SUPPLEMENTATION OF SOYA PROTEIN WITH L-METHIONINE, DL-METHIONINE HYDROXY ANALOGUE AND CERTAIN NATURAL SOURCES OF METHIONINE

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In a recent investigation[14] of the protein values of legume seeds grown in South Africa in significant quantities, soya protein proved to have the highest nutritive value. This finding led the authors to suggest that 'soya might, perhaps in combination with other foodstuffs rich in methionine, furnish a practical basis for the immediate improvement of the Bantu diet'.

The suggestion that soya protein be combined with foodstuffs rich in methionine was based on a knowledge of the amino acid composition of soya protein and on the results of earlier experiments on animals[1,2,6-8,10,17,19,25,27,28,32] and man,[23] which clearly indicated that the protein quality of the pulses is limited by an insufficiency of the sulphur-containing amino acids, i.e. methionine and cystine.

Chemically pure methionine as well as the so-called 'methionine analogues' are available for the supplementation of foodstuffs deficient in methionine. Of especial interest is methionine hydroxy analogue (α-hydroxy-γ-methio-butyric acid), the zinc salt of which was, as early as 1932, found by Block and Jackson[5] to be biologically effective. Their finding has since been confirmed and is being extensively applied in animal feeding.[20,26]

It has been shown that not only the hydroxy but also the keto analogues of most of the essential amino acids can be converted into the corresponding amino acids by the rat.[24] We have not, however, found confirmation in the literature that the results of the animal experiments also apply in the case of humans.

Natural Sources of Methionine

In addition to the chemically pure substances there are several natural sources of methionine which are worthy of consideration.

Maize protein is the first such source which can be expected to improve the biological value of soya protein because of its high methionine and cystine content (about 4·5 G/100 G for South African sifted granulated maize meal protein)[31] and which should itself benefit from the fairly high concentrations of lysine and tryptophan in soya protein.

Sesame, one of the oldest of the vegetable oil crops, at present grown in significant quantities in India, China, Egypt, West Africa, Latin America and the USA (Texas) is another good source of the sulphur-containing amino acids. Block and Bolling[4] have reported a value of 4·4% (methionine + cystine) for sesame protein, which is high in comparison with the value of only 1·7% reported by Bandemer and Evans[3] for soya protein. De Groot and Spanjers[10] observed a protein supplementary effect when mixtures of sesame and 'brown beans' were fed to rats.

Grass seed. The highest figure ever reported for the sulphur-containing amino acid concentration of a native protein is that of 8·4 obtained by Carbiener, Jaeger and Busson[9] for the protein fraction of a West African grass seed, *Digitaria exilis (Kippist) Stapf*. This seed is said to be part of the staple diet of West African natives during the dry seasons and has been suggested by De Groot and Spanjers[10] as a possible supplement for legume seed proteins.

The practical value of proteins containing a high proportion of a particular amino acid for supplementing other proteins deficient in that amino acid will depend on (*a*) the extent to which the amino acid can be liberated in the intestinal tract of the animal, and (*b*) the actual amount of the particular amino acid that can be 'spared' by the supplementing protein over and above that needed for optimal assimilation of the supplementing protein itself.[22]

Assessing Usefulness

Since suitable chemical methods for the determination of digestibility and assimilability are not as yet available, the most accurate assessments of the supplementary effects of pure amino acids, amino acid analogues or proteins in the nutrition of monogastric animals are those based on a biological technique in which rats or other monogastric mammals are employed.

Using as a criterion of response in rats weight gain (in G) per G protein consumed (i.e. protein efficiency ratio or PER), De Groot and Spanjers[10] investigated the supplementary effects of the proteins in milk, meat, fish, potato, wheat, sunflower seed and sesame on the proteins of brown beans. There is also a need for information on the supplementation of soya protein with products rich in methionine. We therefore wish to submit in the present publication the results of an investigation of the supplementary value of 5 different sources of methionine on soya protein. Each study consisted of an investigation of the relationship between protein value and level of supplementation with the methionine source. The supplements investigated were maize, sesame, *Digitaria exilis*, methionine and methionine hydroxy analogue.

