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Further Studies on the Amino Acid Deficiencies of Plant Proteins.

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In a previous paper (1938), it was pointed out that the nature of the amino acid deficiencies in natural protein feeds is an essential factor in the qualitative selection of protein mixtures; that there exists no direct method of determining the nature of these amino acids and that the chemical analyses may reveal to some extent the value of a protein, but are of no actual biological significance unless correlated with the biological requirements of the animal. Mitchell (1938) fully endorsed this statement on the value of chemical analyses. He states "that even with all the chemical and biochemical information before us, the quantitative evaluation of protein diversities in nutrition would not be solved because the information neglects the animal".

It appears that the best method at present to study the nature of the amino acid deficiencies in protein feeds is to supplement different protein feeds with the suspected deficient amino acids. These supplementations are then compared with the unsupplemented protein feeds on young rats by means of the paired feeding method under identical conditions. Since the only difference in these comparisons is the inclusion of a small amount of a specific amino acid, it is reasonable to attribute any significant gain in weight in favour of the supplemented ration, to the inclusion of the amino acid. However, gain in weight alone is a non-specific criterion of nutritive value. Unless therefore in studies of this nature, the gains in weight can be defined or correlated with increased nitrogen storage, the results are inconclusive and have a dual interpretation. Conclusive proof, as to whether the greater gains induced by the amino acid inclusion are due to a better utilization of the nitrogen contained in the protein under experimentation can be obtained either by a complete nitrogen analysis of rats in each pair or by a determination of the biological value of the supplemented and unsupplemented ration. The latter method was adopted in preference to the first, since it fitted into our general programme of protein investigation. Briefly then the method adopted in this Laboratory for the determination of amino acid deficiencies in protein feeds is as follows: The

nature of the amino acid deficiency is first established by means of the paired feeding method. If by statistical analysis, it is found that the supplemented ration produces greater gains than the unsupplemented ration, then the biological value of the same rations containing the same percentage of nitrogen is determined. By this means the greater gain in weight is supported by nitrogen utilization figures and definite conclusions can be arrived at. No difference in growth response in the paired feeding test signifies no supplementation and consequently nitrogen balance studies are conducted only in selected cases.

Most of the plant proteins except sesamemeal and cottonseedmeal have already been supplemented by some or other of the indispensible amino acids (Smuts and Marais, 1939). Due to lack of direct information the same course as previously adopted, namely supplementation by the least expensive and most easily procurable amino acid, was followed. Consequently sesamemeal and cottonseedmeal were each supplemented by cystine and compared with the unsupplemented component in a paired feeding test.

The supplementation of coprameal with 0·20 per cent. cystine showed no improvement in gains in weight over the unsupplemented coprameal ration (Smuts and Marais, 1939). It was therefore decided to test the inclusion of another indispensible amino acid namely lysine in a ration of which the nitrogen consisted entirely of coprameal protein.

Previous attempts (Smuts and Marais, 1938) to establish the nature of the amino acid deficiency of peanutmeal proved unsuccessful. The addition of tryptophane, lysine and cystine produced no significant response in growth. Five out of the six pairs of rats showed greater gains in weight, when cystine was added to the peanut ration. The magnitude of these gains was, however, too small to establish significance. Beach and White (1937) as well as Baernstein produced evidence that arachin, which constitutes four-fifths of the entire globulin of peanut, is deficient in methionine. On the other hand the remaining fraction of the peanut globulin, namely conarachin is, according to the latter author, an excellent protein for growth. It contains apparently sufficient methionine since it compares favourably with casein in growth-promoting properties. Kotasthane and Narayana (1937) conclude that peanutmeal compares better with milk proteins than soyabeans. All these contributions point strongly to a methionine deficiency in peanutmeal. In fact the evidence seemed so convincing that in a previous paper we assumed methionine to be the indispensible amino acid of peanutmeal. In order to verify our assumption, it was decided to supplement peanutmeal with methionine.

Both soyabeanmeal and linseedmeal have been found to be deficient in cystine. These results were established by the paired feeding method as well as by nitrogen balance studies (Smuts and Marais, 1938). However, it appeared that they may be deficient in a secondary amino acid. Consequently tryptophane was added to each of these meals already supplemented by cystine.

