Protein utilization in rats receiving a low-protein diet with various limiting amino acids

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1. A study was made of protein utilization in rats given a variety of low-protein diets containing (g/kg) egg albumin 18, casein 49, gluten 50, or mixtures of either maize protein and gelatine 58, or casein and gelatin 37, each with supplemental methionine. The diets were limiting in leucine, tryptophan or lysine, or in both tryptophan and the sulphur-containing amino acids.

2. Values obtained for net protein utilization (NPU) at these low levels of nitrogen intake were markedly higher than the amino acid score calculated for the different test proteins, except with casein for which the two values were similar. The NPU values agreed more closely with chemical score values based on the content of S-amino acids or isoleucine in the dietary protein.

The nutritional quality of a protein is determined by its amino acid content. The theory and practice of the amino acid score (AAS), recommended by FAO for the evaluation of protein (Report of a Joint FAO/WHO and ad hoc Expert Committee on Energy and Protein Requirements, FAO/WHO, 1973), suggest that the quality of a protein is decided by the essential amino acid present in the least amount, by comparison with a reference pattern. Lack of even one essential amino acid prevents, in principle, utilization of the others. Utilization of such a deficient protein, measured by net protein utilization (NPU) (Miller & Bender, 1955; Rafalski & Nogal, 1964*a*, *b*) should be zero. NPU measurements made by Bender (1965) in young rats using proteins lacking one of the essential amino acids, showed, however, that only those completely without sulphur-containing amino acids or valine gave the zero value predicted by the AAS. Protein not containing lysine had a NPU of 0·38, whilst proteins lacking tryptophan, threonine, histidine or leucine had NPU values from 0·11 to 0·22. Said & Hegsted (1970) investigated the relation between increase in body water content and the amount of dietary amino acid consumed. Their investigations confirmed that only diets without threonine, isoleucine or the S-containing amino acids produced the same response in animals as did protein-free diets. The lowest body-weight losses were seen with diets lacking lysine and leucine.

These studies confirmed the results obtained by Bender (1965) with respect to the sulphur-containing amino acids, and showed that the responses of animals to complete lack or insufficiency of essential amino acids are specific for the particular amino acids used in anabolic processes. The agreement of the results of Bender (1965) and Said & Hegsted (1970), in relation to the sulphur-containing amino acids, and also the disagreement in respect of valine, threonine and isoleucine, permit the supposition that utilization of protein, where little of it is present in the diet, might not be entirely determined by the level of the first limiting amino acid.

The purpose of this present study was to determine protein utilization in rats given low-protein diets at and below maintenance level. Proteins were evaluated in which according to Bender's target mixture, the first limiting amino acid was leucine, S-containing amino acids, lysine or tryptophan; the second limiting amino acid was phenylalanine, methionine or isoleucine; the third limiting amino acid was lysine, threonine, or phenylalanine and S-containing amino acids.

Table 1. *Composition (g/kg) of experimental diets given to rats*

Dietary protein source	Protein	Starch	Sucrose	Lard	Vitamin-mineral mixture*
Egg albumin	18	582	150	150	100
Casein	49	551	150	150	100
Wheat gluten	50	550	150	150	100
Maize-gelatine-methionine	58	542	150	150	100
Casein-gelatine-methionine	37	563	150	150	100

* The vitamin-mineral mixture contained/kg diet: 2909 μg retinol, 42 μg ergocalciferol, 2·9 mg thiamine, 9·7 mg riboflavin, 1·9 mg pyridoxine, 0·05 mg cyanocobalamin, 16·4 mg α-tocopheryl acetate, 0·2 mg biotin, 1·0 mg menadione, 57·9 mg calcium pantothenate, 193·0 mg nicotinic acid, 1·9 mg folic acid, 193·0 mg *myo*-inositol, 579·2 mg *p*-aminobenzoic acid, 579·2 mg choline, 3·5 g sodium chloride, 20·8 mg monocalcium phosphate, 20·0 g potassium citrate, 0·8 g ferrous citrate, 4·8 g magnesium sulphate, 44 mg potassium iodide, 37 mg sodium fluoride, 7 mg manganese sulphate, 3·7 mg copper iodide, 3·7 mg potassium aluminium sulphate; 3·7 mg zinc sulphate.

