Animal Models of Human Amino Acid Responses

David H. Baker

Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801

ABSTRACT The principal differences between experimental animals and humans with regard to amino acid responses are 
1) growing animals partition most of their amino acid intake to protein accretion, whereas growing children partition most of their intake to maintenance; 2) invasive assessment procedures are common in animals but very limited in humans; and 3) humans can describe how they feel in response to amino acid levels or balances, whereas animals cannot. New (pharmacologic) uses of amino acids have been and are being discovered (e.g., cysteine, arginine, leucine, glutamine), and this makes it imperative that tolerance limits be established. Work with pigs suggests that excessive intake of methionine and tryptophan present the biggest problems, whereas excessive intake of threonine, glutamate, and the branched-chain amino acids seems to be well tolerated. J. Nutr. 134: 1646S–1650S, 2004.

KEY WORDS: • excess amino acids • cysteine • arginine • pigs • chickens

This review focuses on amino acid (AA) responses in experimental animals, primarily pigs and chickens, with emphasis on outcomes of excessive intake levels of single indispensable AAs in animals fed normal diets. The ideal protein concept (i.e., ideal AA ratios relative to Lys) has been well accepted in diet formulation schemes for both growing pigs (1–5) and chickens (3,6–9). The 1998 National Research Council Subcommittee on Swine Nutrition (4) presented estimates of ideal AA ratios for protein accretion per se and maintenance per se based on work from the laboratories of Fuller (1) and Baker and colleagues (2,3,5,10–13). The most striking differences between ideal ratios (i.e., percent of Lys need) for maintenance and protein accretion occur with sulfur AAs (SAAs, i.e., Met plus Cys), Thr, and Arg. Thus the proposed Thr:Lys and SAA:Lys ideal ratios for maintenance per se are over twice as high as those for protein accretion per se. For Arg, where in vivo biosynthesis occurs (in mammals), the ideal ratio for accretion is 0.48, whereas for maintenance, it is less than zero. All these dietary ideal ratio estimates for pigs are based on (true) digestible levels of AAs present in a diet.

AA responses in growing animals support the view that crystalline AAs are essentially 100% digestible, i.e., absorbed (14,15), and also that there are inefficiencies in the use of absorbed AAs for protein accretion. Hence, recovery of absorbed AAs in whole-body protein when the level of a given AA is at a growth-limiting level is 80% or less (16–23). This is surprising, but even more vexing are findings that maintenance-requirement estimates based on zero protein accretion are meaningfully lower than those based on zero accretion of the AA under investigation. Indeed, animal studies suggest that at zero protein (or nitrogen) balance, dispensable AAs are in positive balance but indispensable AAs are in negative balance.

This review focuses on tolerances for excess AAs in pigs and chickens. Much of the literature on excess AAs in rats has involved specialized diets that are either low in protein or deficient in one or more AA. Also, pigs and chickens do not practice coprophagy, which is a potential confounding factor in assessment of untoward effects of excess AAs. Thus, studies involving pigs and chicks fed standard diets that contain all AAs at or in excess of requirements are emphasized.

Pig studies

The four bioassays from pigs reviewed in Table 1 (24,25) involved graded additions of δ-Met, L-Thr, or L-Leu to a standard corn–soybean meal (SBM) diet containing 196 g of crude protein (CP)/kg; the Lys trial, however, employed a semipurified diet (142 g of CP/kg) that contained Lys at its required level (11.5 g of CP/kg). The corn-SBM diet contained SAAs and Thr at levels only slightly in excess of their requirements; Leu was present at 170% of its required level. These studies and others (24–26) suggest that excess Met (followed by Trp) is the most growth depressing among the indispensable AAs when plethoric dose levels are added to the diet. Lys, Thr, and Leu, in contrast, are much better tolerated when excess levels are fed. Relative to its baseline (i.e., normal) level in the plasma, Met accumulates to the greatest extent when excess Met is fed, which is perhaps an indication that the capacity of the transsulfuration pathway to metabolize Met has been exceeded. Thr also accumulates in plasma to a significant extent when Thr is fed, which may be an indication of the capacity of the transamination pathway to oxidize Thr.
TABLE 1

