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Plant Proteins III.

The Supplementary Effect amongst Certain Plant Proteins.

By D. B. SMUTS and J. S. C. MARAIS, Section of Nutrition, Onderstepoort.

IT is a generally recognized fact in protein studies, that the nutritive value of a certain protein feed may be significantly enhanced by the inclusion of some other protein feed in the ration. Such a supplementation amongst different proteins naturally becomes of considerable practical importance in assessing the biological values of the proteins most commonly employed in feed mixtures, since it has been shown, that the supplementary action of proteins cannot be considered to occur in a linear fashion. Consequently it cannot be assumed that the mean of the biological values of two proteins at a certain level of feeding, would represent the biological value of a mixture of equal parts of the respective proteins. Conclusive proof as to whether supplementation between two proteins has actually taken place, can only be obtained by a direct determination of the biological value of each protein, as well as the mixture of the two proteins at the same level of intake.

Supplementation amongst proteins is the nett result of a variable amino acid constitution of protein feeds in respect to the indispensable amino acids. A protein fed at a low level of intake may be considered to consist of two distinct entities, one constituting the amino acids necessary for maintaining the integrity of the tissues and the other composed of those amino acids which form an incomplete assortment, and consequently incapable of promoting synthesis of new tissues. These latter amino acids are therefore drawn into the oxidative processes of the body and catabolized. If, however, a second protein, which has a different indispensable amino acid deficiency, is fed in conjunction with the first, it can readily be seen how the fractions of each protein which separately were destined to be deaminized and oxidized can between them make up a complete assortment of those indispensable amino acids to satisfy the requirements of new tissue and hence promote synthesis. In this case the biological value of the mixture of the two proteins will obviously be greater than the mean biological value of the two proteins, since a portion of the two fractions in the respective proteins, which would otherwise have gone waste as far as tissue synthesis is concerned, is now utilized in the body.

It is evident from the above reasoning, that supplementation will not take place between all proteins. If the remaining fraction of each protein in the mixture, after maintenance has been taken care of, should be deficient in the same indispensable amino acid, then naturally there exists no chance of selecting a complete amino acid complex for synthetic purposes and consequently no supplementation is possible. On the other hand it is self evident that the greater the variation of the missing indispensable amino acids in the two fractions, which effect supplementation, the greater are the possibilities of a supplementation. A protein having a biological value of 80 would, therefore, indicate that 20 per cent. of the absorbed nitrogen has not been utilized on account of a deficiency of one or more of the indispensable amino acids in the protein molecule. Another protein having a biological value of 50, shows that 50 per cent. of the absorbed nitrogen has not been used by the body. In the latter case the fraction which can be supplemented is much greater than the first. If, therefore, these two proteins are fed as a mixture and supplementation takes place, then one has to assume that it was possible for the body to select a complete assortment of amino acids from the 20 and 50 per cent. fractions.

In a previous paper [Smuts and Malan (1938)] the biological values of individual plant proteins have been determined. In this study the supplementary effect amongst certain of these proteins is reported on.

As far as the authors are aware no studies have been conducted on the supplementary effect of the proteins investigated in this study. However, since the supplementation can only be accomplished when the limiting indispensable amino acids of a protein are covered by the inclusion of a second protein, it seems appropriate at this stage to refer to some investigations concerned with the amino acid deficiencies of the proteins under discussion. Haag (1931) in a study on the physiological effects of rations restricted principally or solely to plants, claims that lucerne is seriously deficient in cystine for growth in rats. Scoz (1932) found that cystine deficiency limits growth in rats, and that the animal protects the protein sulphur of the organism at the expense of less essential substances. It would appear, therefore, that lucerne is deficient in the indispensable amino acid cystine and that it can consequently only be supplemented by the addition of cystine or another protein containing enough cystine or methionine to cover the cystine deficiency [Jackson and Bloch (1933); Weichselbaum (1935)].

