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Plant Proteins. VI.—The Amino Acid Deficiencies in Certain Plant Proteins.

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ALTHOUGH the majority of the amino acids were discovered prior to 1900, very limited data exist concerning their relative distribution. Consequently there has been little appreciation of the fact that the nutritive value of a protein depends upon the kind and quantities of its component amino acids. It is, therefore, not surprising that the protein requirements are most generally expressed in terms of amounts of digestible protein without any reference to the differences in quality. However, the biological value of proteins, as developed by Mitchell (1929), takes into account the qualitative differences existing amongst proteins, since it measures the magnitude of absorbed nitrogen actually utilized by the body. It is, therefore, a necessary and essential measure in the calculation of the nett efficiency of different proteins for maintenance, growth, etc. Two proteins may contain the same amount of digestible protein, the only difference being, that one has a biological value of 40 while the other has a biological value of 80. Disregarding qualitative differences one would feed exactly the same amount of the two digestible proteins to cover a specific need of an animal. However, when the quality of these two proteins is taken into consideration, it is obvious that only half the amount of digestible protein of the latter protein is necessary to fulfil the same function as the full amount of the former.

While the biological value actually takes into account the deficiencies of indispensable amino acids in the protein molecule, it does not supply direct information as to the nature of such deficiencies. In order, therefore, to accomplish supplementation judiciously, thereby enhancing the quality of deficient proteins, it becomes imperative that the nature of the amino acid deficiencies prevailing in our feeds should be determined.

Unfortunately no direct and suitable method of determining the amino acid content of feeds is available. Chemical analysis is not only handicapped by laborious, and in many ways incomplete, methods of determination, but also by the fact that such figures, while indicative, have no actual biological significance unless they are tested out on animals. As a result the method most generally employed is very indirect and of the "*hit and miss*" type, by which different amino acids suspected to be deficient are incorporated with a protein and the effect studied by differences in growth response under controlled conditions.

Rose (1935, 1937), through his extensive studies, has in recent years considerably augmented the existing list of indispensable amino acids. Apart from threonine (α amino β hydroxy butyric acid) no new indispensible amino acid has been added. However, valine, phenylalanine, leucine and isoleucine, which have in the past been considered dispensable are now classified by Rose (1937) as indispensable, making the problems of determining the amino acid deficiencies in feeds even more complicated.

As far back as 1915 Osborne and Mendel (1915) demonstrated that *l*-cystine supplements stimulated the growth of rats on a casein containing diet of low sulphur. Since then many investigators have contributed confirmatory evidence of the indispensability of cystine. Johns and Finks (1920) found that the addition of cystine to diets containing phaseolin markedly improves the nutritive quality of this protein. Similarly Sherman and Merril (1925) found that cystine enhanced the growth promoting power of whole milk powder overdiluted with starch. Mitchell and Smuts (1932), in their studies on meat protein, showed that while 20 per cent. meat protein was adequate in cystine, a significant stimulation of cystine on growth occurred when this protein constitutes 9 per cent. of the ration. The same workers (1932), as well as Shrewsbury and Bratzler (1933), and Hayward, Steenbock and Bohstedt (1936), found that cystine was the limiting amino acid in soyabeans. Indeed, so marked is the growth response of rats to the inclusion of cystine in an otherwise adequate ration that Sherman and Woods (1925) actually employed it as a method for quantitative determination of this amino acid. Recently, however, Rose (1937), Beach and White (1937), and Baernstein have produced evidence showing that methionine instead of cystine is the indispensable amino acid. Such evidence must await further confirmation, since it may be possible that these two amino acids may have a reciprocal function in nutrition.

By using Zein as the sole protein in a ration for rats, Osborne and Mendel (1914) demonstrated in a decisive manner the indispensability of tryptophane and lysine. The addition of tryptophane to such a ration satisfied the maintenance requirements, but growth was actually only attained when lysine was incorporated.

Several investigators found soyabeans deficient in cystine. However, the supplementary effect of this amino acid on soyabean protein was repeated, since the possibility existed that all species were not necessarily deficient in cystine. Morris and Wright (1933) found peanutmeal deficient in lysine for milk production, while Johns and Jones (1917), by means of chemical analysis, found no such deficiency. Consequently lysine appeared to be a possible limiting factor in the protein of peanutmeal. No indication of any definite amino acid deficiency in linseedmeal and coprameal was available, so that cystine, the most easily available amino acid, appeared to be the most reasonable to try. The outcome of these different tests is reported below.

Experimental.

The protein feeds which were of an average commercial quality, were finely ground and mixed with the rest of the ingredients of the ration so as to give approximately 8-10 per cent. of protein. The amino acids were incorporated at the rate of ·20 per cent., replacing an equivalent amount of protein nitrogen. These 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 used throughout these studies. In some cases more than six pairs of rats were used, but never less. The two rats of each pair received identical amounts of food, the supplemented ration in one case and the unsupplemented ration in the other. Continuous records were kept of all refusals of feed, which necessitated a reduction in the amount weighed out to both members of the pair. Rats were paired according to age, sex, litter and weight. Frequently rats weighing more than the required weight (60 gms.) had to be used due to scarcity of rats. Body weights were taken weekly and the initial and final weights were the average of three consecutive daily weighings.

