The nutritional value of plant-based diets in relation to human amino acid and protein requirements

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The adequacy of plant-based diets in developed and developing countries as sources of protein and amino acids for human subjects of all ages is examined. Protein quantity is shown not to be an issue. Digestibility is identified as a problem for some cereals (millet (Panicum miliaceum) and sorghum (Sorghum sp.)) and generally is poorly understood. Direct measurements of biological value in children are reviewed and scoring is considered. Various existing requirement values for amino acids and especially lysine are reviewed, and it is concluded that stable-isotope studies do not yet provide adequate alternative values to N balance data, which for lysine are robust after recalculation and adjustment. A new maintenance requirement pattern is developed, with higher values than those of Food and Agriculture Organization/World Health Organization/United Nations University (1985) but lower values than the Massachusetts Institute of Technology pattern (Young et al. 1989). Calculations of age-related amino acid requirements are based on most recent estimates of human growth and maintenance protein requirements, a tissue amino acid pattern and the new maintenance amino acid pattern. These values appear valid when used to score plant proteins, since they indicate values similar to or less than the biological value measured directly in young children. When used to score plant-based diets in India, no marked deficiencies are identified. All regions score > 1 for adults, whilst for children scores range from > 1, (Tamil Nadu) from 6 months of age to 0.78 (West Bengal), rising to 0.9 in the 2–5 year old, consistent with reports that high-lysine maize supports similar weight and height growth to that of casein. Inadequate amino acid supply is not an issue with most cereal-based diets.

Lysine: Protein scoring: Plant-based diets: Children

The present paper reviews current knowledge relating to protein quality in the context of the meat or wheat debate. The question posed is: are the meat-free or low-meat plant-based diets consumed currently by minorities of vegetarians in the developed world and the majority of populations of the developing world adequate as sources of protein and amino acids for the various age-groups consuming them? The answer to this simple question is in fact difficult to formulate and subject to controversy due to uncertainty about amino acid requirements. The problem was recognized by Food and Agriculture Organization/World Health Organization (FAO/WHO. 1991) in their review of the assessment of protein quality. They recommended that the scoring pattern to be utilized for older children and for adults could be based on values previously adopted for the preschool child, but this recommendation was only made in the absence of agreement on any other suitable values, and was presented as a strict interim measure until a better set of values could be identified.

The main reason for this problem is, in my view, because of the unsatisfactory nature of our understanding of N, protein and amino acid homeostasis. In this paper I intend to...
identify the extent and nature of the issues, and propose a way forward which, whilst not representing a complete solution, is at least an improvement on the current status quo.

Plant protein sources

Plant protein sources provide 65% of the world supply of edible protein (Young & Pellet, 1994), with cereal grains (47%) and pulses, nuts and oil seeds (8%) as the other major sources. Plant protein sources in the developed countries constitute a lower proportion of intake compared with animal protein (see Table 1), but are the major source in the developing countries, with cereals predominant. Of the cereals, wheat (43%), rice (39%) and maize (12%) are the main contributors (see Rosegrant, 1999).

Plant protein sources can differ from animal sources in terms of digestibility, amino acid composition, the presence of anti-nutritional factors which adversely influence digestibility and safety, and the presence of phytoprotectant factors (such as phyto-oestrogens, see Cassidy, 1996) which may be advantageous in mediating disease protection. This latter factor, together with the perceived need to limit animal fat and hence meat intakes, has resulted in a proposed increase in consumption of plant food sources as part of the healthy diet (Health Education Authority, 1996). Although it is frequently pointed out that plants can provide all human protein needs, it is nevertheless the case that the misconception persists that they are nutritionally inferior to animal proteins. This is the result of both complex social and cultural attitudes towards meat and the scientific tradition of protein-quality evaluation in animals. In fact, the important nutritional question is not whether plant proteins can completely provide for human amino acid needs, because this has been established for all ages; rather, the question is whether this is an easy task in practice, i.e. achievable with relatively unsupplemented low-cost cereal or other staple diets available to poor developing communities, or only possible with the much-higher-cost carefully-selected mixed diets consumed by affluent vegetarians.

### Protein quantity

Animal food sources are generally high in protein, so that there is a clear relationship between protein intake and the proportion of animal foods, especially meat, in the diet. As shown in Table 1, the overall protein : energy (P : E) value of the national food supply falls from 12.1% energy in the USA to 8% in a Sub-Saharan African country like Sierra Leone as the animal protein intake falls from 74 g/d to 6.9 g/d. In the UK the P : E value falls from 14.2% energy in omnivores to 12.7% in the small number of vegetarians (non-meat eaters) identified in the UK food intake survey (Jackson & Margetts, 1993). Thus, protein intakes of vegetarians are likely to be closer to the reference nutrient intake (RNI, Department of Health, 1991) and approximately 20–30% of this small sample were below the RNI.