Morris and Wright (1933) in a study on the protein values for milk production claim that peanutmeal is deficient in lysine and since this amino acid is considered necessary for milk production, peanutmeal ranks low as a protein feed for lactating animals. On the other hand John and Jones (1917) by chemical analysis showed that the protein of peanut is rich in lysine. Quite recently Beach and White (1931) stated that the protein of arachin is deficient in methionine and that L.-cystine was incapable of replacing the former.

McCollum and Simmonds (1916) as well as Mitchell and Smuts (1932) have shown that oats are deficient in the indispensable amino acid lysine. Outhouse and Krause (1934) found that a lysine deficiency with rats causes an inefficient utilization of protein nitrogen and a less active cellular metabolism.

The Paired Feeding Tests.

Paired feeding tests together with the determination of the respective biological values were conducted on the different rations. The rations were compounded in such a fashion that they were, as far as possible, equal in all respects except for the difference in source of protein. In the comparison of peanut plus lucernemeal with lucernemeal plus cystine, 7 per cent. of agar replaced an equal weight of starch in the former ration, while in the comparison of peanut and peanut plus oats no agar was included in the ration. The different proteins were incorporated in the ration so as to furnish approximately 9 per cent. of crude protein. The other constituents included in the ration contributed negligible amounts of nitrogen but supplied the necessary amounts of other nutrients to make the rations complete. The comparison of the rations is given in Table 1.

Table I.

Composition of Rations.

	A.	B.	C.	D.	E.	F.
Peanutmeal	16·5	—	—	7·8	7·8	—
Lucernemeal	—	57·1	55·7	—	28·6	—
Oatmeal	—	—	—	36·4	—	—
Cystine	—	—	·20	—	—	—
Ext. Egg white	—	—	—	—	—	3·8
Sucrose	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
Yeast Extr. 1	10·0	10·0	10·0	10·0	10·0	10·0
Agar 3	—	—	—	—	—	2·0
Codliveroil	2·0	2·0	2·0	2·0	2·0	2·0
NaCl	1·0	1·0	1·0	1·0	1·0	1·0
Salt Mixture 2	4·5	4·5	4·5	4·5	4·5	4·5
Starch	48·0	7·4	8·6	20·3	28·7	58·7
Total	100·0	100·0	100·0	100·0	100·0	100·0
Per cent. Nitrogen	1·45	1·52	1·55	1·42	1·50	·650

(1) Yeast extract was prepared according to the method of (Itter 3, Orent E. R. and McCollum E. V. J. B. C. 108, 2, 571–577, 1935.)

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

(3) The fibre content was equalized by adjusting the agar in the rations compared.

Five to six pairs of rats were used in the different paired feeding tests. These were selected according to age, sex, litter and weight. Each pair received the same amount of food. The initial weights taken over three consecutive days, together with the total gains, total food consumption, and total gain per week of the different comparisons, are summarized in tabular form.

In Table II a summary of the comparisons between lucerne supplemented by ·20 per cent. cystine and lucerne meal is given. In each pair the rat receiving the cystine supplement exceeded its mate in total gain in weight over the experimental period. Such a consistent outcome favouring either one or the other ration would have resulted from chance only twice in 128 trials. The average difference in gain between the six pairs is 13·5 with a standard deviation* of 6·5. According to Students probability table, when Z which is equal to $\frac{M}{\text{St. Deviation}}$ is equal to 2·0 and N=6, the chances are approximately one in 300 that this difference is due to chance. From a comparison of the weekly gains, it is seen that out of the 42 weekly comparisons, the cystine supplemented rat exceeded its pairmate in gains in 29 cases. This experiment, therefore, clearly demonstrates a difference between the two rations, and since the only difference was the inclusion of cystine in one ration, which proved to be superior to the control ration of lucernemeal, it must be concluded that the addition of cystine was instrumental in promoting more rapid growth.