Weight gain and plasma AA concentration in young pigs fed graded levels of individual excess AAs

<table>
<thead>
<tr>
<th>Excess level of</th>
<th>Weight gain</th>
<th>Diet intake</th>
<th>Plasma level of</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (g/kg)</td>
<td>g/d</td>
<td>g/d</td>
<td>supplemental AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SEM</td>
</tr>
<tr>
<td>DL-Met²</td>
<td>0.0</td>
<td>450&lt;sup&gt;a&lt;/sup&gt;</td>
<td>754&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>499&lt;sup&gt;a&lt;/sup&gt;</td>
<td>802&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>454&lt;sup&gt;a&lt;/sup&gt;</td>
<td>717&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>396&lt;sup&gt;b&lt;/sup&gt;</td>
<td>627&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>18.5</td>
<td>270&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>L-Thr³</td>
<td>0.0</td>
<td>368&lt;sup&gt;a&lt;/sup&gt;</td>
<td>590&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>359&lt;sup&gt;a&lt;/sup&gt;</td>
<td>599&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>361&lt;sup&gt;a&lt;/sup&gt;</td>
<td>563&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>9.5</td>
<td>315&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>18.8</td>
<td>322&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>L-Leu&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.0</td>
<td>525&lt;sup&gt;a&lt;/sup&gt;</td>
<td>822&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>502&lt;sup&gt;a&lt;/sup&gt;</td>
<td>804&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>16.4</td>
<td>538&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>32.4</td>
<td>519&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>38.3</td>
<td>350&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>L-Lys&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.0</td>
<td>541&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1057&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>11.5</td>
<td>12.2</td>
<td>553&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>23.0</td>
<td>22.4</td>
<td>498&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>34.5</td>
<td>30.5</td>
<td>443&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>13</td>
<td>38</td>
</tr>
</tbody>
</table>

1 Data from Edmonds and Baker (24,25). Standard 196 g CP/kg corn-SBM diets were fed in the Met, Thr, and Leu trials; a whey-corn-casein semipurified diet (142 g CP/kg, 11.5 g of Lys/kg) was fed in the Lys trial. Within each of the four trials, means within columns with different superscript letters are different (P < 0.05). Some SEM values were not available (NA) due to a heterogeneous variance.

2 Four pens of five pigs were fed each diet for 27 d; average initial weight was 8.1 kg.

3 Three pens of three pigs were fed each diet for 28 d; average initial weight was 7.3 kg.

4 Four pens of five pigs were fed each diet for 27 d; average initial weight was 9.0 kg.

5 Eight pens of three pigs were fed each diet for 28 d; average initial weight was 10.5 kg. Lys was supplemented as L-Lys acetate.

Excess AA mixtures

It is well to remember that any diet that has one or more AA at a deficient level also has an excess AA mixture over and above the AA deficiency. Moreover, protein sources considered near ideal (eg., lactalbumin, egg albumin) have excess AAs. Egg albumin, for example, contains Ile at a level that is almost double that considered optimal for growing chicks and rats. Evidently, the seminal Lys shown in Table 1 (i.e., 34.5 g/kg) did not result in any significant changes in liver, kidney, or gut mucosal arginase or ornithine transcarbamoylase activity (25). Thus, excess Lys in the pig did not antagonize Arg.