Under practical conditions bran is considered an excellent feed for almost every type of animal. From a scientific point of view, it seems as if this general experience is correct. The endosperm protein appears to be the least valuable, while the proteins of the embryo and aleurone cells are the most valuable. Mitchell (1938) found that whole wheat including the epidermis or bran portion has a biological value of 67 as compared with 52 for patent white flour of which the epidermis has been removed. Klein and associates has made a complete study of the whole wheat kernel. Jones (1937) in a study of the nutritional properties of wheat proteins showed by chemical analysis that the cystine, arginine, histidine, lysine and tryptophane content of bran proteins are higher than that of the endosperm proteins. He further showed by feeding experiments that bran proteins gave a better growth with rats than white flour. Osborne and Mendel (1919) state " that wheat proteins considered in their entirety are adequate for promoting normal growth if eaten in sufficient amounts ". By means of feeding experiments wheat has been found to be deficient in lysine. In order to ascertain, if bran is not probably deficient in lysine, this amino acid was added to a bran ration and its effects on the growth of rats in paired feeding tests studied.

Experimental.

All protein feeds were of commercial quality representative of those brands used in practical feeding. These meals were finely ground and mixed with the rest of the ingredients of the experimental ration so as to give approximately 9 per cent. protein. The amino acids were carefully mixed with the rest of the ration so as to ensure homogeneous distribution. The percentage composition of the rations is given in Table 1.

The paired feeding method was utilized throughout these studies. The nitrogen balance sheet method of Mitchell was used for the determination of the biological values. Rats were paired according to age, sex, litter and weight. Body weights were taken weekly and the initial and final weights were the average of three consecutive weighings.

Discussion of Experimental Results.

In Table 2 is given the results on the paired feeding trial of sesamemeal and sesamemeal supplemented with 0·2 per cent. cystine. In a comparison of the weekly gains, as shown in Table 3, rats on sesamemeal supplemented by 0·2 per cent. cystine gained 37 times and those on the unsupplemented ration, only 9 times out of the 48 comparisons.

The supplemented ration gained in total weight over the unsupplemented in 4 out of the 6 pairs of rats. The mean gain between the six comparisons is 23 and the standard deviation 19·8. The value of Z in this case becomes 1·2. Applying Student's probability table for $Z = 1{\cdot}2$ and $N = 6$, the probability that the increase in weight registered by the addition of cystine was a chance effect is 1 in 45. This result, therefore, indicates that the inclusion of cystine has enhanced the growth-promoting properties of sesamemeal. However, the crucial point is whether the inclusion of cystine

TABLE 1.

Percentage Composition of Rations.

Ingredients.	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.	N.
Sesamemeal	23·0	22·6	—	—	—	—	—	—	—	—	—	—	—	—
Wheat bran	—	—	—	—	—	—	—	—	—	—	53·4	52·3	—	—
Coprameal	—	—	—	—	30·2	29·6	—	—	—	—	—	—	—	—
Peanutmeal	—	—	—	—	—	—	—	—	—	—	—	—	15·0	14·7
Cottonseedmeal	—	—	22·2	21·8	—	—	—	—	—	—	—	—	—	—
Soyabeanmeal	—	—	—	—	—	—	23·2	22·8	—	—	—	—	—	—
Linseedmeal	—	—	—	—	—	—	—	—	25·1	24·7	—	—	—	—
Cystine	—	0·2	—	0·2	—	—	0·2	0·2	0·2	0·2	—	—	—	—
Lysine	—	—	—	—	—	0·2	—	—	—	—	—	0·2	—	—
Tryptophane	—	—	—	—	—	—	—	0·15	—	0·15	—	—	—	—
Methionine	—	—	—	—	—	—	—	—	—	—	—	—	—	0·2
Butterfat	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0
Codliveroil	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Harris yeast	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Sucrose	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0
* Salt mixture	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Na Cl	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
Starch (Dextrinized)	52·0	52·2	52·8	53·0	44·8	45·2	51·6	51·85	49·7	49·95	21·6	22·5	60·0	60·1
Total	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0
Per cent. Nitrogen	1·50	1·51	1·49	1·47	1·51	1·52	1·45	1·46	1·50	1·50	1·52	1·53	1·44	1·45

* New salt mixture of Hubbel, R., Mendel, T. B. and Wakeman, A. J. (J. Nutr. 14–273·285–1937).

