Problems in the assessment of nutrient requirements

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Malaria and other parasitic infestations
Malabsorptive disorders common
Diarrhoeal disorders constantly present
Chronic infectious disease endemic (e.g. tuberculosis)
Climatic changes (e.g. monsoon failing)
Crop failure
Dehydration
Sweating of vitamins
Epidemics
Religious customs
Conditions in prisons
Lack of price control
Distribution of foodstuffs breaking down
Black markets
Aid restrictions by Governments
Social disruption
Floods and earthquakes
War

Conclusions

In summary, it is suggested that problems of primary vitamin deficiency in this country are found to a limited extent for three vitamins. Matters are entirely different in large areas of the globe and study in depth of all the factors would require an international conference or publication of a very large volume.

REFERENCES


Problems in formulating simple recommended allowances of amino acids for animals and man

By K.J. Carpenter, Department of Agricultural Science & Applied Biology, University of Cambridge

There seem to be a special set of difficulties in recommending allowances of amino acids. With the vitamins and trace elements the overriding concern is to have an adequate safety factor. Most nutritionists probably believe that a large proportion
of the supplements of these, whether for man or animals, could be dispensed with without ill effect. Yet it is not necessarily sensible to reduce their use, because their cost is relatively low and we cannot be absolutely sure of which are and which are not the conditions in which a marginal deficiency condition could occur.

On the other hand, with energy, which is the most expensive component of the diet to provide, voluntary intakes are largely controlled by homeostatic mechanisms. In animals, where ad lib. feeding is the norm, the metabolizable energy (ME) values (kcal/g) of mixed diets can vary widely without there being corresponding differences in actual energy intake per head. In man there is also such individual diversity in physical activity and energy expenditure (Widdowson, 1962) that any generalized recommended allowances are incapable of precise application to individuals.

The nutrients (i.e. the amino acids) required for body protein synthesis are, therefore, the only ones whose provision takes a considerable proportion of the cost of a balanced diet (20–30% for fast-growing species) and for which there is no intake-control mechanism.

Since, in practice, the feeding of intensively kept pigs and poultry is on a more rational basis and we have so much more experimental data for these species, they will be considered before turning to the special problems of formulating adequate human diets. Ruminant animals will not be considered; their possible requirements for individual amino acids are still the subject of debate.

**Allowances for animals**

*Experience with animals.* The requirement of an animal having a given rate of production is, of course, for so much of a particular nutrient per day. However, intensively kept animals are not usually fed on individually weighed-out rations but are provided ad lib. with mixed diets and allowed to eat the quantity they choose. The practical problem is therefore to make recommendations as to the percentage composition of a mixed diet.

Since intensive diets are usually sufficiently concentrated in calories for their 'bulk' not to limit intake significantly, the main factor controlling consumption is the calorie intake. It then seems reasonable, therefore, that if we specify levels of amino acids per calorie this will ensure the consumption of certain levels per head per day.

Earlier recommendations (e.g. National Research Council: Committee on Animal Nutrition, 1954) were made as '% of the diet'. This made little difference when, in practice, there was little variation in the energy contents of the practical diets used in a particular region for a particular purpose; these, in turn, depended largely on which was the staple feed grain produced in the area. However, re-calculation of amino acid standards on a calorie basis reconciled the apparent large differences in protein and amino acid requirements (as % of the diet) at that time between birds in the USA which received diets based mainly on maize and those in the UK which received diets based mainly on barley (Table 1). Also, nowadays, with the greater appreciation of the economic importance of energy in pig and
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Table 1. An example of energy levels influencing protein standards for laying diets of different types

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dietary energy level (ME) (kcal/g)</th>
<th>Protein found inadequate (%)</th>
<th>Typical daily intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley, weatings etc. (traditional UK)</td>
<td>2.39</td>
<td>12</td>
<td>156 373 18.7</td>
</tr>
<tr>
<td>Maize, soya (USA mid-West)</td>
<td>3.03</td>
<td>15</td>
<td>123 390 18.2</td>
</tr>
</tbody>
</table>


poultry nutrition, and the availability of fat at prices worth consideration, there is greater variation in energy levels even within a region.

If energy levels in diets (kcal ME/g) are raised to a point in which animals no longer cut back consumption so as to maintain only their standard calorie intake (cf. Morris, 1968), it appears that the extra energy consumed is retained almost entirely as fat and that protein synthesis is unaffected. In this circumstance one would expect that amino acid levels would need to be raised only in inverse proportion to average food intake per head, and not in full proportion to the caloric value of the diet. However, such fine points correspond to differences of less than 5% in amino acid levels. Can we determine or test requirements with that degree of precision?