MATERIALS AND METHODS

The Samples and Their Preparation for Investigation

In all the experiments suitable quantities of a single sample of cooked whole soya meal ('Geduld' variety) were used. Preparation of the meal was as follows: The seeds were cracked in a roller mill, winnowed to remove the hulls and soaked overnight in a suitable quantity of tap water. The soaked seeds, together with the soaking water, were transferred to a 'Siko' pot and autoclaved for 20 minutes at 15 lb. steam pressure. The cooked beans plus the remaining soaking water were spread out in thin layers in flat, stainless steel pans, dried in a circulation oven at 50°C, and ground to a meal in a hammer mill. The protein content (Nx5.71) of the dried meal was found to be 37·9% on a moisture-free basis.

In the experiment with soya + maize it was found that the standard ratio of 10 G protein/100 G experimental ration could not be achieved in those rations in which the maize protein content needed to be high in relation to the soya protein content. To make possible the inclusion of those quantities of maize protein which would yield diets containing 10% protein the bulk of the soya meal had to be reduced. This was achieved by reducing the fat content of the soya through hexane extraction in a Soxhlet apparatus. The protein content of the defatted meal was 51·7% on a moisture-free basis.

The maize meal was cooked by autoclaving a mixture of 30 parts by weight of commercial sifted granulated maize meal and 70 parts of tap water for 20 minutes at 15 lb. steam pressure. The cooked porridge was then roller-dried and the dried material was ground to a meal. The protein content (Nx6.25) of the meal was 9·5% on a moisture-free basis.

For the preparation of the sesame meal, the seeds of a commercial sample of sesame (*Sesamum indicum*) were first cracked between a set of laboratory-type stone rollers and then defatted by means of hexane extraction in a Soxhlet apparatus. The defatted material was heated in a 'Siko' pot at 15 lb. steam pressure for 15 minutes, then dried in a circulation oven at 50°C and finally ground to a meal. The meal contained 44·4% protein (Nx5.30; moisture free).

The sample of *Digitaria exilis* was prepared from seeds imported from Gambia, West Africa, by the Plant and Seed Control Division of the Department of Agricultural and Technical Services. Since certified seeds are often treated with dieldrin by the Gambian authorities before dispatch to other countries, the material used in our experiments was first Soxhlet-extracted for about 6 hours with petroleum ether (boiling point 63 - 75°C) as a precautionary measure against possible insecticide poisoning of the experimental animals. The extracted seeds were autoclaved in a 'Siko' pot at 15 lb. steam pressure for 20 minutes, dried at 50°C in a circulation oven and then ground in a hammer mill. The protein content (Nx6.25) of the prepared material was 8·9% on a moisture-free basis.

After preparation, all food materials were packed in clean tins with tight-fitting lids. The soya meal was stored in a deepfreeze and the other materials in the laboratory at room temperature.

In the studies with chemically pure crystalline materials the following were used: BDH Laboratory Reagent Grade L-methionine and the calcium salt of DL-methionine hydroxy analogue from the National Biochemical Corporation. These materials were dispatched from overseas by airmail and stored in a refrigerator until used.

Biological and Analytical Methods

Each experiment consisted of a series of determinations of the digestibility and biological value of soya protein alone and of soya protein supplemented at graded levels with the particular methionine source under investigation.

Protein digestibility and biological value were assessed by a modification[11-13] of Mitchell's nitrogen balance method.[21] Comparable groups of 6 weanling rats (3 males and 3 females per group) were used in each test, 5 groups in the case of maize and respectively 11, 12, 11 and 11 groups in the cases of sesame, *Digitaria exilis*, methionine and methionine hydroxy analogue.

All experimental diets contained approximately 1·6% nitrogen, which means that whenever a nitrogen-containing supplement was included it replaced an isonitrogenous amount of soya protein in the diet. In the case of methionine hydroxy analogue, which is nitrogen-free, the soya nitrogen content of all the diets was kept at the same level (1·6%), the hydroxy analogue added replacing an equal weight of the carbohydrate (dextrin) component of the diet instead of the protein component.