Avian studies

Studies on chickens with excess indispensable AAs added to standard corn-SBM diets (27–29) indicate that both growing chicks and adult laying hens have considerable tolerance for DL-Met and L-Lys even when the Lys is furnished as L-Lys-HCl. Dietary addition of 10 g/kg of excess DL-Met or L-Lys-HCl does not affect the growth rate of chicks (27) or the feed intake and egg production of layers (29). Large excesses (40 g/kg) of indispensable AAs, however, have variable effects on chick performance (Table 2). With plethoric dosing at 40 g/kg, Met, Phe, and Trp are very growth depressing, and the same level of excess Lys (as the acetate salt) is considerably more growth depressing in chicks than in pigs. Conversely, excess Arg is more growth depressing in pigs than in chicks (26,28). In chicks, excess Lys antagonizes Arg (30,31); anorexia, induction of kidney arginase, inhibition of hepatic glycine transamidinase, and urinary spillage of both Lys and Arg are all involved in the outcome of feeding excess dietary Lys to chicks. Growing dogs likewise show evidence of Lys-Arg antagonism when a large excess of Lys (40 g/kg) is fed (32).

In general, the branched-chain AAs are well tolerated when provided in great excess. It should be noted, however, that the basal corn-SBM diet used in these studies contained almost twofold excesses of Leu and Phe plus Try, and both Val and Ile were 20–30% above required levels. All other AAs in the basal diet were near or only slightly above required levels. As in the work on pigs, the 40 g/kg Lys addition was innocuous, and remarkably, when chicks were given a choice between the basal diet (no excess AAs added) and the diet with 40 g/kg Lys, they actually consumed more of the diet containing excess Leu (28). Hargrove et al. (33) likewise found rather amazing tolerance in kittens fed diets with 100 g/kg of supplemental Lys. They did find, however, as has also been shown in studies on chicks (34–37), rats (38,39), and pigs (40), that large excesses of Lys will depress growth, albeit modestly, when experimental assay diets are made just adequate or deficient in Ile and/or Val.

TABLE 2

Performance of chicks fed a large excess of individual indispensable AAs

<table>
<thead>
<tr>
<th>Supplemented AA²</th>
<th>Weight gain g</th>
<th>Food intake g</th>
<th>Gain:food ratio g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>118</td>
<td>175</td>
<td>674</td>
</tr>
<tr>
<td>DL-Met</td>
<td>11*</td>
<td>77*</td>
<td>141*</td>
</tr>
<tr>
<td>L-Phe</td>
<td>33*</td>
<td>105*</td>
<td>312*</td>
</tr>
<tr>
<td>L-Trp</td>
<td>51*</td>
<td>118*</td>
<td>433*</td>
</tr>
<tr>
<td>L-Lys</td>
<td>61*</td>
<td>121*</td>
<td>500*</td>
</tr>
<tr>
<td>L-His</td>
<td>62*</td>
<td>119*</td>
<td>519*</td>
</tr>
<tr>
<td>L-Thr</td>
<td>79*</td>
<td>125*</td>
<td>632</td>
</tr>
<tr>
<td>L-Ile</td>
<td>95*</td>
<td>155*</td>
<td>615*</td>
</tr>
<tr>
<td>L-Arg</td>
<td>108*</td>
<td>165*</td>
<td>671</td>
</tr>
<tr>
<td>L-Val</td>
<td>111</td>
<td>165</td>
<td>671</td>
</tr>
<tr>
<td>L-Leu</td>
<td>120</td>
<td>175</td>
<td>687</td>
</tr>
<tr>
<td>SEM</td>
<td>3</td>
<td>3</td>
<td>17</td>
</tr>
</tbody>
</table>

1 Data are from Edmonds and Baker (28). Triplicate groups of five chicks were fed each diet (230 g CP/kg; corn-SBM) for 8 d; average initial weight was 82 g; *different (P < 0.05) from the positive control (no supplemental AA).