TABLE 2.

Comparison between Sesame Meal and Sesame Meal +0·2 per cent. Cystine.

	1.		2.		3.		4.		5.		6.	
	Sesame	Sesame and Cystine	Sesame	Sesame and Cystine.	Sesame	Sesame and Cystine.	Sesame	Sesame and Cystine.	Sesame	Sesame and Cystine.	Sesame	Sesame and Cystine.
Initial weight, gm.	115	111	109	108	115	116	117	118	104	105	104	104
Final weight, gm.	175	202	154	202	192	190	190	187	170	199	150	187
Gain, gm.	60	91	45	94	77	74	73	69	66	94	46	83
Food Consumption, gm.	663	663	649	649	662	662	663	663	663	663	622	622
Refusals	0	0	13	0	0	1	1	5	0	0	10	0

has rendered the nitrogen of sesamemeal more utilizable for synthetic purposes. In Table 4 the data pertaining to the biological value determination of sesamemeal supplemented by cystine are given. In a previous paper by Smuts and Malan (1938) the biological value of sesamemeal at approximately the same level was determined and found to be 71. In Table 4 the biological value of sesamemeal supplemented by cystine was determined and a value of 80 obtained. Hence there was an increased nitrogen utilization of 9 per cent. This result, together with the paired feeding test, definitely establishes that the cystine addition to sesamemeal enhanced growth through a better utilization of the sesamemeal nitrogen.

TABLE 3.

A Comparison of the Weekly Gains between Sesame and Sesame +0·2 per cent. Cystine.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week.		
							+	−	±
1	−	−	−	−	−	−	0	6	0
2	−	−	+	+	±	−	2	3	1
3	−	−	−	+	−	−	1	5	0
4	−	−	+	+	+	−	3	3	0
5	−	−	+	−	−	−	1	5	0
6	−	−	+	−	−	−	1	5	0
7	−	−	−	+	−	−	1	5	0
8	−	−	±	−	−	−	0	5	1
	Total for Experiment						9	37	2

\+ Indicates greater gain by Sesame meal.
− Indicates greater gain by Sesame meal + 0·2 per cent. Cystine.
± Indicates equal gains.

In the comparison of bran and bran supplemented by lysine, three pairs favour the bran ration and three the supplemented bran ration in total weight over the experimental period. In the weekly comparisons as shown in Table 6 the gains in weight are distributed almost equally amongst the two comparisons. Judging superficially there appears to be no difference in growth between the two comparisons. This assumption is borne out by a statistical analysis of the data. The mean gain in weight over the period of experimentation is 2·5 and the standard deviation 11·3, giving a value of 0·21 for Z. According to Student's probability table the odds are so small that chance alone may have brought about this result. Consequently, it must be concluded that the addition of lysine to bran had no effect on its growth-promoting properties and that it is not a limiting amino acid in bran protein.

TABPE 4.

Biological Value of Sesame Meal +0·2 per cent. Cystine Ration.
Nitrogen Metabolism Data and the Calculation of the Biological Value.

N. Low Period.

Animal No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N. Intake.	Daily Fecal N.	Body N. in Feces.		Food N. in Feces.	Absorbed. N.	Daily Urinary. N.	Body N. in Urine.		Food N. in urine.	Food N. Retained.	Biological Value.	App. Digestibility.	True Digestibility.
							Per Gram. Food.	Per Day.				Per 100 gms.	Per Day.					
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.			
1.....	96	87	91·5	6·0	—	17·7	2·95	—	—	—	25·5	27·9	—	—	—	—	—	—
2.....	94	87	90·5	6·4	—	17·2	2·69	—	—	—	24·5	27·1	—	—	—	—	—	—
3.....	93	84	88·5	6·3	—	17·5	2·78	—	—	—	30·5	34·5	—	—	—	—	—	—
4.....	106	100	103·0	7·0	—	20·3	2·90	—	—	—	20·4	19·8	—	—	—	—	—	—
5.....	125	112	118·5	7·3	—	19·8	2·71	—	—	—	23·6	19·9	—	—	—	—	—	—
6.....	104	92	99·0	8·2	—	20·6	2·51	—	—	—	22·3	22·5	—	—	—	—	—	—

Protein Period.