From Table III it is evident that when peanut is supplemented by oatmeal, the resulting protein mixture is superior to that of peanut alone. Out of the 30 weekly comparisons of gains, 18 favoured the peanut plus oatmeal, while only 9 favoured the peanut ration. The average difference in total gains is 9·8 and the standard deviation 4·7. The ratio (Z) of the mean difference to the standard deviation is 2·1. With Z=2·1 and N=5, the odds according to Students' table, that the addition of oatmeal to peanutmeal was responsible for the more rapid gains experienced on the mixed protein ration, are approximately 143 to 1.

Nitrogen Metabolism Experiments.

The same rations as were used in the paired feeding tests were utilized in the nitrogen metabolism experiments. An extra period consisting of lucernemeal supplemented by peanut in equal proportions in respect to protein was also included. The nitrogen low ration was adjusted for its fibre content by the addition of agar to equal that of the protein ration under investigation. The collection periods were of 7 days duration and were in each case preceded by a preliminary feeding period of 6 days on the same ration. For the biological value determination of each protein a new series of rats were used, so that rats were not kept longer than 26 days on experiment.

* Standard Deviation = $\sqrt{\frac{\Sigma D^2}{n-1}}$

TABLE II.

Summary of Bodyweights, Gains, Feed Records, Number of Refusals, and Total Gains per week on Lucerne and Lucerne plus Cystine Ration.

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.
Initial wt.	64·0	66·0	70·0	63·0	92·0	92·0	53·0	52·0	54·0	49·0	60·0	54·0
Final wt.	140·0	120·0	142·0	117·0	135·0	130·0	122·0	104·0	105·0	89·0	108·0	94·0
Gain in grams	76·0	54·0	72·0	54·0	43·0	38·0	69·0	52·0	51·0	40·0	48·0	40·0
Total Food consumption	509	509	530	530	474	474	493	494	407	407	410	410
Refusals	0	6	0	4	4	1	1	8	0	16	0	15
Total gains per week	7 — 0 — 0		6 — 1 — 0		3 — 2 — 2		4 — 2 — 1		5 — 1 — 1		4 — 1 — 2	

TABLE III.

Summary of Bodyweights, Gains, Feed Records, Refusals and Gains per week on Peanut and Peanut plus Oatmeal Rations.

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		
	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	
Initial wt.	9·50	97·0	92·0	99·0	94·0	87·0	97·0	99·0	97·0	105·0	
Final wt.	130·0	142·0	125·0	140·0	124·0	132·0	124·0	139·0	126·0	137·0	
Gain	35·0	45·0	33·0	41·0	30·0	45·0	27·0	40·0	29·0	32·0	
Total Food consumption	356	356	369	369	334	334	339	339	322	322	
Refusals	5	0	1	1	4	0	4	2	3	2	
Total gains per week	3 — 0 — 3		3 — 0 — 3		2 — 0 — 4		0 — 2 — 4		1 — 1 — 4		9 — 3 — 18

TABLE IV.

Nitrogen Metabolism Data and the Calculation of Biological Values.

