Advances in the Amino Acid Nutrition of Broilers

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ABSTRACT: The optimum amino acid balance for broilers varies with ambient temperature. In particular, the optimum dietary arginine:lysine ratio increases at heat-stress temperatures, probably reflecting a reduced uptake of arginine from the digestive tract. Feed enzymes have generally improved amino acid digestibility but a standardised digestibility procedure is required before digestible amino acids will become the basis of dietary formulation.

Key Words: Broilers, Amino Acids, Heat Stress, Dietary Arginine:Lysine Ratio, Electrolytes, DL-Methionine, 2-hydroxy-4-(methylthio) butanoic acid, Ileal Digestibility, Feed Enzymes, Xylanase, Phytase, Feed Formulation

INTRODUCTION

The supply of amino acids in the form of protein to poultry diets constitutes the most costly component of such diets. Therefore, it is essential to optimize the utilization of these dietary nutrients. This involves the identification of amino acid requirements and the ideal amino acid balance for specific types of production as well as maximising the digestibility, uptake and utilization of amino acids from dietary ingredients. Currently, digestibilities of amino acids determined at the terminal ileum are considered to give good estimates of amino acid availability. The increasing use of specific feed enzymes targeting anti-nutritional factors is likely to change the quantities of amino acids derived from feed ingredients as well as their digestibilities. However, until recently, estimates of amino acid requirements and the definition of optimum amino acid balances were derived from studies conducted in temperate climates or under thermoneutral conditions. Increasing concentrations of commercial poultry are being maintained in regions which experience high temperatures for at least part of the year. It appears that amino acid requirements and optimum amino acid ratios vary with ambient temperature so that data obtained in temperate conditions are not necessarily applicable under heat stress conditions.

The following papers will highlight recent developments in the amino acid nutrition of broilers. In particular, they will concentrate on the effect of ambient temperature on optimum amino acid balance, the role of dietary enzymes on amino acid digestibility and the use of digestibility values in feed formulation.

FACTORS INFLUENCING AMINO ACID REQUIREMENTS DURING HEAT STRESS

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The amino acid requirements of broilers at thermoneutral temperatures have been well documented and “ideal” dietary amino acid patterns established. However, as a result of recent investigations it has become evident that the ideal amino acid balance for 3-7 week old broilers varies with ambient temperature (Brake et al., 1994, 1998). In particular, the optimum dietary arginine:lysine (Arg:Lys) ratio has been shown to increase at high ambient temperatures, probably because of a reduced uptake of arginine from the digestive tract (Brake et al., 1998). Collaborative research at the University of Sydney and at North Carolina State University has specifically targeted this new area of investigation.

Varying the Arginine:Lysine ratio

The Arg:Lys ratio in a diet can be increased either by increasing the dietary concentration of arginine or by decreasing the dietary concentration of lysine. Brake et al. (1998) have shown that the dietary arginine concentration can be increased successfully either by using L-arginine free base as a dietary supplement or by altering the feed ingredients in the diet. Both procedures have been shown to improve the performance of broilers maintained at high temperatures between 3 and 7 weeks of age. The alternative method of decreasing the dietary lysine concentration was recently examined by removing the lysine supplement from the diet of 6-7 week old broilers caged at 30°C and previously fed grower diets between 3 and 6 weeks of age that varied in Arg:Lys ratio from 0.88 to 1.35 in four individual studies. The same finisher diet formulation used in each experiment contained 160 g crude protein/kg and included supplements of L-lysine (0.8 g/kg), DL-methionine (0.8 g/kg), L-threonine (1.0 g/kg) and L-arginine (0.5 g/kg). The amino acid composition of this diet met the NRC (1994) amino acid recommendations for 42-49 d old broilers, including lysine (8.5 g/kg), arginine (10.0 g/kg), methionine (3.2 g/kg) and total sulphur amino acids (6.0 g/kg). Amino acid adjustments to this diet were balanced by alterations to the dietary concentration of solka-floc, an inert cellulose supplement. Mash feed and water were supplied ad libitum and continuous fluorescent lighting was provided.

Removing the lysine supplement from the low-protein finisher diet had no adverse effects on performance in any experiment. Removal of the lysine supplement gave production responses similar to those obtained from the complete diet when grower diets containing...
Arg:Lys ratios of 0.88 to 1.05 were fed prior to the introduction of the finisher diet or the lysine-depleted diet at 42 d. However, when grower diets containing Arg:Lys ratios of 1.15 to 1.35 were fed prior to 42 d, improvements in feed intake, body weight gain and feed conversion were obtained as a result of removing the lysine supplement from the finisher diet. Responses obtained by the removal of lysine from the finisher diet mirrored those observed from the addition of arginine in the two experiments where that treatment was employed. This suggested that the observed effects were due to the Arg:Lys ratio per se. This conclusion was supported by the observation that removal of the methionine supplement in addition to the lysine supplement resulted in poorer performance in some experiments.