1.....	125	130	127·5	10·0	151·0	44·0	2·95	29·5	14·5	136·5	54·5	27·9	35·6	18·8	117·7	86	71	90
2.....	109	119	114·0	9·4	141·9	44·4	2·69	25·3	19·1	122·8	53·6	27·1	30·9	22·7	100·1	82	69	87
3.....	119	127	123·0	10·0	151·0	39·2	2·78	27·8	11·4	139·6	58·0	34·5	42·4	15·6	124·0	89	74	92
4.....	122	132	127·0	10·0	151·0	42·4	2·90	29·0	13·4	137·6	58·0	19·8	25·1	32·9	104·7	76	72	91
5.....	149	149	149·0	9·7	146·5	46·0	2·71	26·3	19·7	126·8	62·0	19·9	29·6	32·4	94·4	74	68	87
6.....	122	125	123·5	10·0	151·0	45·6	2·51	25·1	20·5	130·5	61·2	22·5	28·1	33·1	97·4	75	70	86
														Average...		80	71	89

TABLE 5.

Comparison between Wheat Bran and Wheat Bran +0·2 per cent. Lysine.

	1.		2.		3.		4.		5.		6.	
	Wheat bran.	Wheat bran and lysine.	Wheat bran.	Wheat bran and lysine.	Wheat bran.	Wheat bran and lysine.	Wheat bran.	Wheat bran and lysine.	Wheat bran.	Wheat bran and lysine.	Wheat bran.	Wheat bran and lysine.
Initial weight, gm.	70	71	62	63	87	88	90	87	75	79	74	73
Final weight, gm.	141	137	149	144	147	157	150	167	155	160	162	148
Gain, gm.	71	66	87	81	60	69	60	80	80	81	88	75
Food consumption, gm.	598	598	605	605	589	589	605	605	597	597	609	609
Refusals	2	4	1	6	8	0	3	0	4	3	0	4

TABLE 6.

A Comparison of the Weekly Gains between Wheat Bran and Wheat Bran +0·2 per cent. Lysine.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week. +	−	±
1	±	±	+	+	−	+	3	1	2
2	±	±	−	−	+	+	2	2	2
3	+	+	±	+	+	+	5	0	1
4	+	−	−	−	−	±	1	4	1
5	−	−	−	−	−	±	0	5	1
6	+	−	−	−	±	+	2	3	1
7	+	+	−	−	−	+	3	3	0
8	−	+	+	−	+	−	3	3	0
	Total for Experiment						19	21	8

\+ Indicates greater gain by Wheat Bran.
− Indicates greater gain by Wheat Bran + 0·2 per cent. Lysine.
± Indicates equal gains.

In Table 7 the results of the paired feeding test on coprameal and coprameal supplemented by 0·2 per cent. lysine are reproduced. From the total gains in weight of the six pairs of rats, it will be seen that four of the 6 pairs showed a better gain on the supplemented ration.

However, the magnitude of these gains are extremely small, so much so that no statistical significance could be established. The mean gain in weight is 0·66 and the standard deviation, 4·8, making the outcome of this test completely insignificant. Hence, under these conditions, it appears as if lysine is not a limiting factor of coprameal.

In Table 9 the total gains in weight of the comparison between peanutmeal and peanutmeal supplemented by methionine, are summarized. Four pairs favour the supplemented and two pairs the unsupplemented ration in total gains. By analysing the results statistically, it is found that the mean gain in weight is 3·3 and the standard deviation 6·2. The value of Z becomes 0·53. According to Student's table of probability the odds are approximately 1 in 7 that chance alone may have brought about this result. On this basis it must be concluded that methionine addition did not contribute anything to the growth-promoting properties of peanutmeal.