The moisture contents of samples and experimental diets and the nitrogen contents of samples, diets and excreta were determined according to standard methods. Details have been given in previous publications.[11-13]

RESULTS AND DISCUSSION

The results obtained in the 5 series of investigations for protein digestibility (D), biological value (BV) and percentage nett assimilability (NPU) of the protein at the different levels of supplementation are shown in 5 separate graphs in Fig. 1. The Y-axes represent the observed protein values and the X-axes the level of supplementation in terms of supplement N as a percentage of total N. The regression lines were fitted to the various straight-line segments of the digestibility and biological value data by the least-squares method. The lines for NPU were derived from the lines for digestibility and biological value on the basis of the equation NPU = D% × BV% ÷ 100. To assist the reader in comparing the supplementation level: NPU relationships for the different series all the NPU data are combined in a sixth graph in the lower right-hand corner of Fig. 1.

Further details in regard to the relationship between level of supplementation and biological response are given in Table I, in which are recorded the least-squares regression equations upon which the graphs in Fig. 1 are based, and the degree of significance of the upward or downward trend of each straight line. The latter statistic was calculated according to the method proposed by Terpstra.[29]

L-Methionine

As was expected, supplementation of soya protein with free L-methionine resulted in a significant increase in the digestibility of the soya N + supplement N mixture, the soya protein being only about 92·5% available for absorption but the free amino acid wholly available. Also in accordance with expectation was the rapid increase in biological value with the increase in methionine content. A distinct maximum was reached at a level of approximately 1% L-methionine nitrogen, or a whole soya + L-methionine mixture containing about 0·7% L-methionine. The gain in biological value at an optimal level of supplementation with methionine was considerable, viz. about 15·5 percentage units, the soya protein being thereby promoted to a class superior to that of most other proteins, including the high-quality proteins of meat and milk. An increase in the level of supplementation beyond the optimum (i.e. from 1% up to about 18%) resulted in a decrease