Table 2. *Essential amino acid content of the protein mixtures given to rats in relation to Bender's* (1965) *target mixture* (*i.e. relative amino acid scores* (*AAS*)†)

Amino acid	Composition of target mixture (mg/g)	AAS of dietary proteins: Egg albumin	Casein	Wheat gluten	Maize–gelatine–methionine	Casein–gelatine–methionine
Isoleucine	43	1·697	1·279	0·953	0·465**	0·604**
Leucine	78	1·089*	1·243	0·884	0·743	0·628
Lysine	52	1·250***	1·596	0·269*	0·653	0·980
Methionine	27	1·481	1·037	0·592**	0·814	1·000
Total sulphur-containing amino acids	47	1·404	0·680*	0·787	0·574	0·617***
Phenylalanine	49	1·224**	1·081**	1·040	0·571	0·612***
Threonine	41	1·268	1·170***	0·609***	0·560***	0·634
Tryptophan	10	2·000	1·600	1·000	0·260*	0·440*
Valine	50	1·800	1·380	0·840	0·600	0·740
Histidine	18	1·444	1·666	1·222	0·722	0·777

* First limiting amino acid; ** second limiting amino acid; *** third limiting amino acid.
† Content in test protein: content in reference protein (Bender, 1965).

EXPERIMENTAL

Protein utilization measurements were made using the NPU method of Miller & Bender (1955), as modified by Rafalski & Nogal (1964*a*, *b*). At the protein concentrations used for the experiments, determinations were made as 'NPU zero' (NPU^0) or 'standardized NPU' (NPU^{st}) of Miller & Payne (1961), or using the nomenclature of Rafalski & Nogal (1966) of maintenance NPU (NPU^m), which is a measure of the greatest efficiency with which a dietary protein can be used.

The diets studied were based on Miller & Bender's (1955) protein-free diet, which contains (g/kg): 600 starch, 150 sucrose, 150 lard, 50 vitamin mixture in starch, 50 minerals. To make the low-protein diets, the test mixtures, egg albumin, casein, gluten, maize–gelatine–methionine and casein–gelatine–methionine, were substituted for starch (Table 1).

The experimental work on animals was carried out according to the methods described by Miller & Bender (1955) and Rafalski & Nogal (1966). Wistar rats were housed in groups of four and the number of groups given the test diets were: albumin diets, six; casein diet, nine; gluten diets, ten; maize–gelatine–methionine diet, eight; casein–gelatine–methionine

Table 3. *Relation between quantity of protein in diet, food intake and protein intake, and body-weight change in rats given experimental low-protein diets**

(Mean values for groups of four rats; no. of groups as indicated)

Dietary protein source	No. of groups	Dietary protein content (g/kg)	Protein intake (g/d)	Diet intake (g/d)	Body-wt change (g)
Egg albumin	6	18	2·39	123·6	−31·5
Casein	9	49	11·98	244·5	−24·1
Wheat gluten	10	50	11·60	232·0	−36·0
Maize–gelatine–methionine	8	58	5·82	157·3	−47·2
Casein–gelatine–methionine	8	37	10·68	184·0	−46·4

* For details of composition, see Table 1.

diet, eight. The AAS of the proteins were calculated in relation to the target mixture devised by Bender for Wistar rats (Table 2).

Total nitrogen in the diets was determined by the Kjeldahl method. Body N content of the rats was calculated as N:body water (Rafalski & Nogal, 1964*a*, *b*).

RESULTS

Table 3 shows the relationship between the quantity of protein in the diet and food and protein intake, on a per group basis, during the 10 d experimental period, and the apparent body-weight change. With all the test diets the animals received insufficient protein, regardless of its quality, to allow growth or maintain body-weight. At the start of the experiment each group of four rats weighed 240 g, and during the 10 d experimental period consumed 112·5–244·5 g of an isoenergetic diet (0·019 MJ/g). Thus, individual rats had an intake of 0·053–0·116 MJ/g. According to the National Academy of Sciences (1972), a rat weighing 60 g needs only 0·051 MJ/d to maintain a stable body-weight. The diet received by the rats in our experiment was, therefore, adequate for energy requirements and the maintenance of body-weight.

Table 2 shows the essential amino acid content of the proteins relative to the composition of a target mixture (Bender, 1965). The first limiting amino acids were, for albumin, leucine; for casein, the S-containing amino acids; for gluten, lysine; for the maize–gelatine–methionine mixture, tryptophan; and for the casein–gelatine–methionine mixture, tryptophan. AAS values for these amino acids were 1·09, 0·68, 0·27, 0·26 and 0·44 respectively.