It is interesting in this respect to refer to a previous publication by Smuts and Marais (1938) where peanutmeal was supplemented with cystine. In that case, although 5 out of the 6 pairs favoured the cystine supplementation, the magnitude of the gains was so small that the outcome was completely insignificant. These results would seem to indicate that peanutmeal contains enough cystine and methionine or methionine alone for the growth of the white rat. This would mean that while the greater portion of the globulin in peanut is deficient in methionine, the rest of the protein fraction is capable of supplying the needs of this amino acid for the growth of the rat.

On the other hand, it may be equally possible that minute quantities of methionine such as is present for instance in the conarachin fraction of peanuts, are sufficient in the presence of adequate cystine to promote normal growth. Under these conditions, it must be assumed that cystine can replace a certain portion of methionine in the process of anabolism.

Cottonseed gave the highest biological value of the natural plant proteins thus far investigated (Smuts and Malan, 1938). The indications are that in all probability, it is not seriously lacking in any particular indispensible amino acid. Nevertheless the effect of cystine supplementation was studied. The results on total gains and weekly gains are given in Tables 11 and 12.

TABLE 7.

Comparison between Copra Meal and Copra Meal +0·2 per cent. Lysine.

	Copra Meal.	Copra Meal and Lysine.	Copra Meal.	Copra Meal and Lysine.	Copra Meal.	Copra Meal and Lysine.	Copra Meal.	Copra Meal and Lysine.	Copra Meal.	Copra Meal and Lysine.	Copra Meal.	Copra Meal and Lysine.
Initial weight, gm.	102	100	109	107	80	82	114	116	126	120	48	50
Final weight, gm.	149	150	158	150	113	118	168	164	164	164	80	86
Gain, gm.	47	50	49	43	33	36	54	48	38	44	32	36
Food Consumption, gm.	394	394	394	394	331	336	394	394	394	394	272	272
Refusals.	0	0	0	0	12	8	0	0	0	0	11	10

TABLE 8.

A Comparison of Weekly Gains between Copra Meal and Copra Meal +0·2 per cent. Lysine.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week.		
							+	−	±
1.	−	−	−	+	−	−	1	5	0
2.	−	−	+	±	−	±	1	3	2
3.	+	+	−	+	±	+	4	1	1
4.	+	+	+	+	−	+	5	1	0
5.	+	+	−	±	+	−	3	2	1
	Total for Experiment.						14	12	4

\+ Indicates greater gain by Copra meal.
− Indicates greater gain by Copra meal + 0·2 per cent. Lysine.
± Indicates equal gains.

TABLE 9.

Comparison between Peanut Meal and Peanut Meal +0·2 per cent. Methionine.

	1.		2.		3.		4.		5.		6.	
	Peanut.	Peanut + Methionine.	Peanut.	Peanut + Methionine.	Peanut.	Peanut + Methionine.	Peanut.	Peanut + Methionine.	Peanut.	Peanut + Methionine.	Peanut.	Peanut + Methionine.
Initial weight, gm....	63	64	67	64	62	60	65	66	68	68	63	63
Final weight, gm.....	110	115	135	140	105	95	134	142	130	128	128	138
Gains, gm.....	47	51	68	76	43	35	69	76	62	60	65	75
Food Consumption, gm.	430	430	544	544	423	423	541	541	516	516	543	543
Refusals.....	1	19	1	2	5	24	2	2	2	14	2	0

TABLE 10.

A Comparison of the Weekly Gains between Peanut and Peanut +0·2 per cent. Methionine.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week.		
							+	−	±
1.....	−	±	−	−	−	−	0	5	1
2.....	−	+	±	+	+	−	3	2	1
3.....	±	+	+	−	+	−	3	2	1
4.....	+	−	+	±	−	+	3	2	1
5.....	+	±	+	−	+	−	3	2	1
6.....	−	−	−	−	−	−	0	6	0
7.....	+	−	+	+	±	−	3	2	1
8.....	−	−	+	−	+	+	3	3	0
	Total for Experiment.....						18	24	6

\+ Indicates greater gain by peanut meal.
− Indicates greater gain by peanut meal + 0·2 per cent. Methionine.
± Indicates equal gain.

Table 11.

Comparison between Cottonseed and Cottonseed +0·2 per cent. Cystine.