Results.

The results of the paired feeding experiments are summarized in Tables 2 and 3. Any significance attributed to treatments must naturally be based on the differences in total gains of pair mates over the experimental period. The size of these differences and the consistency with which they appear determine the probability of the conclusions that they favour. The statistical analyses were performed in accordance with the method of Student (1908) for the interpretation of paired experimental data. These calculations are summarized in Table 4. The probability (P) is obtained from the ratio of the mean difference between pair mates to the standard deviation of differences and by the number of observations. The value of P naturally indicates the significance of the outcome of a treatment. Hence if P is equal to ·01, it may for all practical purposes be concluded that the result obtained is due to the treatment applied and not to a fortuitous outcome due to chance. The greater P becomes the more likely it is that the outcome is due to chance alone.

The supplementing effect of tryptophane on peanutmeal has been tested out on 9 pairs of rats. As will be seen from these results tryptophane supplementation had no effect on the growth promoting properties of peanutmeal, since out of the 9 pairs in the comparison 7 favoured the unsupplemented ration, while only 2 favoured the supplemented ration in total gains in weight. In fact, the probability P strongly favours the unsupplemented ration, indicating that the addition of tryptophane had in all probability exerted a depressing effect on the ration.

In the case of peanut meal supplemented by lysine negative results were also obtained. Out of the 11 pairs in the comparison, 6 favoured the unsupplemented and 5 the supplemented ration in total gains in weight. The probability P of ·28 for the comparisons of total gains is so large that chance alone might well have determined the outcome.

TABLE 1.

Percentage Composition of the Rations.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Peanutmeal	15·7	15·3	15·7	15·3	15·5	15·2	—	—	—	—	—	—
Coprameal	—	—	—	—	—	—	33·0	32·0	—	—	—	—
Linseedmeal	—	—	—	—	—	—	—	—	26·6	26·0	—	—
Soyabeanmeal	—	—	—	—	—	—	—	—	—	—	35·2	34·7
Cystine	—	·20	—	—	—	—	—	·20	—	·20	—	·20
Tryptophane	—	—	—	·20	—	—	—	—	—	—	—	—
Lysine	—	—	—	—	—	·20	—	—	—	—	—	—
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
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
Codliver oil	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
Yeast Extract (1)	10·0	10·0	10·0	10·0	—	—	—	—	—	—	—	—
NaCl	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
Agar	—	—	—	—	2·0	2·0	—	—	—	—	—	—
Salt mixture (2)	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5
Starch	48·8	49·0	48·8	49·0	55·0	55·1	39·5	40·3	45·9	46·3	37·3	37·6
TOTALS	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0
Percent. N	1·50	1·49	1·42	1·41	1·45	1·45	1·40	1·40	1·96	1·93	2·04	1·96

(1) Yeast extract was prepared according to the method of Itter S, Orent E. R., and McCollum, E. V. (*J. Biol. Chem.*, Vol. 108, No. 2, pp. 571-577, 1935).

(2) A modified Osborne and Mendel salt mixture described by P. B. Hawk and B. L. Osler, 1931. *Science*, Vol. 74, p. 369.

TABLE 2.

The Supplementing Effect of Tryptophane, Lysine and Cystine on the Proteins of Peanut.

	8 PER CENT. PEANUT PROTEIN.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.
Initial weight gm.	102	105	96	96	73	76	72	72	105	102	84	84
Final weight gm.	134	130	120	115	105	102	104	100	147	142	129	130
Total Gains gm.	32	25	24	19	32	26	32	28	42	40	45	46
Total food consumption gm.	314	314	286	286	258	258	246	246	343	343	343	343

	Pair 7.		Pair 8.		Pair 9.	
	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.
Initial weight gm.	93	95	80	92	75	67
Final weight gm.	129	128	108	116	102	100
Total Gains gm.	36	33	28	24	27	33
Total food consumption gm.	336	336	322	322	323	323

TABLE 2—(*continued*).

	8 PER CENT. PEANUT PROTEIN.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.
Initial weight gm.	79	79	64	63	52	52	49	49	75	76	55	57
Final weight gm.	130	126	122	115	112	119	95	94	132	122	110	118
Total Gains gm.	51	47	58	52	60	67	46	45	57	46	55	61
Total food consumption gm.	427	427	427	427	415	415	337	337	427	427	406	406

	Pair 7.		Pair 8.		Pair 9.		Pair 10.		Pair 11.	
	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.
Initial weight gm.	50	52	50	50	46	48	48	47	63	60
Final weight gm.	66	65	62	67	56	60	67	59	90	88
Total Gains gm.	16	13	12	17	10	12	19	12	27	28
Total food consumption gm.	176	176	151	151	156	156	167	167	172	172

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm.	81	71	76	76	65	62	135	138	67	69	64	64
Final weight gm.	106	91	98	104	90	88	170	175	89	93	96	104
Total Gains gm.	25	20	22	28	25	26	35	37	22	24	32	40
Total food consumption gm.	360	360	356	356	342	342	497	497	372	372	349	349

TABLE 3.

