### Nutritive value of mixed proteins

1. In cereal-based diets for poultry

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1. Two series of protein feeding-stuffs each consisting of a fish meal, meat-and-bone meal, soya-bean meal, groundnut meal and sunflower-seed meal were analysed for total amino acid composition and evaluated, both individually and combined in all possible pairs, as supplements to cereal-based diets for growing chicks by the total protein efficiency (g weight gain/g protein consumed; TPE) procedure. Each pair of feeding-stuffs provided 120 g supplementary protein/kg diet and the diet was made up so that the relative amounts of protein provided by each of the pair of constituents were (w/w): 120:0, 100:20, 80:40, 60:60, 40:80, 20:100 and 0:120 respectively, in addition to 60 g protein/kg provided by cereals.

2. In all but one of the twenty pairs of feeding-stuffs studied the mixtures exhibited a marked synergistic effect in that the TPE value was higher than the appropriately weighted mean of the TPE values obtained with the individual components.

3. Neither chemical score ([amount of limiting amino acid/the chick's requirement for the same amino acid]  $\times$  100) nor essential amino acid index; geometric mean for the ratio, amount of essential amino acid: the chick's requirement for that amino acid, for all ten essential amino acid) calculated from the amino acid composition of the dietary constituents could be used routinely to predict the results of the chick growth test, although chemical score did parallel the TPE values in some instances. In a number of instances, mixtures containing an apparently less favourable amino acid composition than one of the components of the mixture gave a higher TPE value.

4. It seems likely that the relative proportions of a number of amino acids determine the optimum combination of a mixture of proteins. The removal of amino acid deficiencies alone is not sufficient to ensure that a given mixture of proteins produces optimum performance in growing chickens.

Ever since the realization that conventional protein feeding-stuffs differed in their amino acid composition, the possibility of supplementary relationships between them has been acknowledged. The nutritional value of various combinations of protein feeding-stuffs have been studied including, for example, sesame-soya bean (Grau & Almquist, 1944), beef-cereals (Block & Mitchell, 1946), maize-cowpea (Vigna sinensis) (Bressani & Scrimshaw, 1961) and sunflower seed-whale meal (Thomas, Martin, Wessels & Human, 1965). Bressani & Elias (1968) suggested that when two protein sources of differing nutritive value are mixed the nutritive value of the mixture may either fall precisely on a straight line joining the values of the two components, or there may be a synergistic effect between them resulting in a curve. This curve may either flatten as the composition of the mixture approaches that of the better component, or there may even be combinations which are superior in nutritive value to that of the better component. Such mixtures in which the protein utilization is higher than the appropriately weighted means of the values obtained for the individual components are clearly of special interest because they hold out the possibility of achieving considerable savings of high-quality protein concentrates by replacing

them with inferior and cheaper materials in the amount needed to give not merely optimum performance, but performance equivalent to, or even better than, that on the better constituent alone.

The present work was planned to study more thoroughly the result of combining various pairs of conventional protein sources along with a constant cereal component under near-practical conditions where the mixture of test concentrates provided two-thirds of the total dietary protein, and cereals provided one-third of the total dietary protein level was fixed at 180 g/kg in order to avoid the possibility of obliterating the supplementary effects which it was desired to measure.

#### MATERIALS AND METHODS

Two series of protein sources (series 1 and 2), each of five protein concentrates and comprising a white fish meal (FM), a meat-and-bone meal (MM), a soya-bean meal (SB), a sunflower-seed meal (SF) and a groundnut meal (GN), were collected, the first series in 1966 and the second series in 1970. Each of the protein concentrates was purchased from ordinary commercial sources. Cereals for use in the basal diets were purchased similarly and in quantities of 1 t, each batch being thoroughly mixed before sampling for analysis. More than one batch of the different cereals was needed during the course of the experiments. The amino acid composition of the cereals and concentrates was determined by ion-exchange chromatography and the mixtures evaluated biologically by a chick growth procedure, i.e. the determination of total protein efficiency (g weight gain/g protein consumed; TPE).

#### Amino acid analysis

The method used was based on that of Spackman, Stein & Moore (1958). Test materials were hydrolysed with 6 M-hydrochloric acid, with the flask immersed to just below the internal liquid level in a deep sand-bath. The liquid was boiled gently (sand-bath temperature  $110-115^{\circ}$ ) for 18 h and the amino acids were separated, using a separation programme of  $20 \cdot 5$  h, in an AutoAnalyzer (Model NC1; Technicon Instrument Co. Ltd, Basingstoke, Hants) with a  $1.4 \text{ m} \times 6 \text{ mm}$  column of Chromo-Beads B (Technicon Instrument Co. Ltd). Methionine and cystine were estimated in samples oxidized with performic acid (Moore, 1963).

For each material at least two hydrolysates were prepared using the unoxidized material and at least another two hydrolysates were prepared using oxidized samples.

#### Tryptophan

The method of Spies & Chambers (1949) was used for the first series of protein concentrates and the modified procedure developed by Matheson (1974) was used for the second series of protein concentrates. The short-comings of the Spies & Chambers (1949) procedure, which are referred to by Matheson (1974), were apparent when the first series of protein concentrates was being studied and the development of Matheson's (1974) alternative procedure began then. The Matheson (1974) method appears to give somewhat lower values than that of Spies & Chambers (1949).