For albumin, the second limiting amino acid was phenylalanine, and the third limiting was lysine. The AAS for S-containing amino acids was 1·40. In casein, the second and third limiting amino acids were phenylalanine and threonine, with AAS of 1·08 and 1·17 respectively. In gluten, the second and third limiting amino acids were methionine and threonine, with AAS of 0·59 and 0·61 respectively. For the maize–gelatine–methionine mixture, the second and third limiting amino acids were isoleucine and threonine, with AAS of 0·465 and 0·56 respectively.

The second limiting amino acid in the casein–gelatine–methionine mixture was isoleucine (AAS 0·60); equal third were phenylalanine (AAS 0·61) and the S-containing amino acids (AAS 0·62).

Table 4 shows NPU^m values for the five protein mixtures and the AAS of their first limiting amino acid. For all the proteins except casein, the NPU^m values were significantly higher than would have been predicted from the AAS.

The NPU^m values approximated those predicted on the basis of the second and third limiting amino acid content, when the first limiting was tryptophan (Table 5).

Table 4. *Net protein utilization (NPU^m) values and amino acid score (AAS)* values for low-protein diets† given to rats*

Dietary protein source	No. of groups	NPU^m Range	NPU^m Mean	NPU^m SD	AAS	Significance of difference between AAS and NPU	Limiting amino acid
Egg albumin	6	1·38–1·67	1·51	0·119	1·09	$P < 0·05$	Leucine
Casein	9	0·60–0·81	0·69	0·067	0·68	NS	Sulphur-containing amino acids
Wheat gluten	10	0·34–0·46	0·40	0·042	0·27	$P < 0·01$	Lysine
Maize–gelatine–methionine	8	0·43–0·50	0·47	0·024	0·26	$P < 0·001$	Tryptophan
Casein–gelatine–methionine	8	0·48–0·67	0·60	0·063	0·44	$P < 0·001$	Tryptophan

NS, not significant.
* Content in test protein: content in reference protein (Bender, 1965).
† For details of composition, see Table 1.

Table 5. *Net protein utilization (NPU) and amino acid score (AAS)*of the first, second and third limiting amino acids in low-protein diets† given to rats*

Dietary protein source	NPU Present results	NPU FAO/WHO (1973)	Limiting amino acid and AAS First	Second	Third
Egg albumin	1·51	—	leucine (1·09)	phenylalanine (1·22)	lysine (1·25)
Casein	0·69	0·72	Sulphur-containing amino acids (0·68)	phenylalanine (1·08)	threonine (1·17)
Wheat gluten	0·40	0·39	lysine (0·27)	methionine (0·59)	threonine (0·61)
Maize–gelatine–methionine	0·47	—	tryptophan (0·26)	isoleucine (0·47)	threonine (0·56)
Casein–gelatine–methionine	0·60	—	tryptophan (0·44)	isoleucine (0·60)	phenylalanine (0·61) S-containing amino acids (0·62)

* Content in test protein: content in reference protein (Bender, 1965).
† For details of composition, see Table 1.

For albumin, the NPU^m determined was close to that predicted from the S-containing amino acid content (Table 2). With gluten, the NPU^m values found were markedly higher than those expected from the AAS, but lower than might have been predicted from the second and third limiting amino acid content (Table 5).

The NPU^m for egg albumin (1·51) (Table 5) was 1·4 times greater than would have been expected from the AAS and, for all the remaining proteins except casein, the NPU^m values were also greater than would have been expected from the AAS. Thus for gluten, the NPU^m was 1·48 times greater than the values indicated by the AAS, for the maize–gelatine–methionine mixture and for the casein–gelatine–methionine mixture the corresponding values were respectively 1·81 and 1·36 times greater.

An NPU^m of 1·51 for egg albumin was not unexpected as we had obtained values of a similar order in several other experiments. An NPU^m which was 1·4 times greater than that predicted on the basis of the AAS has been obtained also by other authors (FAO, 1970).