	1.		2.		3.		4.		5.		6.	
	Cotton-seed.	Cotton-seed + Cystine.	Cotton-seed.	Cotton-seed + Cystine.	Cotton-seed.	Cotton-seed + Cystine.	Cotton-seed.	Cotton-seed + Cystine.	Cotton-seed.	Cotton-seed + Cystine.	Cotton-seed.	Cotton-seed + Cystine.
Initial weight, gm.....	95	95	116	118	108	109	121	121	127	128	135	132
Final weight, gm.....	167	186	185	186	163	182	185	184	167	190	192	190
Gain, gm.....	72	91	69	68	55	73	64	63	40	62	57	58
Food Consumption, gm.....	664	664	638	638	648	648	648	648	646	646	648	648
Refusals.....	0	2	1	12	0	7	2	3	8	0	8	0

Table 12.

A Comparison of the Weekly Gains between Cottonseed and Cotton seed +0·2 per cent. Cystine.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week.		
							+	−	±
1.....	+	+	−	±	+	+	4	1	1
2.....	−	−	−	±	−	+	1	4	1
3.....	−	−	−	−	−	−	0	6	0
4.....	−	+	+	+	−	±	3	2	1
5.....	−	−	−	−	−	−	0	6	0
6.....	−	+	±	+	−	+	3	2	1
7.....	−	−	−	±	−	±	0	4	2
8.....	−	+	−	−	−	−	1	5	0
	Total for Experiment.....						12	30	6

\+ Indicates greater gain by Cottonseed.
− Indicates greater gain by Cottonseed + 0·2 per cent. Cystine.
± Indicates equal gains.

TABLE 13.

Biological Value of Cottonseed +0·2 per cent. Cystine Ration.
Nitrogen Metabolism Data and the Calculation of the Biological Value.

N. Low Period.

Animal No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N. Intake.	Daily Fecal N.	Body N. in Feces.		Food N. Per Feces.	Absorbed. N.	Daily Urinary N.	Body N. in Urine.		Food N. in Urine.	Food N. Retained.	Biological Value.	App. Digestibility.	True Digestibility.
							Per gram Food.	Per Day.				Per 100 gms.	Per Day.					
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.			
1.....	85	84	85	6·5	—	18·4	2·83	—	—	—	10·5	12·4	—	—	—	—	—	—
2.....	124	126	125	9·3	—	25·5	2·74	—	—	—	14·7	11·8	—	—	—	—	—	—
3.....	137	135	136	9·9	—	30·7	3·10	—	—	—	14·1	10·4	—	—	—	—	—	—
4.....	102	100	101	7·4	—	23·8	3·22	—	—	—	16·6	16·4	—	—	—	—	—	—
5.....	136	135	136	8·9	—	25·0	2·81	—	—	—	13·9	10·2	—	—	—	—	—	—
6.....	129	130	130	9·2	—	25·7	2·79	—	—	—	17·0	13·1	—	—	—	—	—	—

Protein Period.

1.....	113	123	118	9·5	139·7	44·0	2·83	26·9	17·1	122·6	32·7	12·4	14·6	18·1	104·5	85·2	68·5	87·8
2.....	149	160	155	11·0	161·7	48·7	2·74	30·1	18·6	143·1	43·3	11·8	18·3	25·0	118·1	82·5	69·9	88·5
3.....	160	160	160	9·3	136·7	42·8	3·10	28·8	14·0	122·7	31·8	10·4	16·6	15·2	107·5	87·6	61·4	89·8
4.....	133	136	135	9·3	136·7	47·7	3·22	29·9	17·8	118·9	31·8	16·4	22·1	9·7	109·2	91·8	65·1	87·0
5.....	159	168	164	8·9	130·8	44·8	2·81	25·0	19·8	111·0	41·8	10·2	16·7	25·1	85·9	77·4	65·7	84·9
6.....	160	168	164	10·7	157·3	51·9	2·79	29·9	22·0	135·3	48·2	13·1	21·5	26·7	108·6	80·3	67·0	86·0
														Average...		84·1	66·3	86·3

TABLE 14.