### Table 1. Protein and lysine content of diets in relation to estimates of the requirements

<table>
<thead>
<tr>
<th>Country</th>
<th>Protein (%)</th>
<th>Protein:energy</th>
<th>Animal (g/d)</th>
<th>Cereal (g/d)</th>
<th>Pulses and soyabean (g/d)</th>
<th>Rest (g/d)</th>
<th>Lysine (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food balance sheet*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>113</td>
<td>12.1</td>
<td>73.5</td>
<td>65.0</td>
<td>24.6</td>
<td>21.8</td>
<td>12.9</td>
</tr>
<tr>
<td>UK</td>
<td>91</td>
<td>11.0</td>
<td>52.3</td>
<td>57.5</td>
<td>22.9</td>
<td>25.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Turkia</td>
<td>91</td>
<td>10.9</td>
<td>19.0</td>
<td>20.9</td>
<td>52.6</td>
<td>61.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Egypt</td>
<td>87</td>
<td>9.4</td>
<td>12.9</td>
<td>14.8</td>
<td>59.6</td>
<td>68.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Brazil</td>
<td>66</td>
<td>9.3</td>
<td>27.1</td>
<td>41.1</td>
<td>22.6</td>
<td>34.3</td>
<td>10.4</td>
</tr>
<tr>
<td>Nepal</td>
<td>50</td>
<td>10.2</td>
<td>7.3</td>
<td>14.6</td>
<td>34.8</td>
<td>69.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>43</td>
<td>8.4</td>
<td>4.8</td>
<td>11.3</td>
<td>33.4</td>
<td>78.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>34</td>
<td>8.0</td>
<td>6.9</td>
<td>20.3</td>
<td>17.0</td>
<td>50.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Food intake data†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK: Omnivores</td>
<td>74</td>
<td>14.2</td>
<td>44.1</td>
<td>59.4</td>
<td>17.3</td>
<td>23.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Vegetarians</td>
<td>54</td>
<td>12.7</td>
<td>18.1</td>
<td>33.6</td>
<td>18.7</td>
<td>34.8</td>
<td>9.4</td>
</tr>
<tr>
<td>India (mean)</td>
<td>62</td>
<td>11.1</td>
<td>3.4</td>
<td>5.5</td>
<td>47.8</td>
<td>76.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>46</td>
<td>9.7</td>
<td>2.4</td>
<td>5.3</td>
<td>29.0</td>
<td>63.6</td>
<td>6.6</td>
</tr>
<tr>
<td>West Bengal</td>
<td>53</td>
<td>8.8</td>
<td>0.8</td>
<td>1.5</td>
<td>48.0</td>
<td>89.9</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Lysine requirements (at 65 kg body wt)

FAO/WHO (1973)  780
FAO/WHO (1991)  3770
FAO/WHO (1991)  2795
MIT scoring pattern (Young et al., 1989)  4114
MIT calc. (1995) recalculated  1209

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Whether this is a problem is to some extent debatable, but it is probably unlikely, especially in developed countries, given the overall lower morbidity and mortality of vegetarians compared with meat eaters. First, with the adult protein requirement equivalent to \( P : E \) values of about 9 and 7 % energy for the RNI and estimated average requirement respectively (i.e. 0.75 and 0.6 g protein/kg body weight respectively in adults (Department of Health, 1991) with an energy requirement of 1.6 × BMR), the lowest-protein diets shown in Table 1 fall between the estimated average requirement and RNI for adults. Second, in Table 1 all values for protein in terms of food supplies or intake indicate \( P : E \) values higher than breast-milk at 7 % energy since, as shown in Table 2, only a few plant staples have a \( P : E \) value lower than 7 % energy. Indeed, wheat and maize are ‘high-protein foods’ compared with breast-milk if the energy density issue is ignored. Furthermore, on the basis of a metabolic model for the protein requirement, which includes a substantial adaptive component varying with intake, there is by definition a correlation between intake and requirement, so that low intakes are unlikely to become associated with substantial prevalence rates of inadequacies until they fall close to the lower RNI. Most importantly, at least as far as food supply data are concerned, wheat-based food supplies, such as those in Tunisia and Egypt which have only 15–20 % animal protein sources, clearly supply protein at levels well above the human protein requirement. As for infants and children, the wide and successful use of protein at levels close to those of predominantly animal-based food supplies, such as those for the UK. Thus, cereal-based diets, especially those based on wheat, can supply protein at levels well above the human protein requirement. As for infants and children, the wide and successful use of soybean-based infant formulas is evidence that plant-based diets can be adequate for infants. On the other hand, the monotonous diets based on very-low-protein starchy root crops, such as cassava (Manihot sp.) may well supply inadequate protein intakes to ensure adequate height growth (see Waterlow, 1992). In this context it is interesting to note that in such cases (e.g. the stunted Bundi orphanage children described by Malcom (1970) who were fed almost exclusively on the low-protein starch root taro (Colocasia esculenta)), stunting was the only observable symptom of any nutritional inadequacy. They were otherwise healthy, without overt symptoms of kwashiorkor, in support of Golden’s (1997) arguments that kwashiorkor is not a protein-deficiency disease. The issue of whether stunting in children reflects inherent inadequacies of plant-based diets as protein sources, as opposed to other nutrient inadequacies, is outside the scope of the present paper. However, as discussed later, it has been demonstrated that young children fed on one of the hybrid varieties of maize (opaque-2, sugary-2 hybrid) as their only source of protein and energy (but with mineral and vitamin supplementation) grow in height and weight at rates similar to those achieved with casein (Graham et al. 1990). For these reasons, and with the nutritional adequacy question limited to consideration of protein needs, then we can reasonably safely conclude that, with the exception of some starchy roots, plant-based diets available in most parts of the world are capable of providing adequate protein for all ages. Thus, protein quantity is unlikely to be an issue, and the main question of the nutritional adequacy of plants as protein sources is limited to their quality, i.e. digestibility and biological value (BV).

Digestibility

The FAO/WHO (1991) report on protein-quality evaluation considered the methodology for measurement of digestibility, and concluded that values for true faecal digestibility obtained in rat trials correspond closely with human measurements. As reviewed recently (Millward, 1998a), the latter report showed that food sources with high (> 95 %) true faecal digestibility, e.g. a typical US mixed diet (egg, milk and meat), also includes wheat gluten, wheat flour and soyabean-protein isolate. Thus, once plant-cell-wall constituents are removed, the inherent digestibility of plant proteins may be indistinguishable from that of animal proteins. Lower digestibility, i.e. 80–90 % (whole-grain cereals, peas (Pisum sativum), polished rice, soyabean flour, chickpea (Cicer arietinum), and pea-protein isolates) or 50–80 % (whole wheat (Panicia miliaceum), beans, breakfast cereals and developing-country mixed diets), reflect particularly-tough plant cell walls (millet and sorghum), anti-nutritional factors (beans) or processing and heat treatment (breakfast cereals).

Graham and colleagues (Graham et al. 1979; Maclean et al. 1981) working in Lima, Peru have systematically explored protein quality in young children recovering from malnutrition, and reported the digestibility of diets based on individual sources of plants relative to casein (%) as: wheat 100, maize 90, potato 82, rice 82, beans 81, sorghum 57. For beans (Phaseolus vulgaris), anti-nutritional factors resulted in poor digestibilities of energy as well as N, with very low overall utilization (30 % of the values for casein). With sorghum the poor digestibility can be improved by fermentation and processing to some extent. Clearly, for some plant protein sources digestibility can limit nutritional value.