Animal No.	Initial weight grm.	Final weight grm.	Average weight grm.	Daily food intake grm.	Daily N intake mgm.	Daily Fecal N mgm.	*Body N in Feces.* Per grm. food consumed. mgm.	*Body N in Feces.* Per day mgm.	Food N in Feces mgm.	Absorbed N mgm.	Daily Urinary N mgm.	*Body N in Urine.* Per 100 gm. weight mgm.	*Body N in Urine.* Per day mgm.	Food N in Urine mgm.	Food N Retained. mgm.	Biological Values.
N. Low Period for Lucerne plus Peanut Ration.																
30	91·0	92·0	91·5	8·0	—	25·55	3·20	—	—	—	22·94	25·07	—	—	—	—
31								—	—	—			—	—	—	—
32	92·0	92·0	92·0	9·3	—	26·97	2·90	—	—	—	22·48	24·44	—	—	—	—
33	104·0	104·0	104·0	8·0	—	25·55	3·22	—	—	—	24·98	24·02	—	—	—	—
34	83·0	84·0	83·5	7·6	—	25·17	3·31	—	—	—	20·44	24·48	—	—	—	—
35	91·0	91·0	91·0	6·6	—	16·59	2·51	—	—	—	23·74	26·09	—	—	—	—
Lucerne plus Peanut Ration containing 1·50 per cent. Nitrogen.																
30	89·0	97·0	93·0	10·6	159·0	53·80	3·20	33·92	19·88	139·12	64·18	25·07	23·32	40·86	98·26	71
32	99·0	115·0	107·5	10·9	163·5	50·06	2·90	31·61	18·45	145·05	76·64	24·44	26·37	50·27	94·78	65
33	10·0	109·0	105·0	10·4	156·0	55·06	3·22	33·49	21·57	134·43	69·94	24·02	25·22	44·72	89·71	67
34	90·0	95·0	92·5	10·9	163·5	50·50	3·31	36·08	14·42	149·08	74·24	24·48	22·64	51·60	97·48	65
35	95·0	98·0	96·5	10·9	163·5	65·97	2·51	27·36	38·61	124·89	64·24	26·09	25·18	39·06	85·83	69
															Average......	67
N. Low Period for Oatmeal plus Peanut Ration.																
36	120·0	115·0	117·0	6·2	—	24·41	3·94	—	—	—	25·72	21·98	—	—	—	—
37	110·0	112·0	111·0	7·6	—	26·14	3·44	—	—	—	17·16	15·45	—	—	—	—
38	111·0	112·0	111·5	6·7	—	18·29	2·73	—	—	—	23·98	21·50	—	—	—	—
39	110·0	110·0	110·5	8·0	—	25·76	3·22	—	—	—	21·78	19·71	—	—	—	—
40	110·0	105·0	107·0	5·4	—	18·04	3·33	—	—	—	26·70	24·95	—	—	—	—
41	105·0	102·0	103·5	5·7	—	20·24	3·55	—	—	—	23·32	22·53	—	—	—	—

TABLE IV (*Continued*).

Oatmeal plus Peanut Ration containing 1·42 per cent. Nitrogen.