Comparison between Soyabeans +0·2 per cent. Cystine and Soyabeans +0·2 per cent. Cystine +0·15 per cent. Tryptophane.

	1.		2.		3.		4.		5.		6.	
	Soya-beans+ Cystine.	Soya-beans+ Cystine + Trypto-phane.	Soya-beans+ Cystine.	Soya-beans+ Cystine + Trypto-phane.	Soya-beans+ Cystine.	Soya-beans+ Cystine + Trypto-phane.	Soya-beans+ Cystine.	Soya-beans+ Cystine + Trypto-phane.	Soya-beans+ Cystine.	Soya-beans+ Cystine + Trypto-phane.	Soya-beans+ Cystine.	Soya-beans+ Cystine + Trypto-phane.
Initial weight, gm....	67	64	75	72	99	98	105	106	108	103	91	92
Final weight, gm.....	75	65	130	122	100	119	114	107	156	121	138	138
Gain, gm.....	8	1	55	50	1	21	9	1	48	18	47	46
Food Consumption, gm.	323	323	508	508	415	415	317	317	419	419	421	421
Refusals.....	4	32	3	5	13	0	9	10	0	27	0	2

TABLE 15.

A Comparison of the Weekly Gains between Soyabeans +0·2 per cent. Cystine and Soyabeans +0·2 per cent. Cystine +0·15 per cent. Tryptophane.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week. +	Total per Week. −	Total per Week. ±
1.....	±	+	−	+	−	..	2	2	1
2.....	±	−	−	+	+	..	2	2	1
3.....	−	±	+	±	−	−	1	3	2
4.....	+	±	−	+	+	−	3	2	1
5.....	±	−	±	+	+	+	3	1	2
6.....	+	+	−	+	+	+	5	1	0
7.....	+	−	−	..	−	+	2	3	0
8.....	±	±	+	..	+	±	3	0	2
	Total for Experiment.....						21	14	9

\+ Indicates greater gain by Soyabeans + 0·2 per cent. Cystine.
− Indicates greater gain by Soyabeans + 0·2 per cent. Cystine + 0·15 per cent. Tryptophane.
± Indicates equal gain.

TABLE 16.

Comparison between Linseed +0·2 per cent. Cystine and Linseed +0·2 per cent. Cystine +0·15 per cent. Tryptophane.

	1.		2.		3.		4.		5.		6.	
	Linseed +Cystine.	Linseed + Cystine + Trypto-phane.	Linseed +Cystine.	Linseed + Cystine + Trypto-phane.	Linseed +Cystine.	Linseed + Cystine + Trypto-phane.	Linseed +Cystine.	Linseed + Cystine + Trypto-phane.	Linseed +Cystine.	Linseed + Cystine + Trypto-phane.	Linseed +Cystine.	Linseed + Cystine + Trypto-phane.
Initial weight, gm....	99	99	97	93	87	85	84	84	84	86	86	83
Final weight, gm.....	189	180	192	178	158	158	155	147	165	173	148	137
Gain, gm.....	90	81	95	85	71	73	71	63	81	87	62	54
Food Consumption, gm.	579	579	599	599	519	519	520	520	547	547	475	475
Refusals.....	0	6	0	1	8	0	11	12	14	0	14	16

TABLE 17.

A Comparison of the Weekly Gains between Linseed +0·2 per cent. Cystine and Linseed +0·2 per cent. Cystine +0·15 per cent. Tryptophane.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Total per Week. +	Total per Week. −	Total per Week. ±
1.....	±	+	+	+	+	+	5	0	1
2.....	−	+	−	−	−	−	1	0	5
3.....	±	+	+	+	−	+	4	1	1
4.....	+	−	±	+	±	−	2	2	2
5.....	−	−	−	+	−	+	2	4	0
6.....	+	−	−	−	±	+	2	2	1
7.....	+	+	−	+	−	+	4	2	0
8.....	+	−	−	+	±	+	3	2	1
Total for Experiment.....							23	14	11

\+ Indicates greater gain by Linseed + 0·2 per cent. Cystine.
− Indicates greater gain by Linseed + 0·2 per cent. Cystine + 0·15 per cent. Tryptophane.
± Indicates equal gain.