One issue which was arguably not sufficiently considered by FAO/WHO (1991) is that of ileal digestibility, i.e. the fact that more N enters the colon than leaves via faecal N, with the potential for loss of indispensable amino acids (IAA) through their conversion by bacterial metabolism to NH₃ and other N sources, some of which are absorbed. This process means that true digestibility of some amino acids is overstated, with less of the dietary IAA content absorbed than is assumed on the basis of faecal N. The extent to which this loss represents an important problem is difficult to judge. MacFarlane & Cummings (1991) report that in adults on a mixed diet about 4 g N/d is absorbed from the colon, but on the basis of measurements of urea salvage in adults on mixed diets this could all be accounted for by N derived from endogenous urea hydrolysis. Endogenous ileal IAA loss (i.e. that measured in adults on a protein-free diet) is about 22 mg/kg body weight per d (Fuller et al. 1994), much lower than that of a young pig, and equivalent to about 5 % of the IAA intake of subjects consuming the average UK intake. However, for plant protein sources which are poorly digested in the upper intestine and which increase ileal N flow to be fermented in the colon, loss of IAA may be marked.
In addition, Bingham and colleagues (see Bingham, 1997) argue that the conventional view of digestibility does not take into account variation in faecal biomass as a function of fermentable energy supply to the colon. They argue that in normal mixed diets total faecal N excretion does not increase in response to increases in diet protein intake, and that the major determinant of faecal N output is biomass arising from fermentation in the colon by bacteria, in turn dependent on dietary NSP and other fermentable carbohydrate intake. If this somewhat surprising view of digestibility is true, then measures of digestibility in standard rat assays may not be appropriate.

This issue is further complicated by the possibility that the N which is derived from bacterial N metabolism absorbed from the colon can include IAA. Jackson and colleagues (for example, see Meakins & Jackson, 1996) have consistently shown that a major part of urea salvaged in the lower gut and returned to the systemic circulation is not recycled into further urea synthesis but retained, suggesting that the salvaged N includes amino acids. Furthermore, we have shown that in malnourished infants recovering from protein-energy malnutrition 15N can be transferred from urea to the circulating lysine pool in nutritionally-significant amounts (≥ 20 m g/kg; Gibson et al. 1997). Thus, the potential exists for colonic N metabolism to both remove IAA from or add IAA to the dietary supply. This makes conventional calculations of digestibility difficult to interpret. In other words, whilst the poor digestibility of staples like millet and sorghum is clear and measurable as poor faecal digestibility, for other plant-based diets with better faecal digestibility, whether actual digestibility of individual amino acids such as threonine is in fact much worse due to poor ideal digestibility remains to be established. Since ideal digestibility is difficult to measure it is usually ignored, but it may well be a serious issue and could be part of the explanation for a low BV, when it is observed.

Table 2. Protein (% energy) and amino acid contents of human tissues, breast-milk and animal and plant food sources
(Amino acid contents for beef, human tissues and breast-milk are given as mg/g protein, and those for plant food sources as a percentage of the value found in human tissues)

<table>
<thead>
<tr>
<th></th>
<th>P : E (% energy)</th>
<th>Lysine</th>
<th>Threonine</th>
<th>S amino acids</th>
<th>Biological value</th>
<th>Score§</th>
<th>Limiting amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>66·0</td>
<td>91</td>
<td>47</td>
<td>40</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human tissue</td>
<td>–</td>
<td>71</td>
<td>41</td>
<td>30</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-milk</td>
<td>7·0</td>
<td>64</td>
<td>50</td>
<td>36</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soyabean</td>
<td>38·8</td>
<td>92</td>
<td>93</td>
<td>83</td>
<td>102</td>
<td>0·94†</td>
<td>0·86 S amino acids</td>
</tr>
<tr>
<td>Wheat</td>
<td>16·6</td>
<td>36</td>
<td>71</td>
<td>149</td>
<td>94</td>
<td>0·53‡</td>
<td>0·50 Lysine</td>
</tr>
<tr>
<td>Maize</td>
<td>13·5</td>
<td>41</td>
<td>88</td>
<td>97</td>
<td>40</td>
<td>0·41†</td>
<td>0·79 Lysine</td>
</tr>
<tr>
<td>Opaque-2 maize hybrids</td>
<td>13·5</td>
<td>41</td>
<td>88</td>
<td>97</td>
<td>40</td>
<td>0·41†</td>
<td>0·79 Lysine</td>
</tr>
<tr>
<td>Opaque-2</td>
<td>13·5</td>
<td>41</td>
<td>88</td>
<td>97</td>
<td>40</td>
<td>0·41†</td>
<td>0·79 Lysine</td>
</tr>
<tr>
<td>Mazza</td>
<td>14·0</td>
<td>56</td>
<td>107</td>
<td>160</td>
<td>55</td>
<td>1·05‡</td>
<td>0·91 Lysine</td>
</tr>
<tr>
<td>Mazza 2</td>
<td>14·0</td>
<td>56</td>
<td>107</td>
<td>160</td>
<td>55</td>
<td>1·05‡</td>
<td>0·91 Lysine</td>
</tr>
<tr>
<td>Mazza 2</td>
<td>14·0</td>
<td>56</td>
<td>107</td>
<td>160</td>
<td>55</td>
<td>1·05‡</td>
<td>0·91 Lysine</td>
</tr>
<tr>
<td>Sorghum*</td>
<td>10</td>
<td>31</td>
<td>92</td>
<td>97</td>
<td>92</td>
<td>0·65‡</td>
<td>0·44 Lysine</td>
</tr>
<tr>
<td>Potatoes</td>
<td>9·7</td>
<td>77</td>
<td>94</td>
<td>96</td>
<td>113</td>
<td>1·10†</td>
<td>1·1 Lysine</td>
</tr>
<tr>
<td>Rice</td>
<td>7·2</td>
<td>51</td>
<td>90</td>
<td>133</td>
<td>86</td>
<td>0·86‡</td>
<td>0·84 Lysine</td>
</tr>
<tr>
<td>‘Yam’</td>
<td>6·1</td>
<td>58</td>
<td>82</td>
<td>92</td>
<td>102</td>
<td>–</td>
<td>0·82 Lysine</td>
</tr>
<tr>
<td>Cassava</td>
<td>3·4</td>
<td>46</td>
<td>50</td>
<td>96</td>
<td>109</td>
<td>–</td>
<td>0·65 Lysine</td>
</tr>
</tbody>
</table>