ol. 37	,				Ν	Vu	tr	iti	ve	$v\iota$	ılu	e	of	'n	ixe	d pi	rote	in	s	1							291
				(p)	10.442		790.0	0.200	0.105	4.202	2.675	0.313	8-707	34		<i>(q)</i>	10.442	206.9	0.064	0.290	201.0	}	8.942	0.938	6.317	34	
			3	(3)	3.56		3		}	1.41	£6.o	0.34	4.13	12.37	7	ં	3.26	2.00	ł	ł	1	ļ	2.78	20. I	10.£	12.37	
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fish mec				<i>(p)</i>	677.01		706 o	0.2.0	So1.0	5.253	1.489	0.156	662.6	34		<i>(q)</i>	10.442	6.902	0.064	0.200	0.105	150.1	7.446	0.782	6.918	34	
eal and			N	) S	9.56		8	ļ	1	94.1	0.46	61.0	4.42	12.37	ý	(2)	3.56	2.00	I			0.35	2.32	0.85	62.8	12.37	
an m				(9)	Ċ,	) , ;	3	ł	ł	001	20	ł	ł	180		<i>(q)</i>	30	30	ł	۱		20	100	ł	ł	180	
f soya-be				(a)	1.200	1 /05	1.0	. <u>.</u>	3.1	154.5	43.8	4.6	273.5	001		(a)	1.405	0.202	6.1	8.5	3.1	6.08	0.612	0.22	203.5	100	
ixtures o		<i>(p)</i>		Wt (kg)		- +++ o	706.0	002.0	0.10 <u>5</u>	6.304	}	{	9.893	34		( <i>p</i> )	10.442	6.902	0.064	0.290	501.0	101.2	2.950	o-626	7.520	34	292.
2 <b>*</b> M				-											10	) 🤉	95.8	9. 7. 7	ļ	ļ		04.0	1.85	6 <u>9</u> .0	3.57	12.37	see p. 1968).
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sed with		(9)	Metabo	ener (MJ/		ς, ς	4	1	1	2.7	1	1	4	12.5		(a)	1.201	203.0	6.1	8.5	3.1	61.8	0.5/1	18.4	2.122	100	* For d † Wood
of diets u		) protein	n × 6·25)	oution kg)	i	5	2	1	,	20	0	1	1	80		(q)	10.442	206.9	0.064	0.290	301.0	3.151	4.471	0.469	8.106	34	
sition		(b) Crude 1	litroge	contril (g/l			1	1	ł	Ι		1	I	Ĩ		3	3.56	5.00	ł	1	1	90.I	66.1	12.0	3.85	12.37	
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Table 1. C		(a)	Contribution	to diet (g/kg)	1.1.00	302:0	0.04	× • •	3.1	185.4	l	1	0.162	100		(a)	1.402	0.202	6.1	8.5	3.1	7.20	5.181	13.8	238.4	100	
tet.	101				Ingredient	Theat middlings M	atium chloride	teamed bone meal	itamin-mineral premix†	ish meal 31	oya-bean meal 22	ard	faize starch	lietary total	iet		Ingredient arley B38	Theat middlings M47	odium chloride	teamed bone meal	itamin-mineral premix†	ish meal 31	oya-bean meal 22	ard	faize starch	bietary total	

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### TPE method

The method used for the evaluation of series 1 protein concentrates was that described by Woodham (1968) using Rhode Island Red × White Leghorn male chicks. For the evaluation of series 2 protein concentrates the method was modified as described by Woodham & Deans (1973), and male Ross 1 broiler hybrids were used.

In each experiment five mixtures of protein concentrates in addition to the individual concentrates themselves were evaluated as supplements providing 120 g crude protein (nitrogen  $\times 6.25$ )/kg in diets otherwise consisting of a mixture of barley (B) and wheat middlings (M) which together provided 60 g protein/kg diet, in addition to vitamins and minerals. Diets used in each experiment were made isoenergetic by appropriate additions of maize starch and lard. A specimen set of diets (those used for evaluating FM-SB mixtures in series 2) is given in Table 1. In calculating the energy contributions by the maize starch and lard used to achieve the isoenergetic diets, values of 16.16 and 36.80 MJ/kg were used for maize starch and lard respectively.

As an example, a series of FM-SB mixtures contained B protein (30 g/kg) and M protein (30 g/kg), the protein supplements providing 120 g protein/kg, the relative amounts of the components of the mixtures being respectively (w/w): FM 120, FM-SB 100:20, FM-SB 80:40, FM-SB 60:60, FM-SB 40:80, FM-SB 20:100, SB 120. Each treatment was tested on four groups of chicks, each consisting of six chicks, kept in one house, and the complete trial was repeated with groups of chicks kept in a second house. The TPE values for the mixtures are given as the means of the eight individual values.

#### RESULTS

The N content and the total amino acid composition of the basal cereals used in the testing of both series of protein concentrates are given in Table 2, and the composition of all protein concentrates used is shown in Table 3. Three different cereal combinations were used with series 1 protein concentrates and two cereal combinations were used with series 2 protein concentrates, and their respective contributions of essential amino acids are tabulated in Table 7 together with the contributions made by the protein concentrates. The third cereal-basal diet used with series 2 protein concentrates for g protein/kg diet. Each of the other basal mixtures contained equal amounts of B and M protein, each component contributing 30 g protein/kg diet. Each of the protein concentrates contributed 120 g protein/kg diet.

The basal cereals used for evaluating each of the protein-concentrate mixtures were as follows:

		Protein-concentrate mixtures
	Cereal mixture	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
series 1	B986-M988 B2-M3	MM–GN, FM–GN, FM–MM, SF–MM, SB–MM SB–GN, SB–SF, SF–FM, SF–GN
	B980	SB-FM
series 2	B20–M21 B38–M47	SF-GN, MM-GN, SF-MM, SB-MM, SB-GN, SB-SF FM-GN, FM-MM, SF-FM, SB-FM

concentrates*		1	[		2					
Cereal*	Ba	rley	Wi mide	heat llings	Bar	ley	Wheat middlings			
Amino acid	B2	B986	M <sub>3</sub>	M988	B20	B38	M21	M47		
Aspartic acid	56	59	60	75	63	57	60	63		
Threonine	27	35	26	34	38	31	32	30		
Serine	31	39	35	44	45	40	40	40		
Glutamic acid	223	230	191	213	210	210	207	184		
Proline	123	130	73	73	94	115	75	67		
Glycine	42	42	47	52	48	38	53	42		
Alanine	42	44	44	51	50	37	44	41		
Valine	46	56	43	52	57	39	45	32		
Cystine	25	27	23	21	31	26	19	21		
Methionine	14	22	22	15	17	26	17	12		
Isoleucine	34	40	32	35	39	27	34	22		
Leucine	66	76	61	65	78	63	61	52		
Tyrosine	34	25	31	30	38	32	30	27		
Phenylalanine	53	58	<b>4</b> 1	42	52	45	39	34		
Lysine	38	39	43	43	46	34	39	35		
Histidine	19	23	24	27	23	18	23	20		
Arginine	46	54	65	72	59	47	58	56		
Tryptophan	ND	14	ND	18	ND	10	ND	ND		
Recovery of amino acid-N (% total							<b>.</b>			
N analysed)	91.9	101.3	86.1	96.2	98.8	88.2	87.6	77.8		
Total N in sample					_					
(g/kg)	16.6	15.4	22.8	26.1	12.6	15.6	26.9	23.6		
		ND m	at determ	ined						

## Table 2. Amino acid composition (g/kg crude protein (nitrogen $\times$ 6.25)) of basal cereals in diets given to growing chicks

ND, not determined.

\* For details, see p. 292.

