+cystine) for mixtures of protein concentrates, and the protein efficiency ratio (body-weight gain: crude protein intake; PER) and net protein utilization (NPU) of the individual concentrates and of the best mixtures. (-----), Requirement of each amino acid; SF, sunflower-seed meal; SB, soya-bean meal; FM, fish meal; GN, groundnut meal; MM, meat meal. For details of protein concentrates, see Woodham & Deans (1977).

Fig. 3. Chemical score ([amount of limiting amino acid/the rat's requirement for the same amino acid] × 100; CS) (●) and essential amino acid indices (geometric mean for the ratio, amount of essential amino acid: the rat's requirement for that amino acid for all ten essential amino acids; EAAI) (○) for pairs of protein concentrates (PC 1, PC2) mixed in varying proportions to give a total of 100 g protein/kg diet. SB, soya-bean meal; SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; FM, fish meal. For details of protein concentrates, see Woodham & Deans (1977).

Table 5. *Excesses and deficiencies in amino acid content (g/kg diet) for individual protein concentrates* relative to the requirements of the rat ((US) National Research Council, 1972)*

Protein concentrate ... Amino acid	SB	SF	GN	MM	FM
Threonine	−0·9	−1·4	−2·4	−2·1	−0·8
Valine	−0·9	−1·0	−1·9	−0·8	−0·9
Cystine + methionine	−3·3	−1·7	−4·0	−4·1	−3·2
Isoleucine	−0·7	−1·2	−1·6	−3·0	−1·3
Leucine	+0·6	−1·3	−1·4	−1·7	−0·4
Tyrosine + phenylalanine	+0·7	−0·5	+0·9	−2·5	−0·6
Lysine	−3·2	−5·3	−5·8	−3·8	−1·7
Histidine	−0·4	−0·4	−0·8	−1·0	−0·7
Arginine	+1·3	+2·5	+4·0	+1·1	+0·9
Tryptophan	−0·3	−0·3	−0·7	−0·9	−0·6

SB, soya-bean meal; SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; FM, fish meal.

* For details, see Woodham & Deans (1977).

what more evidence for a synergistic effect from the mixtures comparable to that observed with the chick TPE measurements than did NPU values. This can be deduced from the more frequent occurrence of curvature in the growth-response results (Figs 1 and 2). However, for both PER and NPU tests the only striking deviations from a straight line response were shown by three of the SF-containing mixtures (SF–SB, SF–MM and SF–FM). These were the only mixtures for which a non-linear response was predicted by CS (Fig. 3). EAAI values, on the other hand, suggested a linear response for each of the ten pairs of concentrates.

Neither PER nor NPU tests can be called practical tests of protein quality because of the suboptimal levels of protein and of essential amino acids in the diets used. Nevertheless both tests are popular and widely used because of their relative simplicity and because of their ability to rank protein feeding-stuffs in an order of merit which appears to have some relevance to their value under practical feeding conditions. Indeed they have been used by other workers for the evaluation of mixed proteins. Mixtures of maize with various legumes have been particularly studied because of their importance in human feeding in Central America. Bressani and his co-workers have evaluated such mixtures using the PER test (Bressani & Valiente, 1962; Bressani, Valiente & Tejada, 1962) and De Groot & Van Stratum (1963) used both PER and NPU tests to study similar mixtures. Some mixtures of protein concentrates have also been tested, including SB–cottonseed meal, and sesame–cottonseed meal (Bressani & Béhar, 1964). Previous workers have stated that the benefits of such mixing are attributable to complementation effects involving the amino acids contributed by the constituents of the mixtures, but precise links between the PER and NPU values and dietary levels of individual amino acids are not easy to establish from the published work.

Comparison of the conclusions to be drawn from the PER and NPU values reported in the present work with those from the results of the more practical chick TPE test reported separately (Woodham & Deans, 1977), and obtained using the same samples, confirm that there is a broad agreement, while clearly demonstrating that advantages to be obtained by mixing protein concentrates under practical conditions cannot always be predicted by either NPU or PER tests. Furthermore, while CS is a good indicator of the results to be expected from NPU and PER tests, it is rather less useful in predicting results under practical conditions. EAAI values have been shown to be insensitive for predicting the nutritive value of protein mixtures under either practical or experimental conditions.

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