Procedures for determining requirements. The common procedure has been the straightforward one of setting up trials in which all nutrients are supplied at adequate levels with the exception of the one under consideration and the level of this alone is varied in different experimental treatments. An example of this approach is the study of Baldini & Rosenberg (1955) who set out to determine the methionine requirement of broiler chickens at different dietary energy levels. Their data formed an important part of the evidence used by a British Committee to assess the ‘methionine and cystine’ requirements of broiler chicks on a ‘calorie basis’ (Agricultural Research Council, 1963). The original data have been recalculated and are presented graphically in Fig. 1. The standard error of the response measurement that was used was approximately 2.5 and the least significant difference between values was approximately 8. The Committee’s estimate was that the chicks showed a constant requirement of 2.5 g sulphur-containing amino acids/Mcal with all three experimental diets. Certainly one cannot conclude that the estimates with each type of diet differ significantly, but the actual estimates can only be in the region, 2.45–2.55. At this level, approximately 80% of the sulphur amino acids in the diet came from natural constituents of the diet (maize, soya bean and peas); the estimate depends, therefore, on the correctness of the authors’ analysis and on their assumption that the amino acids were all in a fully available form.

The results that have just been considered give an impression of the degree of precision with which it is possible to estimate the requirement for an amino acid within the conditions of one experiment; between publications, estimates vary considerably more. Turning to another example – the lysine requirement of young
The results of feeding experiments that were done with broiler chicks to determine their dietary requirement of the sulphur-containing amino acids (recalculated from Baldini & Rosenberg, 1955). ○, diets with 2.5 kcal metabolizable energy/g; ●, 2.83; △, 3.15. The arrow marks the estimated requirement of the chicks at all three energy levels (Agricultural Research Council, 1963).

*pigs in the weight range, 15–35 kg – the experimental data that were considered most satisfactory by a Working Party set up to report on the nutrient requirements of pigs (Agricultural Research Council, 1967) are summarized in Table 2.

Table 2. Summary of published data used to assess the lysine requirement of young pigs (12–45 kg weight)*

<table>
<thead>
<tr>
<th>Reference</th>
<th>Crude protein (% of dry diet)</th>
<th>DE (kcal/g dry diet)</th>
<th>DE intake/ weight gain/d on satisfactory diet (Mcal)</th>
<th>Weight gain/d on satisfactory diet (kg)</th>
<th>Minimum satisfactory level of lysine (g/Mcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfander &amp; Tribble (1955)</td>
<td>16</td>
<td>3.65</td>
<td>5.44</td>
<td>0.64</td>
<td>2.6</td>
</tr>
<tr>
<td>Jones, Hepburn &amp; Boyne (1961)</td>
<td>18</td>
<td>3.63</td>
<td>2.15</td>
<td>0.42</td>
<td>3.2</td>
</tr>
<tr>
<td>McWard et al. (1959)</td>
<td>22</td>
<td>3.89</td>
<td>3.53</td>
<td>0.61</td>
<td>2.7</td>
</tr>
<tr>
<td>Brinegar, Williams, Ferris, et al.</td>
<td>22</td>
<td>3.83</td>
<td>4.26</td>
<td>0.49</td>
<td>3.6</td>
</tr>
<tr>
<td>and Brinegar, Williams, Loosli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&amp; Maynard (1952)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DE, digestible energy. *Agricultural Research Council (1967). Table 2.3.

The differences in estimated requirements given in the individual publications listed there may have been due, in part, to random variability of particular pigs about an ‘average’ response curve to graded levels of lysine and, in part also, to errors in their estimates of the potency of the test diets as sources of lysine, since...
only a portion of the lysine was in the form of the synthetic amino acid. Another source of variation may have been real differences in the requirements (expressed as g lysine/Mcal dietary digestible energy (DE)) of the animals in the different trials because of differences in genetic growth patterns, environmental conditions, the nature of the diets or the level or both at which they were fed. The Working Party simply took the average of the four estimates, i.e. $3.0\text{ g/Mcal}$ of diet as their final recommendation or ‘best estimate’.