The values obtained for body-weight gain, protein intake and TPE are tabulated in Table 4 (series 1) and Table 5 (series 2) and the TPE values for series 1 and 2 are shown together in Fig. 1. Although the set of mixtures for each pair of protein concentrates was made isoenergetic, because of the variety of protein sources and the changing basal cereals, it was not possible to maintain a single energy level throughout either series. Consequently it will be observed that different TPE values were sometimes obtained for the same concentrate when tested in different experiments. Results for series 2 in which broiler hybrid chicks were used are understandably higher than those for series 1 but the general pattern of the results for series 1 and 2 was remarkably similar. In common with other biological protein-quality tests such as the protein efficiency ratio (PER) and the net protein utilization procedures, the TPE may be influenced by extreme differences in food intake. Our experience coincides with that of Campbell (1963) regarding PER in that the differences found under carefullycontrolled conditions were not so great as to invalidate the comparisons. In four separate estimates of TPE for a single sample of GN for example, protein intake (g/bird per d), weight gain (g) and TPE were respectively: 4.63, 8.27, 1.79; 5.18,

Series of protein concentrates*	 		I					2		
Protein concentrate	 FM	MM	GN	SB	SF	ŕм	MM	GN	SB	SF
Amino acid	P968	P977	P971	P978	P979	31	27	26	22	29
Aspartic acid	91	75	115	119	95	94	70	103	115	91
Threonine	42	31	30	39	38	42	29	26	41	36
Serine	47	37	51	50	45	51	37	44	55	43
Glutamic acid	133	122	194	178	205	135	113	169	181	197
Proline	56	96	48	54	46	69	85	41	49	60
Glycine	95	136	59	40	55	86	130	52	46	57
Alanine	69	80	41	42	46	67	73	38	50	42
Valine	48	46	44	47	46	51	42	41	55	50
Cystine	11.8	6	14.1	12.1	18.4	10	7	13	15	19
Methionine	25	6	10	12	24	18	12	7	9	24
Isoleucine	39	26	36	42	39	42	25	34	52	43
Leucine	67	58	66	74	62	71	58	61	82	62
Tyrosine	29	18	41	34	25	34	21	39	38	27
Phenylalanine	37	35	53	54	43	40	34	50	53	48
Lysine	74	49	37	61	35	73	52	32	69	37
Histidine	19	18	25	26	24	23	20	22	27	26
Arginine	69	69	121	75	87	69	71	100	75	85
Tryptophan	14	12	II	19	18	9	6	8	12	12
Recovery of amino acid-N (% total N analysed)	96·6	92.0	99·6	08.1	92.1	98.4	88.5	88·o	99 <b>.</b> 4	95.0
Total N in sample		-		-			-			
(g/kg)	106.7	90.1	83.7	73.6	60.0	103.6	85.1	72.7	73 <b>.</b> 1	63.7

### Table 3. Amino acid composition $(g/kg \text{ crude protein (nitrogen <math>\times 6.25))$ of protein concentrates in diets given to growing chicks

FM, fish meal; MM, meat meal; GN, groundnut meal; SB, soya-bean meal; SF, sunflower-seed meal. \* For details, see p. 292.

9.31, 1.80; 4.00, 6.98, 1.75; 4.04, 7.15, 1.77. We have no evidence to suggest that the level of supplementary protein (120 g/kg diet) used in the TPE experiments might contribute a dangerously high level of minerals when normal FM and MM are tested (Woodham, 1968). The curves in Figs 1-3 are based on the plotted values and fitted by eye.

In all but one of the twenty sets of values the curved response line indicated an advantage from combining the pairs of protein concentrates. The exception was the values for SB-GN combinations (series 1) where the points were on a straight line. The values for SB-GN combinations in series 2 were on a line which was only slightly curved and it would seem from this result that little advantage is to be expected from combining SB with GN. In the other sets there was clear evidence that combinations were advantageous, but the proportions of the two protein concentrate components which gave the maximum response differed considerably. In the instance of MM-GN combinations, for example, the optimum amounts would seem to be approximately 50:50 (w/w) for both series, whereas for SB-MM combinations optimum protein utilization was achieved when the SB comprised approximately 75% of the mixture. With FM-GN and FM-MM combinations a much more decisive advantage was indicated in one series than in the other though in both

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Table 4. Series 1 protein concentrates<sup>\*</sup>. Intake of crude protein (nitrogen  $\times$  6·25) (g/bird per d), weight gain (g/bird per d), and total protein efficiency (g weight gain/g protein consumed; TPE) for RIR  $\times$  WL chicks given cereal-based diets supplemented with pairs of protein concentrates (PC1, PC2) mixed in varying proportions to contribute 120 g protein/kg diet

Relative amounts of PC1-PC2 (w/w)

	PC1-PC2	Basal cereals*	120:0	100:20	80:40	60:60	40:80	20:100	0:120
Crude protein intake Wt gain TPE	MM-GN	)	4·05 7·05 1·74	4·67 8·75 1·87	4·77 9·12 1·91	5.01 9.98 1.99	4·92 9·64 1·96	4·80 9·07 1·89	4·63 8·27 1·79
Crude protein intake Wt gain TPE	FM-GN		5·18 12·65 2·44	5·41 12·84 2·38	5·38 12·69 2·36	5·46 12·04 2·21	5·38 11·72 2·18	5·50 10·93 1·99	5·18 9·31 1·80
Crude protein intake Wt gain TPE	FM-MM	B986-M988	4 <sup>.8</sup> 3 11.80 2.44	4·78 11·93 2·50	4·77 11·44 2·40	4 <sup>.65</sup> 10 <sup>.84</sup> 2 <sup>.33</sup>	4·39 9·27 2·11	4·14 7·77 1·87	3·69 5·99 1·62
Crude protein intake Wt gain TPE	SF-MM		5·11 10·47 2·05	5.00 10.76 2.15	5·19 11·53 2·22	4·98 10·70 2·15	4·70 9·46 2·01	4·29 7·80 1·82	3·56 5·25 1·47
Crude protein intake Wt gain TPE	SB-MM		4·56 8·84 1·94	4·99 10·16 2·04	4 <sup>.</sup> 93 10 <sup>.</sup> 07 2 <sup>.</sup> 04	4·89 9·71 1·98	4.66 8.61 1.85	4·08 6·81 1·67	3·56 5·16 1·44
Crude protein intake Wt gain TPE	SB-GN		4·32 8·48 1·96	4·27 8·28 1·94	4·27 8·03 1·88	4·14 7·77 1·87	4·18 7·68 1·84	4·12 7·53 1·82	4.00 6.98 1.75
Crude protein intake Wt gain TPE	SB-SF		4·30 8·12 1·90	4·46 8·40 1·89	4·44 8·74 1·97	4·36 8·48 1·94	4`44 8`47 1`91	4·46 8·37 1·88	4·43 8·15 1·84
Crude protein intake Wt gain TPE	SF-FM		4·58 8·21 1·80	4·89 10·46 2·14	5·38 12·47 2·32	5·15 12·66 2·46	5 <sup>.04</sup> 12.57 2.49	5.05 12.54 2.48	5·05 12·45 2·47
Crude protein intake Wt gain TPE	SF-GN	)	4·13 7·39 1·79	4·19 7·53 1·80	4·37 8·26 1·89	4·48 8·49 1·89	4∙35 8∙09 1∙86	4·33 7·83 1·81	4·04 7·15 1·77
Crude protein intake Wt gain TPE	SB-FM	B986	5·40 11·76 2·18	5·39 12·33 2·34	5·27 12·70 2·41	5·17 12·46 2·41	5·25 12·88 2·47	5.08 12.72 2.50	5.00 12.26 2.46

RIR, Rhode Island Red; WL, White Leghorn; MM, meat meal; GN, groundnut meal; FM, fish meal; SF, sunflower-seed meal; SB, soya-bean meal; B, barley; M, wheat middlings.