The Supplementing Effect of Cystine on Rations containing Coprameal, Linseedmeal and Soyabeanmeal.

	8 PER CENT. COPRAMEAL.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm.	115	107	75	75	85	86	79	79	70	68	73	74
Final weight gm.	162	172	137	135	154	150	117	130	134	141	114	114
Total Gains gm.	47	65	62	60	69	64	38	51	64	73	41	40
Total food consumption gm.	512	512	442	442	492	492	405	405	481	481	407	407

	8 PER CENT. LINSEEDMEAL.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm.	99	97	102	104	90	90	95	95	87	88	97	100
Final weight gm.	190	200	188	199	190	187	187	198	180	195	187	192
Total Gains gm.	91	103	86	95	100	97	92	103	93	107	90	92
Total food consumption gm.	555	555	555	555	531	531	555	555	555	555	555	555

	8 PER CENT. SOYABEANS.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm.	108	110	94	92	100	99	84	82	80	80	105	106
Final weight gm.	158	187	150	177	160	175	120	149	131	142	154	178
Total Gains gm.	50	77	56	85	60	76	36	67	51	62	49	72
Total food consumption gm.	361	361	361	361	361	361	352	352	330	330	361	361

TABLE 4.

Statistical Results of Paired Feeding Experiments.

	CYSTINE.				LYSINE.	TRYPTOPHANE.
	Peanut-meal 8 per cent.	Copra-meal 8 per cent.	Linseed-meal 8 per cent.	Soyabean-meal 8 per cent.	Peanut-meal 8 per cent.	Peanut-meal 8 per cent.
Total gain in weight gm.—						
Mean differences M.	+2·3	+5·3	+7·5	+22·8	−1·0	−2·7
Standard deviation S.	4·5	9·3	6·7	7·9	5·8	4·0
Ratio M/S (Z)	·51	·57	1·1	2·9	·17	0·67
Probability (P)	·157	·118	·028	·0007	·281	·041
Comparisons of weekly gains in weight—						
No. favouring supplemented ration	15	25	26	38	27	14
No. favouring unsupplemented ration	16	16	13	5	33	30
No. favouring either ration	5	1	9	5	6	10

This finding is not in agreement with that of Morris and Wright (1933), who found peanutmeal seriously lacking in lysine for milk production. However, it is quite possible that milk production calls for an increase of quantity of this amino acid so that peanutmeal may under these conditions be short of lysine. On the other hand, Johns and Jones (1917), by means of chemical analysis, found no deficiency of lysine in the proteins of peanutmeal.

When peanutmeal is supplemented by cystine the result is negative. Although 5 of the 6 pairs show a greater total gain in weight when cystine is incorporated, these gains are not large enough to make the difference statistically significant. The probability P is equal to ·157, a value which is far greater than the accepted standard of significance. Under these conditions it must be concluded that chance alone could have produced the outcome. If, however, pair 1 is omitted then cystine supplements peanutmeal significantly. After our results had been obtained Beach and White (1937) and Baernstein (1938) showed that metheonine was the limiting amino acid in arachin, one of the proteins of peanut. Conarachin the other protein, is apparently complete. Hence it would appear that methionine is the limiting indispensable amino acid in peanutmeal.

In Table 3 the supplementary effect of cystine on coprameal, linseedmeal and soyabeanmeal has been summarized. Of the 6 pairs of rats on the coprameal and coprameal supplemented by cystine, 3 favoured the supplemented and 3 the unsupplemented ration as regards total gain in weight. In the 42 weekly comparisons of gain in weight, rats, on the supplemented ration, gained 25 times and those on the unsupplemented ration only 16 times. However, the probability P, that the greater gain of the supplemented ration has been a chance outcome is ·118, a value several times greater than the critical value of ·03. It is, therefore, concluded that the difference in total gain cannot be attributed to the effect of cystine.

In a comparison of linseedmeal with linseedmeal supplemented by cystine, 5 out of the 6 pairs favoured the supplemented ration in total gain. The probability (P) that this difference in total gains is due to chance is only ·028, which means that the chances are approximately 34 to 1 that chance alone would have brought about such a result. It is clear, therefore, that cystine has exerted a supplementing effect on the proteins of linseedmeal.

That cystine has a definite, supplementary effect on the proteins of soyabeans is seen from Table 3. Of the 6 pairs in the test all responded to treatment of cystine and outgained by far their pair mates on the unsupplemented ration. The probability P that this result is due to chance is equal to ·0007, which clearly demonstrates that this outcome is exclusively due to the inclusion of cystine in the ration.

Summary and Conclusions.

By means of the paired feeding method, the possible indispensable amino acid deficiencies of peanutmeal, coprameal, linseedmeal and soyabeanmeal have been investigated.

It has been found that peanutmeal is not deficient in cystine, tryptophane or lysine, but may be in the light of investigations which appeared after the work had been completed, deficient in methionine. Coprameal is not deficient in cystine, while linseedmeal and soyabeanmeal are definitely deficient in cystine.

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