Animal No.	Initial weight grm.	Final weight grm.	Average weight grm.	Daily food intake grm.	Daily N intake mgm.	Daily Fecal N mgm.	*Body N in Feces.*		Food N in Feces mgm.	Absorbed N mgm.	Daily Urinary N mgm.	*Body N in Urine.*		Food N in Urine mgm.	Food N Retained. mgm.	Biological Values.
							Per grm. food consumed. mgm.	Per day mgm.				Per 100 gm. weight mgm.	Per day mgm.			
36	118·0	125·0	121·5	7·7	109·3	25·67	3·94	30·34	0·0	109·3	48·8	21·98	26·71	22·09	87·21	80
37	116·0	118·0	117·0	7·1	100·8	24·38	3·44	24·42	0·0	100·8	39·2	15·45	18·98	21·12	79·68	79
38	118·0	127·0	122·5	8·0	113·6	21·81	2·73	21·85	0·0	113·6	50·9	21·50	26·34	24·56	89·04	78
39	122·0	125·0	123·5	6·7	95·1	23·10	3·22	21·57	1·53	93·6	39·8	19·71	24·34	15·46	78·14	83
40	119·0	123·0	121·0	8·0	113·6	25·15	3·33	26·64	0·0	113·6	46·6	24·95	30·19	16·41	97·19	85
41	112·0	122·0	117·0	8·0	113·6	23·61	3·55	28·40	0·0	113·6	47·2	22·53	26·36	20·84	92·76	82
															Average	81
N- Low Period for Lucerne plus Cystine Ration.																
50	127·0	122·0	124·0	6·4	—	26·94	4·21	—	—	—	21·87	17·63	—	—	—	—
51	130·0	132·0	131·0	5·7	—	25·14	4·41	—	—	—	22·48	17·16	—	—	—	—
52	114·0	114·0	114·0	7·8	—	42·20	5·41	—	—	—	22·48	19·71	—	—	—	—
53	110·0	110·0	110·0	6·8	—	35·43	5·21	—	—	—	18·48	16·81	—	—	—	—
54	100·0	100·0	100·0	7·7	—	35·57	4·62	—	—	—	19·09	19·09	—	—	—	—
55	105·0	105·0	105·0	6·8	—	29·87	4·39	—	—	—	19·40	18·47	—	—	—	—
Lucerne plus Cystine Ration containing 1·55 per cent. Nitrogen.																
50	115·0	129·0	122·0	9·0	139·5	66·0	4·21	37·89	28·11	111·39	35·75	17·57	21·44	14·31	97·08	87
51	127·0	140·0	133·5	9·0	139·5	68·0	4·41	39·69	28·31	111·19	32·34	17·16	22·91	9·43	101·76	91
52	108·0	124·0	116·0	9·0	139·5	68·2	5·41	48·69	19·51	119·99	31·27	19·71	22·86	8·41	111·58	93
53	104·0	120·0	112·0	9·0	139·5	65·1	5·21	46·89	18·21	121·29	31·60	16·81	18·83	12·77	108·52	89
54	100·0	90·0	95·0	8·0	124·0	63·4	4·62	36·96	26·44	100·91	36·08	19·14	18·18	17·90	83·01	82
55	72·0	110·0	91·0	9·0	139·5	62·6	4·39	39·51	23·09	116·41	37·82	18·47	16·81	21·01	95·40	82
															Average	87

As will be seen from Table IV the individual biological values of the lucerne peanut mixture varied from 63 to 71 with an average value of 67. Since the biological values of lucerne and peanutmeal as determined in a previous paper [Smuts and Malan (1937)] are 61 and 69 respectively, the value of 67 obtained for the mixture of these two proteins, at approximately the same level, can only be interpreted as indicating a non-supplementary effect.

In the case of lucernemeal supplemented by ·20 per cent. cystine, the individual biological values vary from 82 to 93 with an average figure of 87. This would mean that the addition of ·20 per cent. cystine to lucerne increased the utilization of its absorbed nitrogen by 27 per cent. When peanutmeal is supplemented by oatmeal, in equal proportions in respect to protein, there seems to be a distinct supplementation between these two proteins. The biological value of peanut and of oatmeal as determined in a previous paper (Smuts and Malan 1937) was found to be 69 and 84 respectively. If no supplementation took place one would expect the biological value of the mixture to be 77. This value is lower than the determined biological value of the mixture, namely 81. It seems therefore reasonable to deduce that supplementation has taken place. This conclusion is, furthermore, supported by the outcome of the growth studies in which the protein mixture of peanut plus oats is significantly superior to peanut alone, when fed at the same level of protein intake. Mitchell and Smuts (1932) have shown that oats are deficient in lysine. Since supplementation has taken place between oatmeal and peanutmeal, it seems obvious that peanutmeal must have been able to cover the lysine deficiency in oats. In a later paper it will be shown by means of paired feeding tests, that the addition of lysine did not enhance the growth promoting properties of peanutmeal.

Summary and Conclusions.

By means of the paired feeding method, it has been shown that the addition of cystine to lucerne significantly enhances the growth promoting properties of the latter, and that when peanutmeal is supplemented by oatmeal the resulting protein mixture is superior to that of peanutmeal alone.

Nitrogen metabolism studies conducted on the same rations showed that the incorporation of ·20 per cent. cystine in a lucerne ration definitely increased the biological value of lucerne, that no supplementation exists between peanutmeal and lucernemeal, and that supplementation occurs between peanutmeal and oatmeal.

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