\* For details, see p. 293 and Tables 2 and 3.

instances it appeared from the results with both series that it was advantageous to combine a proportion of the poorer-quality concentrate, GN or MM, with FM, the latter always being the major constituent.

#### DISCUSSION

The results provided useful information regarding the possibilities of sparing good-quality protein concentrates such as FM or SB by inferior, and perhaps cheaper ones. SF may replace half the FM, for example, without any deterioration in chick growth and protein utilization, and even GN can effect a quite considerable saving.

Table 5. Series 2 protein concentrates<sup>\*</sup>. Intake of crude protein (nitrogen  $\times$  6.25) (g/bird per d), weight gain (g/bird per d) and total protein efficiency (g weight gain/g protein consumed; TPE) for Ross 1 broiler hybrid chicks given cereal-based diets<sup>†</sup> supplemented with pairs of protein concentrates (PC1, PC2) mixed in varying proportions to contribute 120 g protein/kg diet

Relative amounts	s of	PC1-PC2	(w/w)	
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	PC1-PC2	<b>B</b> asal cereals	120:0	100:20	80:40	60:60	40:80	20:100	0:120
Crude protein intake Wt gain TPE	SFGN		7·30 14·17 1·94	7 <b>·20</b> 15·94 2·21	7·20 16·48 2·29	6·64 14·87 2·24	6·68 14·79 2·21	6·39 13·71 2·15	6·48 13·83 2·13
Crude protein intake Wt gain TPE	MM-GN		6.07 12.68 2.09	6·51 14·17 2·18	6·90 15·64 2·31	7·15 16·70 2·33	6·92 16·10 2·33	6·77 15·24 2·25	6·39 13·71 2·15
Crude protein intake Wt gain TPE	SF-MM		7·71 18·69 2·42	7:40 18:42 2:49	7·40 18·13 2·45	7:49 17:88 2:39	7:25 17:31 2:39	7:05 16:18 2:29	6·26 13·49 2·15
Crude protein intake Wt gain TPE	SB-MM	B20-1/121	7·58 20·74 2·74	7·57 21·42 2·84	7 <sup>.</sup> 44 20.89 2.81	7·40 19·88 2·69	7:04 18:04 2:57	6·36 15·13 2·38	5·58 12·36 2·22
Crude protein intake Wt gain TPE	SB-GN		8·39 22·54 2·69	8·26 21·73 2·63	8·15 20·82 2·55	8.00 20.31 2.54	7·76 18·98 2·44	7:49 17:42 2:32	6·64 14·10 2·12
Crude protein intake Wt gain TPE	SB-SF		7 <sup>.</sup> 71 19.73 2.56	8.05 21.76 2.70	8·25 22·57 2·74	7·96 21·26 2·67	7·76 20·69 2·67	7:49 18:83 2:51	6·96 16·20 2·33
Crude protein intake Wt gain TPE	FM-GN		6·83 19·73 2·88	7 <sup>.07</sup> 21.42 3.02	7·33 22·24 3·03	7·26 21·32 2·93	7:02 19:08 2:71	6·51 16·23 2·49	5.80 12.11 2.09
Crude protein intake Wt gain TPE	FM-MM		7·41 22·02 3·01	7·39 21·52 2·95	7·32 20·38 2·75	7·28 19·53 2·68	7·01 17·55 2·54	6.65 15.58 2.34	6.02 12.66 2.08
Crude protein intake Wt gain TPE	SF-FM	( B38–M47	7·39 16·76 2·27	8.02 20.83 2.59	8.09 22.64 2.80	8·15 24·02 2·94	7·96 23·51 2·95	7·92 23·61 2·98	7·51 21·92 2·92
Crude protein intake Wt gain TPE	SB-FM	)	7·57 20·42 2·69	7·97 23·80 2·98	7·78 23·93 3·08	7·92 24·65 3·16	7·64 23·99 3·14	7·40 23·30 3·14	7·02 21·79 3·10

SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; SB, soya-bean meal; FM, fish meal; B, barley; M, wheat middlings.

\* For details, see p. 293 and Tables 2 and 3.

† For details of composition, see Table 1.

Mixtures of comparatively poor-quality concentrates which individually give similar low values provide the opportunity of greatly enhanced performance. Mixtures of GN and MM in roughly equal proportions give much better results than either concentrate fed singly, with both series 1 and 2 protein concentrates. A similar effect is noticeable for SF-MM mixtures.

Synergists may be defined as two or more agents which, when combined, produce an effect greater than the additive effect of both when operating alone (Winburne, 1962). Taking both series 1 and 2 protein concentrates, twenty sets of values in all,





Fig. 1. Total protein efficiency (g weight gain/g protein consumed; TPE) of diets with 180 g crude protein (nitrogen  $\times 6.25$ )/kg, containing cereals (60 g protein/kg) in addition to pairs of protein concentrates (PC1, PC2) mixed in varying proportions to contribute 120 g protein/kg; ( $\bigcirc$ ), series 1; ( $\bullet$ ) series 2. SB, soya-bean meal; SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; FM, fish meal; for details of protein concentrates and cereals, see p. 290.

nineteen values exhibited some synergistic effect in that the performance of birds given the mixture was greater than that which would be predicted from the results obtained by feeding the components singly. The fact that mixtures displaying this synergistic effect predominated among combinations of the common and important protein supplements used in this work is very encouraging. Clearly a knowledge of the existence of, and an understanding of the reasons for such complementary effects would permit the optimum utilization of these materials, allowing advantage to be taken of particular market situations regarding availability and price.

Although the results were obtained with two different series of the same types of concentrate and the testing of each series was carried out with two very different types of chicken, the results are strikingly similar for the two series of protein concentrates. However, the possibility of variations of quality within a concentrate type which have already been amply demonstrated (e.g. Boyne, Carpenter & Woodham, 1961; Carpenter & Woodham, 1974) must lead to caution in assuming that the effects of mixing other samples of protein concentrates would necessarily be similar. The conclusion does, however, seem to be inevitable that whenever possible a mixture of protein concentrates should be used to supplement cereals rather than a single material. It would clearly be very useful if the ideal combinations in such mixtures could be predicted, and the most likely way of doing this would be by means of amino acid composition. Is it possible to explain the supplementary effects revealed by the biological results in terms of improved amino acid balance? If so it should be possible to predict the optimum combinations of any two protein concentrates by routine amino acid analysis.