2 Lys supplied as L-Lys acetate; His and Arg supplied in free-base form.
of all indispensable AAs simultaneously in young chicks. Individual deficiencies (60% of requirement) of SAAs, Leu, Lys, and Arg resulted in growth rates superior to deficiencies (60% of requirement) of all AAs, but similar individual deficiencies of Phe plus Tyr, Trp, or Ile produced growth rates inferior to the diet that was deficient in all AAs. The growth responses noted were due almost entirely to differences in voluntary food intake. With the balanced total deficiency, food intake was not reduced relative to the positive control (all AAs at 100% of requirement), but all the individual deficiencies caused a reduction in voluntary food intake with the greatest reductions occurring when Phe plus Tyr, Trp, or Ile were singly deficient. Remarkably, relative to the total AA deficiency, none of the individual deficiencies reduced the gain:food ratio. This indicates that at the levels of excess AA involved (i.e., mixtures of all AAs except the limiting one), no AA antagonisms occurred. One led to conclude from this work that a deficiency of one AA does not produce the same results as an equal deficiency of another AA, primarily because each individual deficiency involves a unique AA profile over and above the deficiency. And some AA mixtures over and above individual deficiencies are more noxious than others to voluntary food intake. Cieslak and Benevenga (42–44) arrived at conclusions similar to those of Sugahara et al. (41) in rat studies involving excess AA mixtures added to Lys- or Thr-deficient diets. In fact, they concluded that “all AA deficiencies are not alike.”

Studies on chicks (45,46), rats (47), and kittens (48,49) lead us to the conclusion that indispensable AAs are inefficient precursors of dispensable AAs. In felds, some of the problems with using excess indispensable AAs to meet the need for dispensable AAs may reside in the relative toxicity of Met that is present in the indispensable AA mixture (48). It seems likely, however, that this may be primarily a feld phenomenon. In most species but not rats or cats, glutamic acid or diaminonitric citrate can meet the entire need for nonspecific amino nitrogen (45,50–55). Although less efficient as amino donors than glutamate, other dietary (or metabolic) nitrogenous compounds (e.g., nucleic acids, some purines and pyrimidines, urea) can also provide nitrogen for biosynthesis of dispensable AAs (45,54,55).

Efficiency of AA utilization

Considerable difference of opinion exists regarding whether the efficiency of use (for protein accretion) of AAs above maintenance is constant (16–23,36,57) or variable (58–60) in growing animals fed graded levels (from zero to near optimal) of a limiting AA. Other work suggested constant utilization efficiency of a complete AA mixture (16) or of various intact proteins (61,62) when graded levels of each were fed. As pointed out previously, the efficiency above maintenance for retaining ileal digestible AAs is different for different indispensable AAs (18,19–23). Batterham (18) thus estimated efficiency (above maintenance) values of 75, 64, 45, and 38% for retaining (recovering) Lys, Thr, Met, and Trp, respectively, in whole-body protein of growing pigs. Heger et al. (63) also found Trp to have the lowest utilization efficiency among the indispensable AAs. This suggests that even when these AAs are consumed at levels below requirement, true digestible (absorbed) levels of these AAs are retained with surprisingly low rates of efficiency. Why? Is the need for glucose via glucoseoxygenogenesis superseding the AA needs for protein synthesis?

Our data on chicks (17,20–23) and pigs (19) together with the data on pigs from Batterham et al. (56), Adeola (57), and Heger et al. (63,64) point to the conclusion that efficiency above maintenance of using individual (limiting) AAs is constant over a wide range of intake levels of the limiting AA. Thus, from zero or near-zero intake to 80–90% of the requirement, efficiency does not decrease with increasing intake but instead remains constant.

Pharmacologic uses of AAs

Although convincing efficacy data are often elusive, pharmacologic uses of AAs are a fertile area of research. Pharmacologic applications of Cys and Arg are used as illustrations here. Both of these AAs have a multitude of functions beyond their roles in protein synthesis.