It is difficult to know what else they could have done since their terms of reference required ‘an allowance’ of each nutrient for each class of pig. It would, of course, be rash to count on $3.0$ being just ‘right’ and on the errors of those who give lower estimates being the same as those who give higher ones. Yet, to the extent that the ARC recommendations are followed by feed compounders, the financial implications are quite large. In the UK, lysine is usually the first cost-limiting amino acid in pig diets and raising the lysine level by 10% will typically increase the cost of the diet by approximately £0.65 per ton. If the specifications for all the 2.5 million tonnes of compound pig diets that are sold each year in the UK were raised in this way the total cost would be £1.6 million; alternatively there could be a saving of a similar order if the specifications were to be reduced by 10%. Surprisingly enough, the American estimates for pigs in the middle of the same weight range are lower even than this, at approximately $2.2\text{ g lysine/Mcal}$ (National Research Council: Committee on Animal Nutrition, 1968).

With such sums at issue the true nature of the problem deserves more careful examination. There is no reason to think that a protein level providing for less than optimal growth rate causes suffering of any kind, so that the question is one of economics. In other words, although the cheaper of two diets may give a slightly lower average performance, the question is whether or not the saving is greater or less than the fall in returns.

We need, therefore, to know what is the response curve to an amino acid just below its requirement point. It is extremely difficult to be sure of this; experimental data are usually of the type shown in Fig. 2 and, in view of the standard deviation attached to each actual response measurement, one can fit a hypothetical model response of either type ‘A’ or ‘B’ without either being demonstrably a ‘wrong’ model. Even though, for one strain of animal and one set of conditions, the response curve may be of type ‘A’, if, with different strains and different conditions, the requirement differs slightly, the mean response curve for the total population will become of type ‘B’. In this event, with an asymptotic response, it is clearly uneconomic to push up standards to a point at which there is no extra return for the extra cost involved (cf. Fisher, 1967).

In practice there is a further uncertainty for the diet formulator as to the true analysis of his ingredients, and it is sometimes suggested that a safety margin be added to allow for this. Bell & Voldeng (1968) believe that some of the differences between various estimates of the lysine requirements of pigs may be explained by differences in the digestibility of the diets used. Combs (1968) has suggested the multiplication of analytical values by an ‘availability factor’ for each class of in-
Fig. 2. An example of the same data fitted with two 'models', A and B. The vertical bars indicate the standard deviations.

gredient. Although the principle is clearly correct, Miller (1970), after a critical review of available data, concludes that Combs' present factors are not soundly based. Further consideration of this aspect is not possible here.
Another serious factor is the changing requirement for the concentration of amino acids in the diet as animals get older. Thus, it has been estimated that growing pigs at 90 kg live weight need two-thirds the level of lysine in their diet of that they needed at 20 kg (Agricultural Research Council, 1967). Since it is also uneconomic to have a large number of separate diets in use at an animal enterprise, any diet that is adequate at the beginning of its period of use will have a wasteful excess at the end.

One might expect that there would be similar, large differences in the dietary amino acid levels required by hens laying at different rates and Combs (1962) has suggested a procedure for calculating these differences, based on separate estimates of methionine and calorie requirements for maintenance and production respectively. However, this has not been confirmed by experiment. Indeed, it would seem from the results of Fisher & Morris (1967) that the amino acid concentrations required by pullets during the end of their year's lay, when production is at a reduced level, are as high as when it is at its peak.

This is a very suggestive finding. Whether or not a hen lays an egg on a particular day depends on whether it ovulated the day before, which in turn depended on a pituitary stimulus. Since egg numbers are the most important factor influencing the production of egg tissue, we are really concerned with the protein requirement for full pituitary activity in this direction, given the drain on amino acid pools by the protein synthesis in progress. Normally the most severe effect of very low dietary protein levels is the increased occurrence of 'missed' ovulation days. Perhaps, at the end of the first laying year high protein levels may actually be needed to stimulate the pituitary activity. Certainly it seems that we cannot assume that the hen can be treated as a 'black box' whose requirements today can be computed purely from the analysis of its products yesterday.

How many amino acids? Although at least ten amino acids are essential (or semi-essential) for pigs and poultry, most computer formulations have been constructed to meet only three specifications – for lysine, 'methionine+cystine' and total nitrogen respectively. And experience has shown that when this has been done, adequate levels of the other amino acids have automatically been provided. This is because the nutritional value of the proteins in all the main classes of feedingstuffs is limited by their contents of either lysine or the sulphur-containing amino acids. Now that both synthetic lysine and synthetic methionine are cheap enough to be considered seriously in formulation, this situation may not continue. It may then be necessary to consider isoleucine, tryptophan, threonine and arginine. Unfortunately, the requirements for these amino acids have been investigated less.