### Chemical score (CS) and essential amino acid index (EAAI)

Block & Mitchell (1946) proposed scoring protein foods on the basis of the essential amino acid which was in greatest deficit when compared to a reference protein, and whole-egg protein has been frequently chosen as the standard. The essential amino acid in greatest deficit is the limiting amino acid and CS, therefore, is given by the expression:

 $\frac{\text{amount of limiting amino acid (\%)}}{\text{amount of the same amino acid in egg (\%)}} \times 100.$ 

In most instances the limiting amino acid will be lysine, or methionine + cystine.

Oser (1951) suggested that it would be more reasonable to use an expression allowing for the provision of all the essential amino acids and not merely the most limiting amino acid. He proposed using the geometric mean of the values for the ratio, amount of essential amino acid: amount of that amino acid in egg, for all ten essential amino acids and this expression he termed EAAI.

It has been stated recently that because it is based on a single limiting amino acid, CS will tend to underestimate biological value and consequently EAAI is to be preferred, even allowing for the fact that such measurements are based on the analysis of protein hydrolysates and do not take into account differences in protein digestibility and amino acid availability (UN Protein Advisory Group, 1974). However, the EAAI concept has not been universally accepted and despite the UN Protein Advisory

1977

Requirement							
g/kg diet	g/kg dietary protein						
5.0-2.5	28-29						
4.8-5.0	27-28						
6.02.1	38-39						
5.8	32						
< 4.8	< 27						
< 10.5	< 58						
10.0-11.5	61-62						
8.7	48						
< 3.4	< 19						
< 7.6	< 42						
< 1.4	< 7.8						
	friction for the second state of the second						

# Table 6. The essential amino acid requirements of broiler chickens between 14 and 28 d of age\*

\* From Woodham & Deans (1975).

Group (1974) pronouncement it is still widely felt that the extent to which a protein may be utilized is solely dependent upon the limiting amino acid (e.g. Bender, 1973).

The choice of reference protein is clearly critical. While egg protein may be satisfactory for materials which are to be evaluated for human consumption it seems sensible to suppose that a set of hypothetical 'target' values might be more appropriate in many instances. The FAO reference pattern (FAO, 1965) may be quoted as an example. In the present series of experiments using chickens the most appropriate reference would seem to be a hypothetical protein providing exactly the calculated requirements of the chick for each of the essential amino acids. These 'requirement' values (Table 6) were specially determined under the condition of the experiments described here and have been published fully elsewhere (Woodham & Deans, 1975). They are in general agreement with values obtained by other workers using conventional diets, providing that the values are expressed as a percentage of the dietary protein content rather than as a percentage of the diet (Woodham & Deans, 1975).

CS and EAAI were calculated for all the mixed diets tested in the present work. In calculating the latter all eleven of the amino acids known to be essential for the optimum growth of the chicken were taken into account, methionine and cystine being treated additively as one amino acid, as were tyrosine and phenylalanine. The amino acid composition of the cereals and protein concentrates used in preparing the mixtures are given in Table 7 and the contributions of lysine and of methionine + cystine by mixtures of cereals with the individual protein concentrates are given in Table 8. The calculated values for CS and EAAI as well as the limiting amino acids in each of the mixtures tested are shown in Table 9 (series 1 protein concentrates) and Table 10 (series 2 protein concentrates). The values for CS for both series of protein concentrates are shown in Fig. 2.

The EAAI for all mixtures tested were between 95 and 100 and the differences between mixtures were very small. This was due to the fact that in these near-practical formulations most of the essential amino acids were provided at levels greater than https://doi.org/10.1079/BJN19770034 Published online by Cambridge University Press

				Seri	es 1						
		Cereals			Prote	in concer	trates				
	B986+	B2+		FM	MM	GN	SB	SF			
	M988	$M_3$	B986	968	977	971	978	979			
Threonine	2.3	1.8	2.3	5.0	3.7	3.6	4.2	4.6			
Glycine	3.0	2.9	2.7	11.4	16.3	7·1	4·8	6.6			
Valine	3.0	3.0	2.7	5.8	5.2	5'3	5.6	5.2			
Cystine + methionine	2.7	2.6	3.0	4.4	1'4	2.9	3.5	5.1			
Isoleucine	2.5	2.5	2.6	4.7	3.1	4.3	5.0	4.7			
Leucine	4.5	4.1	4.9	8.0	7.0	7.9	8.9	7.4			
Tyrosine + phenylalanine	5.0	5.1	5.3	7.9	6.4	11.3	10.0	8.2			
Lysine	2.8	2.7	2.6	8.9	5.9	4.4	7:3	4.5			
Histidine	1.0	1.4	1.2	2.3	2.2	3.0	3.1	2.9			
Arginine	4.0	3.2	3.4	8.3	8.3	14.5	9.0	10.4			
Tryptophan	1.1	0.2	0.0	1.7	1.4	1.3	2.3	2.2			
	Series 2										
	Cereals				Prote	in concer	ntrates				
	B20	B38		FM	MM	GN	SB	SF			
	M21	M47		31	27	26	22	29			
Threonine	2.1	1·8		5.0	3.2	3.1	4.9	4.3			
Glycine	3.0	2.4		10.3	15.6	6.3	5.2	6.8			
Valine	3.0	2.1		6.1	5.0	4.9	6.6	6·0			
Cystine + methionine	2.2	2.6		3.4	2.3	2.4	2.8	5.5			
Isoleucine	2.2	1.2		5.0	3.0	4.1	6.5	5.2			
Leucine	4.2	3.5			7.0	7.3	9.8	7.4			
Tyrosine + phenylalanine	4.8	4.1		8.9	6.6	10.7	10.0	9.0			
Lysine	2.6	2.1		8.8	6.5	3.8	8.2	4.4			
Histidine	1'4	1.1		2.8	2.4	2.6	3.2	3.1			
Arginine	3.2	3.1		8.3	8.5	12.0	<u>9</u> .0	10.5			
Tryptophan	0.6	0.2		1.1	0.2	1.0	2.3	1.4			

Table 7. Amounts of essential amino acids (g/kg diet) contributed by cereal combinations (barley (B) and wheat middlings (M)) each providing 60 g protein/kg diet, and five protein concentrates each providing 120 g protein/kg diet

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

Table 8. Amounts  $(g|kg \ diet)$  of lysine and cystine + methionine contributed by basal cereal combinations (barley (B) and wheat middlings (M)) with the individual series 1 and 2 protein concentrates\*

•	Lysi	ne	Cystine + methionine			
Series of protein concentrates Cereals	1 B986–M988	2 B38–M47	I B986–M988	2 B38-M47		
Protein concentrate						
Fish meal	11.2	10.8	7.0	5.9		
Meat meal	8.7	8.3	4.1	4.8		
Sunflower-seed meal	7.0	6.2	7.8	7.7		
Soya-bean meal	10.1	10.3	5.9	5.4		
Groundnut meal	7.2	5.9	5.6	4.9		

\* For details, see p. 292.