* Sorghum, Sorghum sp.; yam, Dioscorea sp.; Cassava, Manihot sp.
† Calculated relative to breast-milk from N-balance data reported by Bressani et al. (1973), measured in young children recovering or recovered from malnutrition.
‡ Calculated from similar data from Maclean et al. (1981).
§ Scoring pattern of 3–6-month-old child (See Table 3).
Direct measurement of biological value

Traditionally, rat growth assays have been used to answer this question of assessing protein quality. Since the rat growth requirement pattern (Benevenga et al. 1994) is, with the exception of the S amino acids, similar to that of rat mixed body proteins (Davis et al. 1994), rat growth trials effectively compare the composition of food proteins with that of tissue protein, because the amino acid pattern for growth must provide at least the IAA content of the tissue growth plus any extra to provide for any inefficiency of utilization or any non-growth MD. For the most part, rat growth trials have identified limiting IAA which are consistent with the differences between the amino acid compositions of plant and animal proteins. However, the relevance of rat growth trials in human nutrition can be questioned both in terms of the quantitative importance of tissues to overall human needs, and from the perspective of whether amino acid requirements for growth differ from those for maintenance.

In fact, as shown in Table 3, while tissue growth represents the major part of the protein requirement in the first few months of life, maintenance becomes the major part after 6 months. Thus, as might be expected in rapidly-growing young children, such as those recovering from malnutrition, differences in dietary protein quality of various unsupplemented plant protein sources are observed. From N-balance data reported from such studies (for example, see Bressani et al. 1973; Maclean et al. 1981), the BV can be calculated relative to breast-milk. Such values are shown in Table 2. Clearly, wheat, sorghum and maize have a low BV, especially in the faster-growing children in the Maclean et al. (1981) studies. However, it is clear that the relatively small increases in lysine from wheat to rice have had a marked impact on the BV, whilst for potato and the best maize hybrid the BV is comparable with that of breast-milk. This is the case even though lysine levels are still lower than those in human tissues, and such foods would have had a low BV in rat growth trials. Since in the children the maintenance requirement is a much larger proportion of requirement when compared with that of the growing rat, the higher BV compared with rat studies could reflect a difference in the amino acid requirement for maintenance compared with growth.

As for adults, as reviewed elsewhere (Millward et al. 1989), although the lower BV of wheat gluten compared with beef is observed in short-term N-balance studies (Young et al. 1975), differences in the BV between plant and animal proteins are much less apparent than those found

### Table 3. Revised age-related amino acid requirement and scoring patterns

<table>
<thead>
<tr>
<th>Ages (months)</th>
<th>0–1</th>
<th>1–3</th>
<th>3–6</th>
<th>6–12</th>
<th>12–24</th>
<th>24–60</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein requirements (g/kg body wt per d)*</td>
<td>1.43</td>
<td>0.81</td>
<td>0.43</td>
<td>0.26</td>
<td>0.16</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Amino acid requirements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance (mg/kg body wt per d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>18.1</td>
<td>35.0</td>
<td>68</td>
<td>47</td>
<td>33</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Leucine</td>
<td>26.2</td>
<td>75.0</td>
<td>135</td>
<td>87</td>
<td>58</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td>Lysine</td>
<td>18.6</td>
<td>72.2</td>
<td>135</td>
<td>87</td>
<td>58</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td>Total SAA</td>
<td>55.0</td>
<td>127</td>
<td>135</td>
<td>87</td>
<td>58</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td>Total AAA</td>
<td>19.7</td>
<td>72.2</td>
<td>135</td>
<td>87</td>
<td>58</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td>Threonine</td>
<td>15.7</td>
<td>42.5</td>
<td>74</td>
<td>49</td>
<td>33</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Valine</td>
<td>35.7</td>
<td>72.2</td>
<td>135</td>
<td>87</td>
<td>58</td>
<td>46</td>
<td>38</td>
</tr>
</tbody>
</table>

| Growth (mg/g protein) |_requirements (mg/kg body wt per d) | | | | | | |
| Isoleucine | 3.5 | 0.6 | 0.4 | 0.3 | 0.2 | 0.1 | 0.0 |
| Leucine | 7.0 | 1.2 | 0.8 | 0.6 | 0.5 | 0.4 | 0.3 |
| Lysine | 14.5 | 2.5 | 1.8 | 1.5 | 1.3 | 1.2 | 1.1 |
| Total SAA | 44.5 | 7.7 | 5.8 | 5.0 | 4.5 | 4.3 | 4.3 |
| Total AAA | 20.0 | 3.5 | 2.7 | 2.4 | 2.1 | 2.0 | 2.0 |
| Threonine | 30.0 | 5.0 | 4.0 | 3.5 | 3.2 | 3.1 | 3.1 |
| Valine | 56.5 | 10.1 | 8.0 | 7.0 | 6.5 | 6.3 | 6.3 |

| Amino acid scoring patterns | (mg/g protein) | | | | | | |
| Isoleucine | 32.2 | 35.0 | 34 | 34 | 33 | 33 | 33 |
| Leucine | 47.0 | 75.0 | 67 | 64 | 59 | 56 | 53 |
| Lysine | 33.2 | 71.0 | 60 | 56 | 50 | 45 | 41 |
| Total SAA | 28.6 | 30.0 | 30 | 29 | 29 | 29 | 29 |
| Total AAA | 35.2 | 70.0 | 60 | 56 | 50 | 46 | 43 |
| Threonine | 28.0 | 41.0 | 37 | 36 | 34 | 32 | 31 |
| Valine | 6.6 | 12.8 | 11 | 10 | 9 | 9 | 8 |

SAA, S amino acids; AAA, aromatic amino acids.