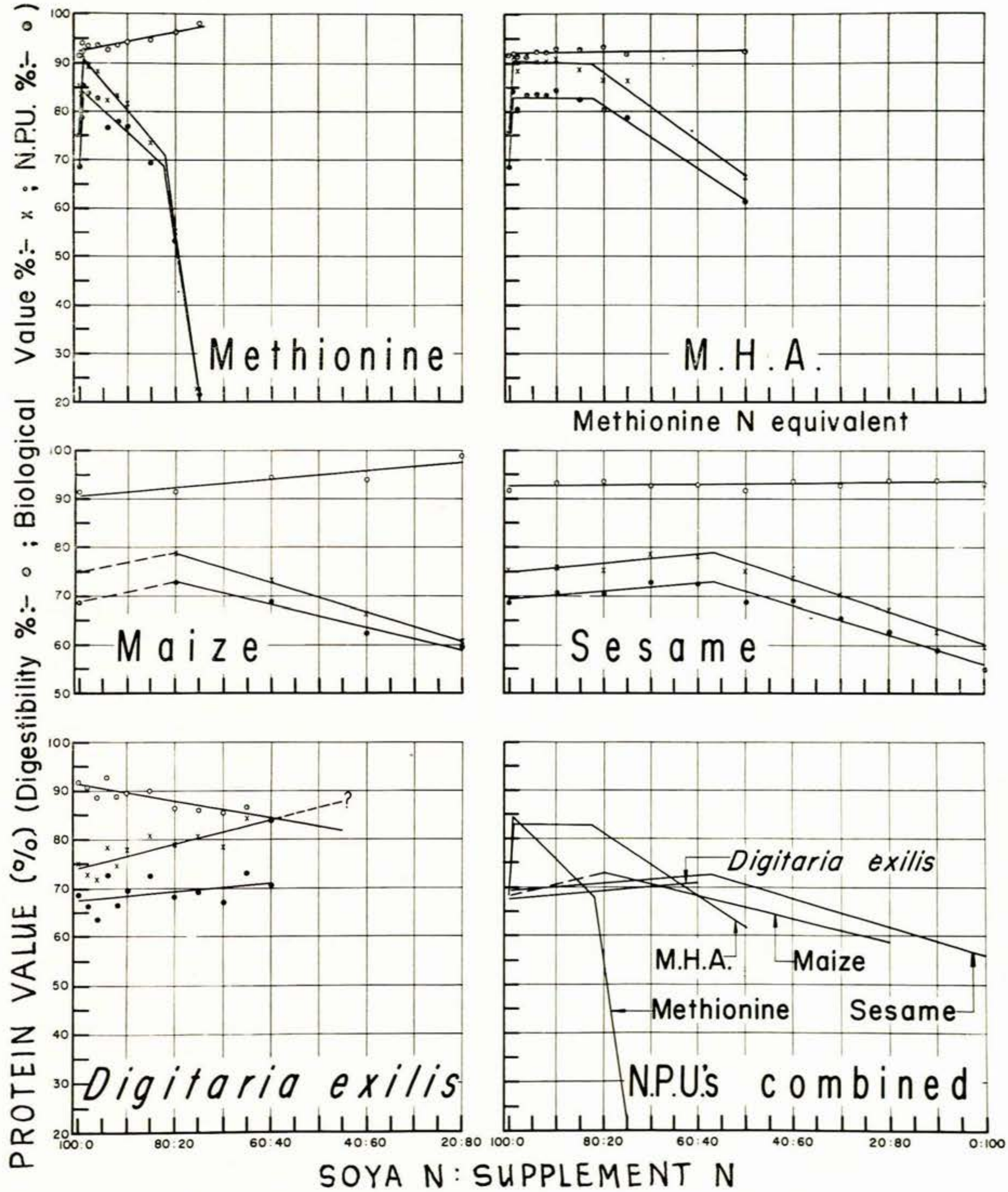

Fig. 1. The relationship between protein value (digestibility, biological value and NPU) and the level of supplement nitrogen in soya supplemented at graded levels with certain sources of methionine. Each point on the graph represents the mean of the results of 6 individual determinations.

TABLE I. THE RELATIONSHIP BETWEEN PROTEIN VALUES (DIGESTIBILITY AND BIOLOGICAL VALUE) AND THE PERCENTAGE SUPPLEMENT N IN CERTAIN SOYA N+SUPPLEMENT MIXTURES

Food mixture	*Range of concentration (%) of supplement N in soya N+supplement N mixture*	*Protein value (Y) in terms of % supplement N in mixture (X)*	*Level of significance of upward or downward trend (P%; two-tailed)*
		Digestibility %	
Soya+L-methionine	0 — 25·0	Y=92·5+0·194 X	<0·01
		Biological value %	
	0 — 1·0	Y= 75·0+16·900 X	<0·006
	1·1— 18·0	Y= 91·8— 1·180 X	<0·006
	18·1— 25·0	Y=186·0— 6·540 X	0·4
		Digestibility %	
Soya+DL-methionine hydroxy analogue	0 — 50·0*	Y=91·8+0·017 X	25·4
		Biological value %	
	0 — 1·0	Y= 75·0+16·400 X	0·4
	1·1— 17·5	Y= 90·3— 0·032 X	95·6
	17·6— 50·0	Y=102·2— 0·713 X	0·22
		Digestibility %	
Soya+Maize	0 — 80·0	Y=90·5+0·086 X	<0·006
		Biological value %	
	0 — 20·0	Y=75·0+0·200 X	52·8
	20·1— 80·0	Y=85·2—0·312 X	0·012
		Digestibility %	
Soya+Sesame	0 —100	Y=92·5+0·006 X	97·4
		Biological value %	
	0 — 43·5	Y=74·8+0·088 X	13·9
	43·6—100·0	Y=92·4—0·324 X	<0·00[illegible]
		Digestibility %	
Soya+Digitaria exilis	0 — 40·0	Y=91·1—0·170 X	<0·006
		Biological value %	
	0 — 40·0	Y=74·0+0·245 X	<0·006

*Since methionine hydroxy analogue is nitrogen-free these figures represent methionine N equivalents.

in biological value apparently owing mainly to a 'dilution' of the assimilable protein nitrogen fraction by methionine nitrogen.