Table 9. Series 1 protein concentrates<sup>\*</sup>. Limiting amino acid (LAA), essential amino acid index (EAAI)<sup>†</sup> and chemical score (CS)<sup>‡</sup> for cereal-based diets supplemented with pairs of protein concentrates (PC1, PC2) mixed in varying proportions to contribute 120 g protein/kg diet Relative amounts of PC1-PC2 (w/w)

	PC1-PC2	Basal cereals*	120:0	100:20	80:40	60:60	40:80	20:100	0:120
LAA EAAI	MM-GN	}	C+M 96·9	C+M 97 <sup>.2</sup>	C+M 97'4	C+M 97 <sup>.6</sup>	C+M 97'7	LYS 97 <sup>.</sup> 9	LYS 98.0
CS			70.0	75.0	79'3	83.6	87.9	85.6	82.7
LAA	FM-GN							LYS	LYS
EAAI CS			100 100	100 100	100 100	100 100	100 100	99.2 99.2	98.0 82.7
LAA	FM-MM		-			C+M	C+M	C+M	C+M
EAAI CS		B986-M988	100 100	100 100	100 100	99 <sup>.</sup> 7 96 <sup>.</sup> 5	98·8 87·9	97·9 79·3	96 <sup>.</sup> 9 70 <sup>.</sup> 6
LAA EAAI CS	SF-MM		LYS 9800 8014	LYS 98·4 83·7	LYS 98·7 86·9	LYS 99 <sup>.1</sup> 90 <sup>.2</sup>	C+M 98·6 91·9	C+M 97·8 81·3	C+M 96·9 70·6
LAA EAAI CS	SB-MM	)	100	C+M 99'7 96'5	C+M 99·2 91·3	C+M 98·7 86·2	C+M 98·1 81·0	C+M 97·5 75·8	C+M 96·9 70·6
LAA EAAI CS	SB-GN	}	100 100	C+M 99·9 99·1	C+M 99·8 98·2	C+M 99 <sup>.</sup> 6 97 <sup>.</sup> 4	LYS 99·0 92·7	LYS 98·4 87·1	LYS 97 <sup>.</sup> 7 81.6
LAA EAAI CS	SB-SF		100	100 100	 100 100	LYS 99 <sup>.</sup> 7 97 <sup>.</sup> 1	LYS 99 <sup>.2</sup> 91.1	LYS 98·6 85·2	LYS 97 <sup>.</sup> 9 79 <sup>.</sup> 3
LAA EAAI CS	SF-FM		LYS 97 <sup>.</sup> 9 79 <sup>.</sup> 3	LYS 98·9 88·3	LYS 99·8 97·3	100 100	 100 100	100 100	100 100
LAA EAAI CS	SF-GN	)	LYS 97 <sup>.</sup> 9 79 <sup>.</sup> 3	LYS 98·0 79·6	LYS 98.0 80.0	LYS 98·0 80·4	LYS 98·1 80·8	LYS 98·1 81·2	LYS 97·7 81·6
LAA EAAI CS	SB-FM	B986	100 100	 100 100	100	100 100	100 100	 100 100	

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

\* For details, see p. 290 and Tables 2 and 3.

† (Amount of limiting amino acid/the chick's requirements for the same amino acid) × 100.

‡ Geometric mean for the ratio, amount of essential amino acid: the chick's requirements for that amino acid, for all ten essential amino acids.

requirement (see Table 11) and it was clear that in such diets the EAAI could give no useful indication of differences in quality. This was borne out by the observation that large changes in TPE were not reflected in the EAAI. CS, on the other hand, did vary considerably as the proportions of protein concentrates in the mixtures were changed (Fig. 2). Differences in the amino acid composition of corresponding pairs of protein concentrates used in series 1 and 2, notably the GN, MM and SB, were reflected in the CS but in general it was clear from Fig. 2 that changes in amino acid composition brought about by mixing protein concentrates were fairly similar for the two series studied. Table 10. Series 2 protein concentrates<sup>\*</sup>. Limiting amino acid (LAA), essential amino acid index (EAAI)<sup>†</sup> chemical score (CS)<sup>‡</sup> for cereal-based diets § supplemented with pairs of protein concentrates (PC1, PC2) mixed in varying proportions to contribute 120 g protein/kg diet

	PC1-PC2	Basal cereals*	120:0	100:20	80:40	60:60	40:80	20:100	0:120
LAA EAAI CS	SF-GN		LYS 98·1 80·9	LYS 98·0 79 <sup>.</sup> 7	LYS 97·8 78·6	LYS 97 <sup>.</sup> 7 77 <sup>.</sup> 4	LYS 97·6 76·3	LYS 96·7 75·1	LYS 95·8 74·0
LAA EAAI CS	MM-GN		C+M 97 <sup>.</sup> 7 82 <sup>.</sup> 4	C + M 97·8 82·7	C+M 97·6 83·1	C+M 97 <sup>.2</sup> 83 <sup>.4</sup>	LYS 96·8 83·2	LYS 96·3 78·6	LYS 95·8 74·0
LAA EAAI CS	SF-MM	D. Mar	LYS 98·1 80·9	LYS 9 <sup>8·5</sup> 84·3	LYS 98·8 87·8	LYS 99 <sup>.</sup> 2 91 <sup>.</sup> 2	LYS 99 <sup>.</sup> 4 94 <sup>.</sup> 7	C+M 98∙9 90∙6	C+M 97 <sup>.7</sup> 82 <sup>.</sup> 4
LAA EAAI CS	SB-MM	B20-M21	C+M 99 <sup>.</sup> 3 92 <sup>.</sup> 7	C+M 99·1 91·0	C+M 99 <sup>.0</sup> 89 <sup>.</sup> 3	C+M 98·8 87·5	C+M 98·6 85·8	C+M 98·4 84·1	C+M 97'7 82'4
LAA EAAI CS	SB-GN		C+M 99 <sup>.</sup> 3 9 <sup>2.</sup> 7	C+M 99 <sup>.2</sup> 91.3	C+M 99·0 90·0	C+M 98·9 88·6	C+M 97 <sup>.</sup> 9 87 <sup>.</sup> 2	LYS 96·9 82·5	LYS 95 <sup>.</sup> 8 74 <sup>.</sup> 0
LAA EAAI CS	SB-SF	}	C+M 99 <sup>.</sup> 3 92 <sup>.</sup> 7	C + M 99·9 99·3	100 100	 100 100	LYS 99•6 95•6	LYS 98·9 88·2	LYS 98·1 80·9
LAA EAAI CS	FM-GN		100 100	100 100	C+M 99 <sup>.</sup> 7 97 <sup>.</sup> 2	C+M 99 <sup>.2</sup> 94 <sup>.</sup> 4	LYS 98·0 87·1	LYS 96·7 77·7	LYS 94.7 68.2
LAA EAAI CS	FM-MM	D-9 M	100	C+M 100 99·6	C+M 99 <sup>.7</sup> 96.5	C+M 99 <sup>.</sup> 4 93 <sup>.</sup> 4	C+M 99 <sup>.1</sup> 9 <sup>0.3</sup>	C+M 98·5 87·2	C+M 96·5 84·1
LAA EAAI CS	SF-FM	D38-147	LYS 97:4 75:1	LYS 98·4 83·4	LYS 99 <sup>.</sup> 2 91 <sup>.</sup> 7	LYS 100 99 <sup>.</sup> 9	100 100	100 100	 100 100
LAA EAAI CS	SB-FM	)	C+M 99`5 94`4	C+M 99·6 95·8	C+M 99'7 97'2	C+M 99 <sup>.</sup> 9 98.6	 100 100	 100 100	 100 100