* From Dewey et al. (1996).
† Values computed from regression equations reported by Hegsted (1962) with addition of 0.3 g N losses assuming 60 kg body weight.
‡ Obligatory oxidative loss (Millward & Rivers, 1988) adjusted for utilization as described by Young et al. (1989).
¶ Modified from Table 11 of Dewey et al. (1996) with cysteine assumed to be 50% concentration of methionine, and tryptophan assumed to be the same as that in beef.
in children, and they are not observed in longer-term studies. Furthermore, the marked inter-individual variability precludes quantitative studies, and has certainly prevented a simple and accurate solution being found to the significance of the low lysine levels in plant-based diets in adult nutrition. This has led to direct assessment by protein scoring, which requires agreement about the scoring pattern and the requirement levels.

The adult lysine requirement

Values for lysine intakes are shown in Table 1. Food-supply data indicate >4-fold differences in per capita lysine supplies between countries consuming high and low levels of animal protein. Similarly, food-intake data for the UK show approximately 2-fold differences between omnivores and vegetarians. Whether these differences are important and influence the BV of plant proteins in human nutrition depends on the MD for lysine and all other amino acids, and this is a complex, unresolved and controversial question.

A number of proposed values for the lysine requirement are shown in Table 1. The value from the FAO/WHO/United Nations University (1985) is higher than those for older children and for adults. As discussed elsewhere (Millward, 1994), the FAO/WHO (1991) report on protein-quality evaluation rejected the FAO/WHO/United Nations University (1985) adult value (and IAA requirement values for older schoolchildren) as flawed, was unable to identify any other appropriate adult scoring pattern, and proposed that the scoring pattern for the preschool child should be utilized for older schoolchildren and for adults as a strict interim measure. This value (58 mg/kg per d) was five times higher than the adult value of FAO/WHO/United Nations University (1985), and judged against this value food supplies in many developing countries are lysine deficient (see Pellett, 1996), as is the food intake of the UK vegetarian community and the whole of India (see Table 1). In my view it was a mistake to adopt this preschool pattern for older children and adults. As discussed elsewhere (Millward, 1994) this value was derived from studies (only partly reported and not peer reviewed) in children who had been malnourished and, as judged by the N accretion rates, may have been still repleting lean tissue. This would account at least in part for the high values.

Young et al. (1989) derived a requirement on theoretical grounds with some support from stable-isotope studies (30 mg/kg per d), and these values have been the subject of considerable debate (see Millward, 1994). Finally, Ball at Guelph and Pencharz at Toronto have published what are referred to as the Toronto break point studies in which a requirement of 43 mg/kg was identified (Zello et al. 1993; Duncan et al. 1996). These values are discussed later.

Clearly, these values vary considerably, and judgement about the adequacy of the various intakes shown in Table 1 will depend on the choice of requirement value. This requires consideration to be given to the question of why it seems so difficult to decide on a value for lysine, or any other amino acid requirement.

Difficulties in defining amino acid requirements

Recently, it has been argued in detail (Millward, 1998) that it is an inherently difficult problem to define the dietary requirements of human adults for IAA for three major reasons. The first of these reasons is adaptation, i.e. a variable MD for amino acids which is set by the habitual intake. The second reason is methodology, with no entirely satisfactory practical N- or amino acid-balance method or other measure of dietary adequacy. The third reason is lack of quantifiable unequivocal metabolic indicators of adequacy which can validate balance measurements. These issues will only be briefly mentioned here.

Nature of the metabolic demand for amino acids and protein

As reviewed recently (Millward, 1998), the obligatory MD for dietary protein is to provide amino acid precursors for growth and maintenance, i.e. for an expansion of body protein and other N-containing material, and for the replacement of amino acids irreversibly transformed for whatever reason. The growth component of the MD is straightforward and determined by the growth rate and amino acid pattern of tissue protein deposited. However, as already discussed, growth needs are low at all ages after early infancy, with maintenance dominating the MD, and the problem in understanding the MD of maintenance is a major reason for the difficulty in defining the requirement. It can be shown that the MD for amino acids is best understood in terms of a two-component model; one component is obligatory, the other adaptive. The limited human data relating to the obligatory MD are consistent with extensive animal data showing the maintenance amino acid pattern to differ from that for growth, with lower levels of lysine and leucine in the maintenance pattern (Millward, 1997). The effect of this finding is that the rate-limiting amino acids in dietary proteins can differ between maintenance and growth, the example derived from rat experiments being that lysine limits wheat for growth but not for maintenance.

The adaptive component is best understood as an increased rate of oxidation of many of the IAA in response to levels in the habitual diet above minimal needs, which occurs independently of acute protein intake during post-absorptive and postprandial states of the daily feed–fast cycle at rates in excess of obligatory consumption (see Fig. 1), and which adapts only slowly to a change in intake. The implication of this increase is that the increasing catabolic losses with increasing protein intake represent an actual MD generated in response to the presence of protein in the diet at levels in excess of minimal metabolic needs. The impact of this component on the overall MD is difficult to predict due to a complex interaction between habitual protein intake (amount and quality) and (a) the

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extent of post-absorptive oxidation rates and consequent losses of tissue protein to fuel the MD, (b) the extent of postprandial oxidation rates and tissue repletion and (c) the recycling of those IAA, such as lysine and threonine, with pool sizes large enough to allow this to occur. Although the time required for complete adaptation is not fully understood, it would appear to involve weeks and possibly months.

The practical implication of this adaptation component is that any attempt to measure the requirement in terms of the adequacy of any intake will depend on the completeness of adaptation to that intake.

Methodological difficulties

The second difficulty is methodology, with no entirely satisfactory practical N- or amino acid-balance method, or other measure of dietary adequacy. Following several critical reviews of N-balance studies (Hegsted, 1976; Young, 1986) and with the proliferation of stable-isotope studies, N-balance studies have not only become less fashionable, but have been rejected as of little value, or even performed and published but ignored when results conflict with those from stable-isotope studies (for example, see Marchini et al. 1993). In fact, close analysis of stable-isotope methodologies indicates that they are by no means free from difficulties. They are usually extremely complicated to interpret, so that few investigators fully understand their limitations, and as a result they can be frankly misleading. This is discussed extensively elsewhere (Millward & Rivers, 1988; Millward et al. 1991; Fuller & Garlick, 1994; Waterlow, 1996; Millward, 1986b), and one example serves to illustrate the difficulty.