An interesting feature of these results was that from the level of about 18% methionine nitrogen onwards there was a more acute deflection in the curve, i.e. a greater depression in the downward trend of the biological value with an increase in the supplementation level. We observed a concomitant decrease in food intake and a general deterioration in the health status of the experimental animals as the methionine N intake rose above the 18% level. In view of the findings of various workers who have studied the pathology of amino acid excesses it would appear that this phenomenon is due to the combined effect of methionine toxicity and further dilution of the available protein, causing among other effects anorexia and tissue breakdown which result in increased urinary nitrogen excretion and an apparent decrease in biological value. In addition to the non-specific weight loss and anorexia caused by excessive methionine intake, Klavins and Peacocke[18] have mentioned pancreatic acinar damage, alterations in salivary glands, renal tubular dilatation and increased iron deposition in the liver, spleen and bone marrow as changes induced specifically by methionine when it is consumed in toxic quantities.

DL-Methionine Hydroxy Analogue

The results obtained with the DL-methionine hydroxy analogue agree closely with those obtained with L-methionine in respect of both supplementary value and toxic effects. There was, however, no appreciable increase in the digestibility with increased supplementation and no 'diluting effect', the analogue being nitrogen-free and therefore incapable of causing such effects. The toxic effect, which came into operation when the supplementation level exceeded the 18% margin, appeared to be less drastic than that obtained with methionine, the latter difference being probably also due to the absence of a diluting effect.

The finding that the hydroxy analogue is for all practical purposes as effective a soya protein supplement as is L-methionine in the nutrition of the rat is of practical importance in view of the rather prohibitive cost of L-methionine compared with the relatively low cost of the analogue. However, it should be emphasized that before supplementation with the hydroxy analogue can become a practical possibility in human nutrition, our findings should first be confirmed in studies on human subjects. We hope that the improvement of the biological value of soya protein to the extent of about 15 percentage units, demonstrated by us to result from the addition of only a small quantity of the hydroxy analogue, will serve as an incentive for such studies.

Sesame

The digestibility figures and biological values obtained in the soya + sesame series are typical of the effect which would be obtained by feeding a mixture of 2 proteins which are equal in digestibility but different in biological value, and at least one of which is capable of furnishing an essential amino acid in which the other is deficient. As was pointed out by Mitchell[22] '. . . the biological value of a mixture of proteins is not necessarily the mean of the biological values of the individual proteins, each value being weighted according to the proportion in which the protein occurs in the mixture. We may consider each protein fed . . . as consisting of 2 fractions, the one including the maximum proportion of amino acids that can be used to replenish or enlarge the supply of nitrogenous substances in the tissues, the other including the remaining proportion of amino acids destined to be deaminised, because it does not contain the complete assortment of amino acids essential for synthesis into complexes needed by the tissues. If 2 proteins are fed together to an animal, those fractions of each which would otherwise be deaminised may together contain a complete assortment of amino acids, permitting a part of the combined fractions to be used for synthetic purposes. In such a case, the biological value of the mixture would be greater than the weighted mean of the biological values of both.'

In the present series there was no significant upward or downward trend in protein digestibility as a result of an increase in the sesame protein content of the mixture, indicating that the 2 protein fractions in question were approximately equal in digestibility. The data (see Fig. 1 and Table I) suggest that maximum assimilability is obtained with a combination consisting of about 56·6 parts of soya nitrogen and about 43·4 parts of sesame nitrogen. When fed separately, these quantities of nitrogen would in each case be assimilated to the extent of D% × BV% ÷ 100, and it can be seen that on this basis they would yield a total of only $\frac{(69{\cdot}2 \times 56{\cdot}6)}{100} + \frac{(55{\cdot}9 \times 43{\cdot}4)}{100} = 63{\cdot}4$ parts of assimilable nitrogen. However, at the suggested optimal combination the percentage assimilability (NPU) was found to be about 72·9, which indicates a gain of about 9·5 percentage units due to a supplementary effect. From a practical viewpoint it would appear, therefore, that the most efficient whole soya meal + defatted sesame meal mixture is one consisting of approximately 62% whole soya and 38% defatted sesame, the exact ratio depending on variations in the nitrogen content of the 2 components. According to our results 100 parts by weight of such a mixture contains about 46 parts of protein of which about 33 parts are assimilable.