Relative amounts of PC1-PC2 (w/w)

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

\* For details, see p. 290 and Tables 2 and 3.

† (Amount of limiting amino acid/the chick's requirements for the same amino acid) × 100.

‡ Geometric mean for the ratio, amount of essential amino acid: the chick's requirements for that amino acid, for all ten essential amino acids.

§ For details of composition, see Table 1.

Comparison of Figs 1 and 2 showed that in some mixtures changes in nutritive value as shown by the TPE measurements may be approximately predicted from the amino acid composition. Reasonable agreement between CS and TPE values were shown in the instances of MM-GN, FM-MM, SF-FM and SB-GN. However, in no instance was the agreement absolute, and in particular it was not possible to predict the increased TPE values obtained by mixing a small quantity of MM with SB or GN with FM. The clear and reproducible improvement obtained by such



Fig. 2. Chemical scores ([amount of limiting amino acid/the chick's requirements for the sameamino acid]  $\times$  100; CS) for diets with 180 g crude protein (nitrogen  $\times 6.25$ )/kg, containing cereals (60 g protein/kg) in addition to pairs of protein concentrates (PC1, PC2) mixed in varying proportions to contribute 120 g protein/kg; ( $\bigcirc$ ), series 1; ( $\bigcirc$ ), series 2. SB, soya-bean meal; SF, sunflower-seed meal; GN, groundnut meal; MM, meat meal; FM, fish meal; for details of protein concentrates and cereals, see p. 290.

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Table 11. Excesses and dej	ficiencies	s* in essential	amino acid	content (g/	kg diet)	of cereal-based	l diets + c	ontaining in	dividual f	rotein conce	ntrates 5	30
and pairs of protein concen	ntrates,	yielding the h.	ighest total	protein eff	iciency (g	weight gain	g protein	consumed;	TPE) for	each pair (s	eries 2 +	4
protein concentrates <sup>†</sup> only)	_											
Protein concentrate	SB	SB-SF	$\mathbf{SF}$	SF-GN	ND	GN-MM	MM	MM-FM	FM	FM-GN	GN	

						A		A		W	Vc	0	DE	IAI	M	ANI	)	P	•	S.		D	EA	N	s							I	97	7
	GN		0 0	+3.0	0.0 0	6.0	+0.8	+ 0.3	+3.6	-2.8	+ 0.3	+ 7-5	1.0+	2.09		GN		- 0.3	+3.6	0.0	6.0	+ o.8	+0.3	9·E+	-2.8	+0.3	+ 7·5	1.0+	2.12					
	FM-GN		40.0	+0.+	8.0+	-0.2	+1.4	1.1+	+3.1	+0.4	5.o+	+5.0	+0.2	3.03		SB-GN		+1.5	+2.9	9·I+	-0.4	+2.9	+2.8	+3.8	9·I+	6.0+	+4.5	+1.4	2.69					
	FM	91	0. <b>1</b> + ·	+ 7.7	1.1+	1.0+	<b>1</b> .1.4 +	+ 1.5	+2.8	+2.1	+0.2	+3.8	+0.2	2.88	10.£	SB		+1.5	6.2+	•1•6	+.0-	+2.9	+2.8	+3.8	9·I+	6.0+	+4.5	+1.4	2.69	2.74	al.			
	MMFM	y	0.1+	+ 7.7	1.1+	1.0+	+1.7	+ 1.5	+2.8	+2.1	+ o.2	+3.8	+0.2	2.88		MM-SB		+1:2	+4.9	+ 1.3	10.5	+2.3	+2.2	+ 3.0	+1.2	4.0.+	+ 4.4	1.1+		2.84	I: FM. fish me			
	MM		- -	+13.0	0.0	0.1 –	£.o –	0.0	10.2	10.1	1.0+	+4:0	0.0	60.2	2.08	MM		1.0+	0.81+	0.0	0.1 -	£.o -	0.0	- 0.2	+.0 	1.0+	+4:0	0.0	2.15	2.22	I. meat mea			
	GN-MM		1.01	+8.3	0.0	6.01	+0.2	1.0+	41.7	9.1 -	+0.2	+5.8	0.0	2.33		SF-MM		40.4	+ 5.9	+0.8	+ 1.3	+ 1.5	+0.3	+ 1.5	8.1~	4 0.2	+ 5.4	+0.+	2.49		hur meal · MN	975).		
	GN		0 0 1	+3.0	0.0	6.0 -	8.0+	+0.3	+3.6	-2.8	+ 0.3	+7.5	1.0+	2.15	2.13	$\mathbf{SF}$		6.0+	+4'2	0.1 +	6.1+	6.1+	+0.4	6.1+	12.5	+0.8	+ 5.7	+ o.2	2.42	2.27	GN around	& Deans (1		
	SF-GN		5.o+	+40	9.0+	• I <del>•</del>	9·1+	+0.4	+2.4	-2.4	9.0+	+ 6.3	+0.4		5.29	FM-SF		+1.4	+ 7.0	1.1+	+0.4	41.7	+1.3	+3.6	0.0	+0.5	+4.2	70.7		2.98	r-seed meal.	by Woodham	Гable 1.	
	$\mathbf{SF}$	_	6.0+	+4'2	+1.0	6.1+	6.1+	+0.4	+1.9	-2.2	+ 0-8	+ 5.7	+0.5	2.33	<b>4</b> 6.1	FM		+1.6	+ 7.7	1.1+	1.0+	41.7	+1.5	+2.8	+2.1	+0.5	+3.8	+ 0.7	3.10	2.92	SF sunflowe	nents given l	osition, see '	<u>9</u> 0.
	SB-SF		+1.3	+3.7	+1.4	+1.2	+2.2	+2.0	+3.1	+0.3	6.0+	+4.9	1.1+	2.74		SB-FM		+1.6	+5.3	+1.4	1.0-	+2.3	+2.1	+3.3	+1.8	40.4	+4.1	+ o.8	3.16		-hean meal - S	ve to requiren	etails of comp	etails, see p. 2
	SB	1	+ 1.5	+2.9	+1.6	+0-	+2.9	+2.8	+3.8	+ <b>1</b> -6	6.0+	+4.5	+1.4	2.26		SB		+ 1.5	<b>6.</b> 2+	9.1+	+.0-	+2.0	+2.8	+3.8	+ <u>1</u> .6	6.0+	+4.5	+ I - <del>1</del>	69.2		SR sov	* Relati	† For d	t For d
-	Protein concentrate	Amino acid	Inreonine	Glycine	Valine	Cystine + methionine	Isoleucine	Leucine	Tyrosine + phenylalanine	Lysine	Histidine	Arginine	Tryptophan	ليقدر	lan	Protein concentrate	Amino acid	Threonine	Glycine	Valine	Cystine + methionine	Isoleucine	Leucine	${f T}$ yrosine + phenylalanine	Lysine	Histidine	Arginine	Tryptophan	rrprj	lan				