This example is the studies of lysine requirements based on the indicator oxidation method (Zello et al. 1993; Duncan et al. 1996). This is an apparently simple approach in which subjects receive meals with decreasing amounts of lysine during which the oxidation of another amino acid is measured, in this case infused $[1\text{-}^{13}\text{C}]$phenylalanine. The principle is that when the lysine intake is inadequate to support protein deposition amino acids are diverted into oxidation, and this is indicated by increased phenylalanine oxidation. Thus, a plot of phenylalanine oxidation $v.$ lysine intake reveals a two-component curve with a break point which defines the intake below which net protein synthesis in the postprandial state is compromised by lack of lysine. At this intake, identified in these studies as either 37 mg lysine/kg body weight per d (Zello et al. 1993) or 45 mg lysine/kg body weight per d (Duncan et al. 1996), amino acid oxidation increases. This approach derives from studies in growing animals where the requirement is relatively simply defined as accretion of protein. When adopted for human subjects to assess maintenance needs the assumption is made that there is a direct relationship between overall daily balance and maximum net deposition during feeding. Whilst even this assumption is in fact quite complex, for such studies to be of value what is reported as amino acid oxidation rate needs to be a true reflection of the actual rate. In fact, in these studies the phenylalanine oxidation rates were extremely low, of the order of 2–3 µmol/kg body weight per h in subjects consuming approximately twenty times this amount of phenylalanine and tyrosine. The reason for this low value is an incorrect calculation method. The $[1\text{C}]$CO$_2$ production rate was converted into phenylalanine oxidation from the enrichment of plasma $[1\text{C}]$phenylalanine rather than $[1\text{C}]$tyrosine, the actual precursor for $[1\text{C}]$CO$_2$. In
the first paper (Zello et al. 1993) the authors quote values from which tyrosine enrichment can be calculated. I have done this calculation, and obtained a more realistic measure of total phenylalnine plus tyrosine oxidation. In fact, this error does not alter the break point other than to indicate a somewhat higher value, closer to 50 mg/kg body weight per d as far as can be calculated from these (admittedly mean and approximate) values.

Further inspection of these studies seems to indicate a fatal flaw in the design. The subjects received generous amounts of lysine at 60 mg/kg body weight per d throughout the studies, apart from the infusion days when the lower lysine meals were fed. Given that the maintenance requirement includes an adaptive component, this design would have generated a high requirement for lysine, so that it is not at all surprising that the study indicates values of between 30 and 50 mg/kg body weight per d. This would also explain why in the second study (Duncan et al. 1996) with the same experimental design no effect of previous protein intake on the break point was observed, since with a lower protein intake (0.8 g/kg body weight per d v. 1.0 g/kg body weight per d used by Zello et al. 1993) the observed requirement was not lower (45 mg/kg body weight per d). Dietary supplementation with additional lysine (and tyrosine) before the feeding studies would have prevented any adaptive influence of a lower protein-lysine intake on lysine oxidation and requirements.

The implications of the Toronto requirement values for 65 kg adults (Zello et al. 1993; Duncan et al. 1996) are shown in Table 1. Two values are shown: the average values quoted by Duncan et al. (1996) for these two studies for mean (43 mg/kg body weight per d) and safe requirements (i.e. mean and 2 std. 63 mg/kg body weight per d). Not only is the whole of India severely lysine deficient, but the intakes of the UK vegetarian population are only barely adequate (see Table 1), and this is a group much more likely to be conforming to the healthy diet guidelines, with lower rates of morbidity and mortality than omnivores. If the safe allowances were adopted, then the intakes of UK vegetarians would all be markedly below them. Such implications do point to the requirement value measured in this way as having little practical value and not representing an actual measure of minimum requirements.

**Indicators of adequacy**

A lack of quantifiable unequivocal metabolic indicators of adequacy which can be used to validate balance measurements is the third major difficulty in identifying requirements. Protein turnover in rats varies markedly in measurements is the third major difficulty in identifying a way forward

My own view is to take notice of a recent statement by Nevin Scrimshaw (1996). The potential for error and bias has led some to reject nitrogen balance data in their entirety. It is foolish to accept poor data or to fail to recognise the limitations of good data, but it is equally inappropriate to reject reliable data as long as limitations are recognised.

On this basis my own approach is to revisit the reliable N-balance data, recalculate the balances on a per kg body weight basis taking account of a reasonable estimate of miscellaneous losses, 5 mg N/kg per d (see Millward & Roberts, 1996), and to use a logarithmic regression to estimate the intake for zero balance as a measure of minimum maintenance needs, as suggested by Hegsted (1963). Whilst the N-balance data reviewed by FAO/WHO (1973) are quite variable in their quality, there are robust data for lysine.

The study by Jones et al. (1956; Fig. 2) involved fourteen women studied using semi-synthetic diets in which 95 % of N came from pure amino acids and ammonium citrate at various levels in a total of over fifty different balances with adequate but not excessive energy (136-157kJ (33-38 kcal)/kg per d). I have recalculated all the data on a per kg body weight basis, including 5 mg miscellaneous losses/kg per d, assuming balance varies as log, lysine intake. I have also included the data for the control periods when 25–30 mg lysine was fed, which Hegsted (1963) did not include in his analysis of these data. In fact the zero-balance intercept is not markedly different when based only on the submaintenance periods or on the entire data set. The intercept indicates a requirement of 18.6 (95 % CI 14.1, 27.3) mg/kg. Since subsequent studies of longer (30 d) balance periods indicated balance could be achieved at 11 mg/kg (Clark et al. 1962), I would assume that the value shown in Fig. 2 is greater than the minimum value achievable with further adaptation. Thus, the mean value of 19 mg/kg is a reasonable estimate of the lysine requirement.