Maize

In similar fashion it can be shown that there was a supplementary relationship between soya and maize protein, causing an additional yield of 6·8 NPU units when soya and maize nitrogen were mixed in the ratio 80 : 20. Scrutiny of the results obtained in this series shows, however, that the number of combinations tested was perhaps too limited to permit a definite conclusion as to the precise combination of soya + maize protein that can be expected to be optimal in protein value. Nitrogen mixtures containing less than 20% maize nitrogen, which were not tested, might conceivably yield higher supplementary gains. Furthermore, in the supplementation of soya with maize protein at graded levels, one would not expect the protein value to rise to a maximum in a linear fashion, the reason being that 3 essential amino acids (methionine, tryptophan and lysine) are involved in a process of mutual supplementation of the 2 proteins in question. Unless the methionine deficiency of soya happens to be exactly equivalent to the tryptophan and lysine deficiency of the maize, the rise in protein value can be expected to occur in 3 separate stages. In the first stage deficiencies in all 3 of the above amino acids would be met simultaneously, causing a rapid increase in protein value up to a point where optimal supplementation is reached for one of these amino acids. The rate of increase should thereafter diminish stepwise into a second and a third stage as optimal supplementation is reached first for one and then for the other of the remaining 2 limiting amino acids.

Digitaria exilis

According to the results *Digitaria exilis per se* proved to be of very little value as a source of methionine, in spite of the very high methionine content of this seed.[9] There was a significant increase in biological value with a rise in the supplementation level, but, unfortunately, there was a concomitant decrease in digestibility, the nett effect in terms of supplementary gain being as a result too small to be of practical significance. Food technological investigation of the protein component of *Digitaria exilis*

TABLE II. CALCULATED WEIGHTS OF CERTAIN FOODSTUFFS REQUIRED TO MEET THE FAO RECOMMENDED DAILY ASSIMILABLE NITROGEN ALLOWANCE OF 3·36 G FOR A CHILD, 2 YR. OLD AND WEIGHING 13 KG.

Food or combination of foods	*N content of food on dry basis (%)*	*Soya N: supplement N*	*NPU or percentage assimilability of N*	*Type of feeding: S = separately; C = in combination*	*Assimilable N content on a dry basis (%) = NPU × N content % ÷ 100*	*Dry weight of food (G) yielding 3·36 G assimilable N*	*Decrease in weight of food required due to supplementary effect (G)*
Cooked whole soya meal	6·63	100 : 0	68·5*	S	4·54	74	—
Cooked defatted sesame meal	8·38	0 : 100	55·9	S	4·68	72	—
Cooked, sifted, granulated maize meal ..	1·52	0 : 100	53·5†	S	0·81	415	—
Soya meal + sesame meal	4·13 + 3·16 = 7·29	56·6 : 43·4	72·9	C	5·31	63	
				S	4·60	73	10
Soya meal + maize meal	3·15 + 0·79 = 3·94	80·0 : 20·0	72·8	C	2·87	117	
				S	2·59	130	13
Soya meal + *Digitaria exilis*	1·61 + 1·08 = 2·69	60·0 : 40·0	70·6	C	1·90	177	—
Soya meal + L-methionine	6·58 + 0·07 = 6·65	99·0 : 1·0	85·2	C	5·67	59	
				S	4·51§	74	15
Soya meal + DL-methionine hydroxy analogue	6·58 + 0·07 = 6·65‡	99·0 : 1·0‡	83·9	C	5·58	60	
				S	4·51§	74	14

*Mean of values given by the 5 separate pairs (digestibility and BV) of regression equations recorded in Table I.
†Estimated through extrapolation on the basis of the regression equations given in Table I.
‡Since the hydroxy analogue of methionine is nitrogen-free, this figure represents methionine nitrogen equivalents.
§In the calculation of the assimilable N contents of these mixtures the percentage assimilability of separately-fed amino acids or that of their corresponding analogues was considered to be zero.

might, however, lead to the development of special techniques for the extraction of the protein and the preparation of nutritionally effective protein hydrolysates for the supplementation of methionine-deficient foodstuffs.