It has been stated that 'it is no longer satisfactory merely to specify minimal levels of essential amino acids in defining diets for animals since growth may be retarded by excesses, as severely as by deficiencies of specific amino acids' (D’Mello, Hewitt & Lewis, 1967). There is, of course, no doubt that such effects can be demonstrated experimentally with chicks, and further investigation is needed as to whether or not the effects could be significant with the range of amino acid concentrations and total protein levels that might be encountered in practical diets. The effect of high
lysin levels in increasing the requirements for arginine (cf. D’Mello & Lewis, 1970a) is irrelevant under the usual conditions in which lysine is an ‘expensive’ component and not included at levels significantly above the minimal, but the second series of interactions between leucine, isoleucine and valine (D’Mello & Lewis, 1970b) could be more of a problem since the levels of these amino acids are not normally calculated. So far, no account has been taken of this possibility in practical formulation except indirectly by way of limitation on the levels of inclusion of uncommon ingredients or material such as feather meal, with an unusual amino-gram.

Allowances for man

Economists and others attempting to assess the adequacy of present human diets in a particular region or to plan the reorganization of resources with a view to their future improvements, are at the present time unable to find clear recommendations of a type that will allow them to make their calculations with respect to protein, taking into account both quantity and quality. With other nutrients, such as the vitamins, they can obtain data for the content of each vitamin in the various dietary ingredients, calculate the sum total of each contributed by all the components of the diet and match this with the figures recommended as ‘requirements’ or ‘allowances’ for each vitamin.

If the requirements for protein were divided into individual requirements for each of the essential amino acids, as is usual with domestic animals, there would be no problem (at any rate in calculation) as to whether or not the standards were being met. However, FAO (1965) in their report on ‘human’ protein requirements did not follow this system; nor has a more recent British Committee (Panel on Recommended Allowances of Nutrients) (Department of Health and Social Security, 1970). This was, presumably, because they believed that data for individual amino acid requirements were not yet adequate.

As an alternative, measurements of the nitrogenous losses of people in different age and activity groups were reassessed, together with the nitrogen content of their tissue growth, and their requirements were then calculated in terms of a hypothetical ‘ideal’ protein which would be used for protein synthesis with 100% efficiency. This approach has already been discussed by the Society (Miller & Payne, 1969).

Since the requirement for ‘ideal protein’ is the sum of the individual requirements for the essential amino acids (and for additional nitrogen, in a form suitable for synthesis of the non-essentials), the problem is to know what amino acid composition it must have. FAO (1965) reviewed a number of alternatives but came to no conclusion. Only ‘methionine + cystine’ or ‘lysine’ seem to be limiting in practical mixtures; Payne (1969) has recently reconsidered both the old and new data and has suggested figures of 5·0% ‘methionine and cystine’ and 5·3% ‘lysine’ (i.e. % of the protein or g/16 g N).

There has been a vigorous debate as to what correction, if any, should be made
for the effect of high dietary concentrations of protein being less effective as sources of amino acids in meeting requirements because of a partial diversion of them for use as energy sources (Miller & Payne, 1961; Hegsted, 1964; Carpenter & Anantharaman, 1968). However, with the exception of infants less than 1 year old, the estimates of requirement are low, and the chemical score of mixed diets seems never to fall below 50, so that in any marginal diet the effect of protein concentration on amino acid requirements would be agreed to be practically negligible. If one then, as a working hypothesis, adapts the data of the Panel on Recommended Allowances of Nutrients (Department of Health and Social Security, 1970) for minimum protein requirements and uses the amino acid values suggested by Payne (1969), results of the type illustrated in Table 3 are obtained.

Table 3. Examples of calculated daily human requirements for protein and selected amino acids

<table>
<thead>
<tr>
<th>Energy requirement* (kcal ME)</th>
<th>Protein* (g)</th>
<th>Methionine +cystine† (g)</th>
<th>Lysine† (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child aged 4 years, weighing 16.5 kg</td>
<td>1600</td>
<td>25</td>
<td>1.03</td>
</tr>
<tr>
<td>Boy aged 11 years, weighing 32 kg</td>
<td>2500</td>
<td>36</td>
<td>1.48</td>
</tr>
<tr>
<td>Man aged 18–35 years, weighing 65 kg</td>
<td>2700–3600</td>
<td>45</td>
<td>1.86</td>
</tr>
<tr>
<td>Woman aged 55–75 years, weighing 53 kg</td>
<td>2050</td>
<td>36</td>
<td>1.48</td>
</tr>
</tbody>
</table>