Is this a sensible value? In fact it is not inconsistent with animal data, and is possibly slightly higher, being equivalent is an extensive literature on such responses (see Young & El-Khoury, 1996). Such work clearly does afford some insight into dietary adequacy, although such studies are only indirect measures, and can only provide at best supportive evidence of balance studies. However, even these relatively simple measures are not entirely unambiguous. Meredith et al. (1956) reported lysine concentrations in the fed and fasted state in subjects receiving various levels of lysine for 2 weeks, and Young & El-Khoury (1996) recently quoted these findings in support of the lysine requirement in the Massachusetts Institute of Technology scoring pattern of Young et al. (1989). When expressed as the molar proportion the values indicate that lysine levels fall with feeding when intakes are between 30 and 60 mg/kg body weight per d. However, when expressed as simple concentrations the fall with feeding does not occur until much lower levels are fed, between 10 and 20 mg/kg body weight per d. Thus, even these simple measurements can be used to support a high or a low lysine requirement. Identifying requirements is not a straightforward matter.

**A way forward**

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to 52 mg/kg body weight\textsuperscript{67} compared with values ranging from 23 to 36 mg/kg body weight\textsuperscript{67} for the rat and pig (Millward, 1997). Furthermore, this value has been validated.

Two long-term studies with bread-based (wheat) diets have been reported. In the Minnesota study (Bolourchi \textit{et al.} 1968) a diet in which 900 g/kg protein was supplied by wheat was fed for 50 d, supplying about 1 g protein/kg body weight and 18 mg lysine/kg body weight, with energy intakes designed to maintain weight against a high level of physical activity in the subjects (223 kJ (54 kcal)/kg); body weight, N balance and fitness were maintained. In the North Carolina study (Edwards \textit{et al.} 1971) a lower-protein wheat and potato-based diet supplying 0.6–0.7 g protein and 24 mg lysine was fed for 60 d, and this induced maintenance of weight and N balance.

With reference to Table 1, this revised value for the lysine requirement indicates that for adults the low-meat or no-meat cereal-based diets in India and Sub-Saharan Africa supply adequate lysine, and of course UK vegetarians are more than adequately supplied.

\section*{Plant-based diets and children}

The important issue is undoubtedly that of plant-based diets and children. My proposal (set out in Table 3) would be to adopt a factorial approach to calculating amino acid requirements. The starting point is the most recent values for protein requirements, which are based on a new estimate of maintenance and a growth requirement derived from known rates of tissue protein accretion adjusted for a 70\% efficiency of utilization (see Dewey \textit{et al.} 1996). A growth requirement amino acid pattern can be derived from the known composition of human tissues with minor adjustments for the S amino acids and tryptophan. As for the maintenancerequirement pattern, the N-balance data for the other IAA are not as robust as these lysine data, but reasonable estimates can be derived. Adjusted values for isoleucine, leucine, threonine and valine can be generated from the regression equations reported by Hegsted (1963), which are between 1.4 and 2.2 times the FAO/WHO/United Nations University (1985) values. For tryptophan the re-adjusted value is similar to the higher value reported by Rose (1957), which was adopted for the FAO/WHO/United Nations University (1985) requirement value, so this value is unchanged. For methionine plus cysteine the balance intake regression reported by Hegsted (1963) was so shallow that adjustment for unmeasured losses results in an approximately 3-fold increase, from 13 to 28 mg/kg body weight. This is an unrealistically high value. Since the obligatory oxidative loss for the S amino acids is 13.5 mg/kg body weight per d (Millward & Rivers, 1988), it seems sensible to adopt an intake likely to deliver this value, i.e. 16 mg/kg body weight, as suggested by Young \textit{et al.} (1989). For phenylalanine+tyrosine Hegsted (1963) only reanalysed a study based on phenylalanine in the presence of tyrosine. However, Tolbert & Watts (1963) subsequently reported requirement values of up to a maximum of 1184 mg phenylalanine/kg body weight per d, fed with no tyrosine and using positive N balance as their criterion. Thus, this value (19.7 mg/kg body weight per d for 60 kg body weight) has been adopted, i.e. 40\% higher than the FAO/WHO/United Nations University (1985) value.

With these two patterns for growth and maintenance, age-dependent values for amino acid requirement patterns and

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig2}
\caption{Nitrogen balance studies of Jones \textit{et al.} (1956). Values recalculated as balance per kg using individual body weights and adjusting for 5 mg N/kg additional losses. Values shown include sub-maintenance values (x) analysed by Hegsted (1963) and control values (o) also reported by Hegsted (1963). The regression of balance v. intake for all values is described by balance = 7.45 ln intake – 21.8 mg N/kg, with intake for zero balance at 18.6 mg lysine/kg. The regression of the sub-maintenance data is described by balance = 7.857 ln intake – 22.4 mg N/kg per d, with intake for zero balance at 17.2 mg lysine/kg. Values at zero balance are for maintenance lysine requirements calculated by two methods for the 40 kg pig (Fulker \textit{et al.} 1989) scaled to 70 kg from values reported as kg body weight\textsuperscript{0.75}.}
\end{figure}
the corresponding scoring patterns (amino acid requirement/protein requirement) can be derived (Table 3) from the average requirement values for protein (Department of Health, 1991).

Validation of new requirement patterns for children
To validate these patterns a comparison can be made between predicted values for the BV of plant foods and the measured values (see Table 2). The amino acid contents of the plant food sources have been scored using the requirement pattern for a 5–6-month-old child. As discussed previously, the BV of potatoes, soybeans, various hybrid strains of maize, oats, rice, wheat and sorghum were measured relative to breast-milk. It is clear that in nearly every case the predicted value is similar to or less than the measured value, even though the scoring pattern used is that for younger children than those in the study. This value is appropriate, since they were exhibiting some catch-up and growing more rapidly than expected for their age. The fact that the scoring patterns do predict what is observed does provide a measure of validation.