Practical Applications

The practical significance of the results discussed above can best be described in terms of the weights of the various foods and combinations of foods required to meet specific protein needs. As a basis for the calculation of such weights we have selected FAO's[15] recommended daily allowance of 21 G assimilable protein (or 3·36 G assimilable nitrogen) for a young child, 2 years of age and weighing 13 kg. This selection is not entirely an arbitrary one, since according to data compiled[30] the incidence of protein-calorie malnutrition (kwashiorkor) is highest in the age group of 1 - 3 years.

In Table II are given the dry weights of the individual foods and of various combinations of these foods which will yield approximately 3·36 G of assimilable nitrogen. We have listed the weights of the food combinations that will meet the nitrogen requirement when the 2 foods are fed (*a*) together and (*b*) separately. The difference between (*a*) and (*b*) gives an indication of the supplementary effect.

It can be seen that in the case of maize meal an exceptionally large quantity is needed to furnish the daily requirement. In view of the fact that maize is generally consumed as a porridge which contains at least 70% water, it is clear that it will be difficult if not impossible for a 2-year-old child to consume daily a sufficient amount of porridge to meet all its protein requirements. It appears, however, that this problem could be solved by adding to the maize either soya alone or soya supplemented with a food material which is a source of the first limiting amino acid of soya, methionine.

In conclusion it seems pertinent to emphasize that the results reported above have a bearing on only one aspect of nutrition, viz. the improvement of the *protein value* of soya and the possible use of either soya alone or soya supplemented with other food materials in the improvement of the protein value of defective diets. In the supplementation of protein-deficient diets it should, however, be borne in mind that the supplement may contain other ingredients in addition to the protein. There may be ingredients present which have a harmful effect. Gilbert and Gillman[16] have for instance shown that the feeding of maize meal supplemented with food yeast, soya meal, Steenbock-40 mineral mixture and vitamins A, E and D causes nephrocalcinosis in rats. This finding was confirmed by one of us (D.B.d.B.—unpublished data) who considers the stone formation to be the result of a calcium-phosphorus imbalance caused by the addition of the salt mixture and the soya meal.

SUMMARY

An investigation is described of the supplementary effects at different levels of supplementation of L-methionine, DL-methionine hydroxy analogue, sesame protein, maize protein, and the protein component of the West African grass seed *Digitaria exilis*, on soya protein.

Each of the 5 studies was based on a series of biological assessments of the digestibility and biological value of the protein component of soya alone and of soya supplemented at graded levels with one of the 5 materials mentioned above. The experimental animals were albino rats of which 6 were included in each test group. The nitrogen content of all test diets was about 1·6%, i.e. about 10 G protein/100 G diet.

The percentage digestibility of a particular mixture of soya protein N + supplement N was found to be equal to the sum total of the digestible fractions of the 2 individual nitrogen components. The biological value was, however, subject to the influence of supplementary, 'diluting' and toxic effects, the first causing an increase and the second and third causing decreases in biological value.

Of the supplements investigated, methionine and methionine hydroxy analogue were the most efficient in supplementing soya protein, small additions being capable of elevating the biological value to a level superior to that of many high-quality animal proteins, such as those of milk and meat. Except in the case of *Digitaria exilis* protein, the results indicate that supplementation with the methionine-rich food proteins tested also causes an appreciable gain in protein value, the extent of the gain depending on the ratio of soya N: supplement N.

The levels (in terms of supplement N as a percentage of total N) at which optimal supplementary gains were observed, were L-methionine, 1; DL-methionine hydroxy analogue, 1 methionine N equivalent; sesame, 43·4. In the case of maize the maximum was at 20%, but the available data are considered too limited to afford accurate assessment of the optimal level.

It is concluded that, with certain necessary precautions, either soya protein or correctly supplemented soya protein can be used for the improvement of protein deficient diets.

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