ME, metabolizable energy.
*From Department of Health and Social Security (1970), Table 3. The protein had been calculated from the net nitrogen requirement (i.e. the mean nitrogenous losses on a nitrogen-free diet and the nitrogenous gains in growth) × 6.25 (to give crude protein) × 1.2 (to allow for individual variability) × 100/70 (which allows for mixed dietary proteins having a net protein utilization value of no more than 70 even when lysine and sulphur amino acid levels are specified, as in the present table).
†The amino acid requirements are calculated as the net nitrogen requirements × 6.25 × 1.2 × 100/90 (to allow for 10% indigestibility of the proteins) × 5.0/100 (for 'methionine+cystine', or 5.3/100 for lysine).

In general, any calculated requirements of amino acids by humans over 1 year old can be met by a much lower level of dietary protein than is actually consumed by people in Western cultures and by most other people whose diet is not severely limited by poverty. As a consequence of this, committees are reluctant to use them as a basis for their recommendations. Thus, the British Committee (Department of Health and Social Security, 1970) has recommended a daily intake of 65 g protein for sedentary, middle-aged men weighing 65 kg in the United Kingdom, while recognizing that their actual calculated minimum requirement (for protein of average quality) is only 43 g. The '65' comes from their adoption of the principle that in all human diets at least 10% of the calories should be in the form of protein.

There are two possible arguments for this recommendation. The first is that the ability of such ‘minimal calculation’ diets to sustain a lifelong, physically vigorous and mentally active life has not been tested. The second is that the Committee did not wish even to seem to advocate a change to diets of lower protein content. They
probably had in mind the position of institutional caterers trying to provide an adequate diet at minimal cost. Since high-protein dishes are, in practice, provided by the more expensive but generally more attractive 'animal protein' items, the appeal of the diets provided might suffer if the higher protein levels were not specified by an official panel. In this respect the recommendation includes an element of compassion, like a medical certificate giving a little brandy at Christmas-time to an old man.

Still other arguments underlie the calculations in the 'Provisional Indicative World Plan for Agricultural Development' (FAO, 1970), and in 'International Action to Avert the Impending Protein Crisis' (United Nations Organization, 1968; Table 5 in particular). Here the regional 'need' for protein at a future date is defined as that supplied by the foods which the average man in different developing countries is likely to demand (in the sense of 'supply and demand') if his income increases in the future as predicted by economists. This can lead to misunderstanding in non-specialist discussion of the 'World Protein Gap' between what is available and what is needed. Where the supply is adequate to meet the physiological demand for nutrients for protein synthesis, but still insufficient to meet the economic demand for 'desirable' foods such as meat and eggs, it would seem entirely inappropriate either to calculate the amino acid content of these 'desires' and express them as allowances, or to think that one would be filling this kind of gap by changing cereal varieties or by the fortification of staple foods with synthetic amino acids.

Although it is encouraging for scientists to see their problem taken up by statesmen and publicists in a simplified manner we must attempt to explain the true position, as we see it, even at the expense of appearing finicky.

I am indebted to Mr P. R. Payne for a discussion of the basis of recommended human allowances.

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Problems in the determination of the trace element requirements of animals

By C. F. Mills and R. B. Williams, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Statements of the trace element requirements of man and domesticated animals have appeared in publications of the (US) National Research Council (1960, 1964, 1966, 1968a, b, 1970), the (UK) Agricultural Research Council (1963, 1965, 1967) and in authoritative reviews such as those of Underwood (1962, 1966). In presenting these statements the authors repeatedly emphasize the provisional nature of their conclusions, the many factors which limit the value of a single statement of requirement and the scarcity of published information relating physiological performance to trace element intake. This review examines some of the practical problems behind the determination of trace element requirements and considers the value and limitations of statements of requirement under practical and experimental conditions.

Several different approaches have been employed to assess trace element requirements, namely (1) studies of relationships between dietary concentration and physiological response, (2) studies of input/output relationships in balance trials, (3) factorial assessments of requirements based upon body composition studies coupled with estimates of endogenous excretion, (4) estimates derived from the analysis of field data obtained in association with studies of suspected deficiency disorders. Each of these approaches is subject to hazards of execution and interpretation and, undoubtedly, the greatest among these are the the problems caused by adventitious contamination during the conduct of the experiment.