DISCUSSION

Like Pellett & Kaba (1972) and Williams, Curtin, Abraham, Loosli & Maynard (1954), we have assumed that the amino acid composition of the rat body and the amino acid requirement are constant for any defined age and physiological state. According to the theory of chemical score (Block & Mitchell, 1946), utilization of dietary amino acids depends on the essential amino acid, the dietary quantity of which is in the greatest deficit compared to its content in egg protein. Instead of egg protein we adopted a target mixture (Bender, 1965) as the standard for determining AAS in proteins tested. The biological quality and utilization of proteins were measured by NPU corresponding to Miller & Payne's (1961) NPU^o and NPUst.

In the opinion of Block & Mitchell (1946) and of Bender (1965), AAS should be numerically equal to the biological value (BV) for proteins of biological quality higher than 0·40, and measured in the equilibrium state of N balance and at maintenance of rat body-weight. With proteins of NPU < 0·40 the NPU values depend on which essential amino acid is limiting (Bender, 1965).

Our results for proteins of NPU > 0·40, estimated at less than maintenance levels of intake, indicate that the NPU values, except for casein, were higher than the chemical score of the first limiting amino acid. We obtained NPU values that were higher than AAS for leucine in egg albumin, for lysine in wheat gluten and for tryptophan which limited the quality of the remaining two protein mixtures tested. Our findings support those of Miller & Payne (1964) who reported that NPU^o and NPUst values for whole egg, casein and wheat gluten were higher than their AAS, when their quality and utilization were determined at and below maintenance. Only with casein was NPU numerically equal to AAS or its limiting S-amino acids. Our finding for casein is consistent with results of Bender (1965) and Said & Hegsted (1970) for proteins other than casein, similarly limited by S-amino acids.

Bender (1965) estimated utilization (NPU and BV) of a mixture of pure amino acids with diets containing about 100 g protein equivalent/kg. Said & Hegsted (1970) similarly used diets containing (/kg) 100 g protein equivalent as a mixture of amino acids containing varying amounts of the essential amino acid under investigation. In our work we used diets containing smaller quantities of protein (18–58 g/kg diet). The limiting amino acid in particular proteins was constant.

We determined whether the limiting amino acids in our test proteins, calculated by reference to Bender's (1965) target mixture, were also the first limiting amino acids in the standard proteins: whole egg, human-milk protein and cow's-milk protein. By all these standards (FAO, 1970), the first limiting amino acids were: leucine for egg albumin, S-amino acids for casein, lysine for wheat gluten, tryptophan for both protein mixtures. The order of the second and third limiting amino acids however varied, depending on the standard used.

Although the NPU values of all the proteins except casein exceeded the chemical score of the first limiting amino acids, they approached the value of the chemical score of the second and the third limiting amino acids. NPU values were similar to chemical score of isoleucine and phenylalanine in the mixture of maize with gelatin and methionine, and isoleucine and phenylalanine as well as S-amino acids in the mixture of casein with gelatin and methionine. The NPU of egg albumin was close to the chemical score of the S-amino acids.

AAS takes into consideration only the amino acid content in the diet, whereas NPU may be affected not only by amino acids from the diet but also by those from body proteins, which enter the metabolic amino acid pool independently of the kind of diet consumed. At low levels of protein intake, amino acids derived from the tissues can play a bigger part in the metabolic amino acid pool and in maintaining NPU at a higher level than the AAS value. If NPU is higher than would be anticipated from AAS of the dietary proteins (apart from those limited by S-amino acids), we may suppose that the dietary protein was beneficially supplemented by amino acids derived from the body, causing its improved utilization. This phenomenon seems to be observed mainly in the state below maintenance of body-weight with proteins of NPU both higher and lower than 0·40. With dietary protein of well balanced amino acid composition (e.g. egg albumin) body protein is spared by reductions of endogenous loss, and NPU values of greater than 1·00 are obtained.

The closer agreement of AAS and NPU for proteins limiting in S-amino acids might result if both the dietary protein and the body protein were limited by the content of S-amino acids (Miller & Payne, 1964; Pellett & Kaba, 1972; FAO, 1970). In the rat there is a large requirement for S-amino acids for synthesis of hair protein, which continues at the cost of other tissues in rats fed at below maintenance levels and under conditions of growth retardation. Demand for these S-amino acids of body origin for hair protein synthesis is so great that no sulphur is available to supplement the dietary protein. This would explain why the utilization of a protein devoid of S-containing amino acids is zero (Bender, 1965; Said & Hegsted, 1970).

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