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Fig. 3. The content of lysine and of sulphur amino acids in mixtures of protein concentrates with cereals, and the total protein efficiency (g weight gain/g protein consumed; TPE) of the individual protein concentrates and of the best mixtures. (---), Requirement of each amino acid; SF, sunflower-seed meal; FM, fish meal; SB, soya-bean meal; GN, groundnut meal; MM, meat meal. For details of protein concentrates and cereals, see p. 290.

additions was not due to a complementary effect involving the provision, by one component of the mixture, of amino acids which were lacking in the other component. Consideration of the situation with regard to mixtures of SF and GN illustrates the difficulties. The GN in series 1 protein concentrates was superior to that in series 2 in that it had a markedly higher lysine content. Replacement of SF by GN in the series 1 protein concentrates the replacement caused a progressive reduction in the SS value. However, for both series 1 and 2 protein concentrates all the mixtures were limiting in lysine. Despite this in both series 1 and 2 protein concentrates the

intermediate mixtures were superior in nutritive value to the diets containing only GN or SF.

The publications referred to previously (Block & Mitchell, 1946; Bressani & Elias, 1968) all attribute effects such as those demonstrated here to over-all improvements in the amino acid composition achieved by each component contributing something lacking in the other. This is not the explanation in the present series of experiments. In the mixtures studied the only amino acid which was limiting was lysine or the sulphur amino acids and differences in nutritive value were even found in some instances between mixtures in which the provision of all essential amino acids was adequate. For example, all series I SB-FM mixtures had CS values of 100 but there were marked differences in TPE between them. Similar situations have been noted previously. For example, most of the 'TPE' diets containing FM described by Carpenter & Woodham (1974) provided more than the calculated requirements of the chick for amino acids, but differences in TPE were nevertheless found.

An attempt has been made to show diagrammatically the relationship between TPE and dietary lysine and cystine + methionine levels for both series of protein concentrates (Fig. 3). From this Fig. the actual levels of these amino acids in each mixture used can be obtained by dividing the line joining the constituents of the pair of concentrates concerned into six equal portions. The TPE of the best mixture of each set has been inserted on the line. All mixtures in the upper right quadrant of Fig. 3 contain adequate lysine and cystine + methionine. Those in the lower left quadrant of Fig. 3 are deficient in both, while those in the upper left and lower right quadrants of Fig. 3 are lacking in lysine and in cystine + methionine respectively. It is clear at once that while high TPE values tend to occur chiefly in the upper right quadrant of Fig. 3 as might be expected, they do not do so exclusively. Similarly, comparatively low TPE values tend to occur in the lower left quadrant of Fig. 3 but are found elsewhere also. Selection of combinations with the highest CS could be very misleading. In both series of protein concentrates for example, a diet containing SB as the only supplement to cereals would on the basis of amino acid composition appear to be superior to any combination of SB with MM, yet in fact the replacement of one-third of the SB by MM gave superior performance in growing chicks. Clearly CS must be considered an imperfect indicator of quality, albeit perhaps the best one based on amino acid composition which is available at present. Attempts to produce a more satisfactory measure by making allowance for excesses as well as deficiencies in the provision of various amino acids, essential and non-essential, have failed, possibly because excesses of some amino acids may be more deleterious than quantitatively similar excesses of others. It might be objected that differences in the digestibility or availability of the amino acids in the pairs of protein concentrates might account for some of the discrepancies noted but as CS is always calculated from total amino acid composition it was decided that in the present work the usefulness of CS and EAAI should be assessed under the conditions in which they would normally be used. The effect of taking availability into account was however tested by allocating extreme values to the samples and re-calculating CS on this basis. As an example the FM lysine was assumed to be 90 % available and that of GN 75 % available

(Carpenter & Woodham, 1974). The resulting curve obtained for the CS of FM-GN was of course displaced downwards, but the configuration was unaltered.

The amounts by which individual essential amino acids exceed or fall short of the requirements listed in Table 6 are shown in Table 11 for each of the diets containing single protein concentrates (series 2 only) and also for the best mixture, i.e. the mixture which gives the highest TPE for each pair. Examination of this table reveals that in some instances, e.g. SB-SF, the improved performance in the best mixture may be attributable to the fact that each component is making good a deficiency in the other. On the other hand it would seem that such an explanation cannot be invoked for the improvement of FM and SB on partial replacement with GN and MM respectively, and in such instances it will be noticed that there is a reduction in the excesses of some of the essential amino acids. If the improvement in chick growth is to be attributed to an improvement in amino acid composition this must mean that the reduction in the levels of a number of amino acids has resulted in a better over-all amino acid balance. If this hypothesis is correct then any measure such as CS which is solely dependent upon the level of the first limiting amino acid must be inadequate. A high TPE value appears to be associated in our experiments not only with the avoidance of amino acid deficits but also with minimizing surpluses. For example, the replacement of FM by SB to give the highest TPE value of any diet of our series of protein concentrates cannot be explained by the removal of any deficiencies in the FM-containing diet but it will be noted that the glycine level of the latter has been markedly reduced.

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