Adequacy of plant-based diets in developing countries for children
The extent to which the low-lysine cereal-based diets of developing countries are adequate for children can be determined by scoring with these age-related scoring patterns (see Fig. 3). I have taken data recently published by Pellet (1996) for amino acid intakes in regions of India reported as preliminary data from the Indian National Food Intake Survey. These diets are probably as low in meat as any in the world. I have taken for the average value for India a diet in which 77% of protein was derived from cereal, supplying lysine at 39 mg/g protein (9·3 MJ (2254 kcal)/d, P : E 11·1% energy); the highest-lysine diet, i.e. Tamil Nadu, a diet with 64% of protein from cereal, supplying lysine at 44 mg/g protein (7·8 MJ (1871 kcal)/d, P : E 9·7% energy), and the lowest-lysine diet, i.e. West Bengal, based on 90% of protein from cereal, supplying lysine at 39 mg/g protein (10·5 MJ (2426 kcal)/d, P : E 8·8% energy). I have scored these diets with age-dependent scoring patterns for 6–12-month-old children and older children. I have also included the UK vegetarian group identified in the UK food intake survey (see Jackson & Margetts, 1993), with approximate calculations of their lysine data reported by Pellett (1996). Clearly, as might be expected, the score for the UK vegetarian diet is >1 for all age-groups since, although meat-free, it did contain dairy products. The score for the Tamil Nadu diet is 1·1 for all age-groups. The score for the mean value for India is 0·86 at 6–12 months, rising to 1 in the 2–5-year-old group. The score for the West Bengal diet is only 0·78 for the 6–12-month-old group and is inadequate for all preschool children (0·90 at 24–60 months). Clearly, the diets of all regions are adequate for adults. Thus, when weaned, Indian children in West Bengal will need either extra milk or pulses if the energy density of the diet does not allow them to increase intakes sufficiently to provide the extra intake. The main point is that the extent of the lysine deficit, when identified, is not very marked in any age-group.

Further evidence that would support these new scoring patterns comes from a more recent study by Graham et al. (1990) of young children recovering from malnutrition and growing at 2–3 g/kg body weight per d after the most rapid phase of catch-up growth had passed. They received the high-lysine maize variety (opaque-2, sugary-2; 43 mg lysine/g protein, which is similar to the values observed in the Tamil Nadu diet) as their only energy, fat and protein source, which was as effective as casein in supporting both weight and height growth when supplemented with minerals and vitamins (Fig. 4).

Fig. 3. Amino acid scores of plant-based diets in India and the UK for children and adults. Scores derived from intake data and scoring patterns shown in Table 3, with lysine as the limiting amino acid in each case. Intakes for the UK were calculated from the protein intakes of food groups reported by Jackson & Margetts (1994) and the lysine contents of food groups reported by Pellet (1996). Indian intake data were those reported by Pellet (1996). (a), UK vegetarian; (b), Tamil Nadu; (c), Indian (mean); (d) West Bengal.

Fig. 4. Growth of recovering malnourished children on maize or milk protein. Recovering malnourished children aged 1–2 years fed on isonitrogenous diets supplying 0·42 MJ (100 kcal) metabolizable energy/kg per d, based on either milk protein or whole ‘quality’ maize (opaque-2, sugary-2 hybrid. (From Graham et al. 1990.)
Conclusions
Within the overall meat v. wheat debate there are several important nutritional issues relating to microminutrition and mineral nutrition which need to be addressed. However, in the present paper I have dealt with one issue, i.e. the adequacy of plant food sources of protein and amino acids in the human diet. It is clear that human amino acid needs are complex and not entirely understood, and I think this is the main reason for the current debate about the magnitude of human amino acid requirements. It is also clear that there remains an inadequate body of reliable measures of such requirements. However, the current fashion for the complete rejection of the large body of N-balance data in adults and children is in my opinion a wholly inappropriate response, especially given that no adequate alternative data have yet emerged from stable-isotope studies. Assuming that lysine is the limiting amino acid in plant-based diets, we can be reasonably confident that the analysis reported here is accurate. On this basis it is clear that meat-free, largely plant-based diets available in developed countries can supply protein in the amount and quality adequate for all ages. In developing countries the protein issues relate mainly to low protein levels in some staples (taro, yams and cassava etc.), but cereal-based diets, especially those based on wheat and maize, supply protein levels considerably above the requirement level. Digestibility does present a problem for many mixed plant-based diets and some cereals (e.g. millet and sorghum), and this may be a major problem for children in the developing countries. More importantly, digestibility is less well understood in terms of colonic N homeostasis than commonly assumed, and more work is certainly required in this area. As for BV, this appears only relevant in infants and young children weaned on unsupplemented cereal diets, a practice which is already recognized as ill-advised. However, even in such cases, the extent of the problem appears less marked than commonly assumed. This is not to underestimate the potential problems relating to poor microminutrient and mineral bioavailability of plant-based diets in infant nutrition. However, whatever the inadequacies of such diets, amino acid supply should not be used as the argument to promote increased intake of animal foods. Calls for lysine fortification and increased animal production to specifically supply more animal protein (for example, see Young & Pellett, 1990; Pellett, 1996) can be accurately. On this basis it is clear that meat-free, largely plant-based diets available in developed countries can supply protein in the amount and quality adequate for all ages. In developing countries the protein issues relate mainly to low protein levels in some staples (taro, yams and cassava etc.), but cereal-based diets, especially those based on wheat and maize, supply protein levels considerably above the requirement level. Digestibility does present a problem for many mixed plant-based diets and some cereals (e.g. millet and sorghum), and this may be a major problem for children in the developing countries. More importantly, digestibility is less well understood in terms of colonic N homeostasis than commonly assumed, and more work is certainly required in this area. As for BV, this appears only relevant in infants and young children weaned on unsupplemented cereal diets, a practice which is already recognized as ill-advised. However, even in such cases, the extent of the problem appears less marked than commonly assumed. This is not to underestimate the potential problems relating to poor microminutrient and mineral bioavailability of plant-based diets in infant nutrition. However, whatever the inadequacies of such diets, amino acid supply should not be used as the argument to promote increased intake of animal foods. Calls for lysine fortification and increased animal production to specifically supply more animal protein (for example, see Young & Pellett, 1990; Pellett, 1996) certain unjustified.

References