Cys is used to make specialized (high-Cys) body proteins such as metallothionein and Cys-rich intestinal protein. Also, when Cys is oxidized to cystine, it impacts structural integrity to proteins including enzymes. In its precursor role, Cys functions in glutathione, taurine, and (via sulfate production) 3'-phosphoadenosine-5'-phosphosulfate biosynthesis. Cys also functions as a reducing agent. For example, adding L-Cys to a diet containing a toxic level of pentavalent organic As (i.e., the kind of As found as a food contaminant) greatly enhances the toxicity, because it reduces minimally toxic pentavalent organic As to highly toxic trivalent organic As (65). Cys interactions with trace metals are also well known. Thus, when Cys binds to certain trace elements such as Cu, Co, or Se (66,67), reduced gut absorption occurs. Cys and its methylated derivatives (dimercaptopropanol, D-penicillamine) are therefore used clinically to treat the Cu toxicity problems of Wilson’s disease (67–71). In contrast, trace elements such as Zn and Fe are absorbed from the gut more efficiently when L-Cys is added to the diet (72–74). Another Cys derivative, N-acetyl-L-Cys, which is fully active as an L-Cys precursor (75,76), is increasingly used for therapeutic applications in clinical situations involving sepsis, respiratory diseases, and various autoimmune deficiency diseases (77,78). It is noteworthy that the above listed pharmacologic effects of oral L-Cys or N-acetyl-L-Cys cannot be duplicated by isomolar levels of L-cysteine, L- or DL-Met, or the DL-hydroxy analog of Met, although L-cysteine and glutathione do have some antioxidant and mineral chelation activity (66,67).

Animal studies show that Cys is the rate-limiting AA in endogenous protein synthesis (79). Hence, previous work showing increased nitrogen retention when Met alone is given to animals fed a protein-free diet appears due entirely to Met being metabolized to Cys. A plethora of animal growth studies show that the dietary Met requirement is only half as large when surfeit Cys is present in the diet as when Met alone is used to meet the SAA requirement. Are humans different in this regard? Vernon Young has presented arguments that they may be different (80), whereas work from the laboratories of Paul Pencharz and Ron Ball (81) suggest that Cys can indeed spare the Met requirement in humans. It is well to remember that cyst(e)ine (i.e., Cys and cystine) is the most poorly digested AA present in proteins (4,82,83), and heat treatment of proteins reduces cyst(e)ine digestibility even further. Thus, heat processing results in increased disulfide formation, and protein-bound cystine is less digestible than protein-bound Cys (84). Moreover, heat treatment of proteins can also cause some of the Cys to be converted to the cross-linked SAA lanthionine, which has little, if any, SAA bioactivity (85).

Arg is getting increasing attention for a variety of conditions such as endothelial dysfunction, wound healing, trauma, burn injury, small-bowel resection, renal failure, cancer, and diabetes (86–89). The discovery in the late 1980s that Arg is a precursor of nitric oxide and that nitric oxide can be produced in macrophages, endothelial cells, and many other cells (90–93)
has led to renewed interest in the biochemistry and nutrition of Arg in both animals and humans. In normal (i.e., healthy) individuals, however, it is hard to envision a scenario where Arg would ever become deficient. The food supply is rich in protein-bound Arg, and in vivo biosynthesis alone is sufficient to meet the protein synthesis and urea cycle needs for Arg in healthy adults (94), similar to the situation in adult gravid and nongravid swine (95,96). Hence, there may be aspects of pharmacologic dosing of Arg in its free AA form that differ from Arg supplied metabolically or consumed as protein-bound Arg.

**Final thoughts**

Animal studies of AA tolerances are useful, but they also have limitations when extrapolated to humans. Rodents, avians, and pigs consume food in many meals throughout the day, whereas humans are considered “meal eaters.” Obviously, taking single AAs without food versus taking them with a large meal could have hugely different consequences. Also, taking a single AA supplement alone is different from taking that same supplement with other AAs or taking it in the form of a peptide or a protein. More work is needed, therefore, on AA versus peptide versus protein sources of AAs in terms of tolerance and safety considerations.

**LITERATURE CITED**