Out of the six pairs in the comparison four showed an increased weight on the supplemented ration. The mean difference in total weight is equal to 9·6 and the standard deviation 10·2, giving a value equal to 0·94. The odds that the increase in weight was due to the cystine supplementation are 22 to 1. Consequently it must be concluded that the addition of cystine to cottonseed meal had no effect on the growth-promoting properties of the latter. In Table 13 the biological value of cottonseedmeal plus cystine was determined. As will be seen from these figures an average value of 84 was obtained. In a previous paper Smuts and Malan (1938) found a biological value for cottonseedmeal at approximately the same level of intake of 81. These two values are very nearly the same with no apparent significant difference in the nitrogen utilization. It appears therefore as if cystine is not a limiting amino acid in cottonseed protein.

Both soyabeans and linseedmeal have previously been found to be deficient in cystine. Consequently the possibility of a secondary amino acid deficiency was tested out. Both these protein feeds were therefore supplemented by tryptophane in addition to cystine and compared with the cystine supplementation. The results on soyabeans are given in Table 14 and 15 and that of linseedmeal in Tables 16 and 17. In neither case did the tryptophane addition enhance the growth promoting properties. In fact with linseedmeal the tryptophane addition had, if anything, a slight depressing effect.

Summary and Conclusions.

By means of paired feeding tests, it has been shown that sesamemeal is deficient in cystine and that cottonseedmeal is not deficient in this amino acid. Bran and coprameal are apparently not deficient in lysine, while the supplementation of peanutmeal with methionine did not enhance its growth-promoting properties. Tryptophane does not appear to be a secondary amino acid deficiency in either soyabeans or linseedmeal.

By means of nitrogen balance studies, it was shown that the increased weights obtained by the supplementation of sesamemeal with cystine are due to a better utilization of the sesamemeal nitrogen. The unsupplemented biological value previously determined by Smuts and Malan was 71 and the supplemented biological value as determined in this paper 80. In thecase of cottonseed supplemented by cystine, the biological value of 84 is only slightly higher than 81, and does not signify a significantly better utilization of nitrogen of the supplemented over the unsupplemented cottonseedmeal.

REFERENCES.

BAERNSTEIN, H. D. (1938). The nutritive values of various protein fractions of peanut. *J.B.C.*, Vol. 122, No. 3, pp. 781-790.

BEACH, E. F., and WHITE, A. (1937). Methionine as a limiting factor of arachin. *Scientif. Proc. J.B.C.*, Vol. 119, No. 8.

JONES, D. B. (1937). Wheat—its proteins and nutritional properties. *Cereal Chem.*, Vol. 14, No. 6, pp. 771-782.

KLEIN, A. B., HARROW, L., PINE, L., AND FUNK, C. (1926). The nutritional value of various layers of the wheat and corn kernel. *Am. J. Physiol.*, Vol. 76, p. 237.

KOTASTHANE, W. V., AND NAYARANA, N. (1937). The proteins of groundnut. *Proc. Indian. Acad. Sci.*, (B), Vol. 6, pp. 376-386.

MITCHELL, H. H. (1938). The biological value of proteins and a criticism of the methods of its determinations. *Reale, Acad. D'italica.*, Vol. 26, pp. 1-32.

MITCHELL, H. H., AND CARMAN, G. G. (1926). The biological value of the nitrogen of mixtures of patent white flour and animal foods. *J.B.C.*, Vol. 68, p. 183.

OSBORNE, T. B., AND MENDEL, L. B. (1919). The nutritive value of the wheat kernel and its milling products. *J.B.C.*, Vol. 37, pp. 557-601.

SMUTS, D. B., AND MARAIS, J. S. C. (1939). The amino acid deficiencies in plant proteins. *Onderstepoort J. Vet. Sci. and Anim. Indust.*, Vol. 11, No. 2, pp. 407-416.

SMUTS, D. B., AND MALAN, A. I. (1938). The biological values of lucernemeal, sesamemeal, peanutmeal, coprameal, cottonseedmeal and oatmeal. *Onderstepoort J. Vet. Sci and Anim. Indust.*, Vol. 10, No. 1, pp. 207-219.