Effect of dietary electrolytes

The two dietary electrolytes of most significance are sodium chloride (NaCl) and sodium bicarbonate (NaHCO₃). The importance of NaCl relates to the fact that supplementation levels vary in different poultry producing regions depending on whether or not dietary formulations are designed to meet minimum chloride specifications. The importance of NaHCO₃ relates to its use as a sodium source divorced from chloride when dietary chloride levels are minimised and to its separate use as a bicarbonate source during heat stress (Balnave and Gorman, 1993). The effect of NaCl on the responses of heat-stressed broilers to varying dietary Arg:Lys ratios was reported by Brake et al. (1998). Broilers at 31°C fed diets containing 1.2 g NaCl/kg showed significant improvements in weight gain and feed conversion with increases in dietary Arg:Lys ratio. However, these responses were not evident with broilers fed diets with 2.4 g NaCl/kg. The data for weight gain and feed conversion indicated that the optimum dietary Arg:Lys ratio decreased at the higher dietary NaCl concentration.

The effect of NaHCO₃ on the responses of heat-stressed broilers to varying dietary Arg:Lys ratios is not so obvious. Balnave and Brake (1999) found that the primary response to increasing dietary Arg:Lys ratios in broilers receiving diets containing 16 g NaHCO₃/kg was increased feed intake with corresponding improvements in weight gain at Arg:Lys ratios of 1.15 and 1.25. Although the increases in feed intake and weight gain were not statistically significant they were numerically large with improvements of 8.7% and 6.6% in feed intake and 8.6 and 5.7% in weight gain. Since NaHCO₃ does not appear to influence the blood pH in heat-stressed broilers (Teeter et al., 1985; Branton et al., 1986), there is no effect on the ileal digestibility of lysine or arginine (Balnave and Oliva, 1991) and does not appear to have a direct effect on plasma amino acid patterns (Balnave and Brake, 1999), the later authors suggested that at high temperatures NaHCO₃ may act by supplying bicarbonate ions to buffer the arginine, a strongly charged cation, in the blood. The pKa of the guanidinium group of arginine is 12.48 compared with a pKa for the epsilon amino group of lysine of 10.53.

Responses in methionine activity sources

There has been considerable controversy during the past three decades concerning the relative efficacies of DL-methionine (DLM) and various methionine hydroxy analogues, most notably 2-hydroxy-4-(methylthio)butanoic acid (HMB, Alimet®). Examination of the diets fed in different studies during this time suggested that the dietary Arg:Lys ratio may have influenced the determined relative efficacies of DLM and various methionine hydroxy analogues (Balnave et al., 1999). This hypothesis was confirmed by these latter workers when DLM and HMB were compared at equimolar equivalence using broilers maintained at 32°C. The primary response appeared to be related to a relatively lower feed intake in heat-stressed broilers fed DLM compared to HMB at higher dietary Arg:Lys ratios. Thus, a high dietary Arg:Lys ratio was found to improve the relative efficacy of HMB relative to DLM at 32°C. The Arg:Lys ratio had no influence on the relative efficacy of HMB and DLM at thermoneutral temperatures (22°C).

THE ROLE OF DIETARY ENZYMES IN IMPROVING AMINO ACID DIGESTIBILITY

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The inclusion of enzymes, with predominantly xylanase activity, in wheat-based broiler diets to increase energy utilisation and overcome the problems associated with “low-ME” wheat is now a routine practice. Phytase feed enzymes are meeting more general and increasing acceptance where their primary role is to increase the availability of phytate-bound phosphorus of plant-sourced feed ingredients. Somewhat fortuitously, both xylanase and phytase feed enzymes additionally enhance amino acid digestibility. The positive effects of xylanase on the apparent ileal digestibility of amino acids in wheat- and wheat-based diets have been demonstrated (Hew et al., 1998; Bedford et al., 1998; Ravindran et al., 1999d). Phytase has been shown to increase amino acid digestibility in broilers fed complete diets based on maize (Kornegay et al., 1998), a sorghum/wheat blend (Ravindran et al., 2000) and a range of individual feed ingredients (Ravindran et al., 1999a).

Enzyme Combinations

The effects of supplementing a diet containing low-metabolisable energy wheat with phytase (600 FTU/kg) and xylanase (1238 EXU/kg), singly and in combination, were investigated by Ravindran et al. (1999a). Individually, phytase increased (P<0.05) the ileal digestibility of lysine and threonine and xylanase increased (P<0.05) the digestibility of histidine and threonine (Table). The two enzymes increased (P<0.05) the overall apparent ileal digestibility of essential amino acids to a similar extent; phytase by...
3.7% and xylanase by 3.5%. However, there was a more pronounced response of 7.3% when the two enzymes were used in combination, and the apparent ileal digestibility of all amino acids, except isoleucine, were significantly increased (P<0.05). Moreover, the combination of enzymes increased (P <0.05) the apparent ileal digestibility of arginine, histidine, leucine, lysine, phenylalanine, threonine and valine to a greater extent than either component enzyme.

**Mode of action of feed enzymes**

In wheat-based diets, it appears that these two quite different exogenous enzymes have complementary modes of action in increasing the apparent ileal digestibility of amino acids. There is a physical association between the fibre component and phytate in wheat, as about 75% of phytate is present in the soluble fibre fraction (Frolich and Asp, 1985). Conceivably xylanase and phytase reciprocally facilitate access to their respective substrates, which may contribute to the additive responses observed. While the capacity of xylanase to reduce gut viscosity is established, xylanase has been shown to increase the apparent ileal digestibility of amino acids without affecting gut viscosity (Ravindran et al., 1999d). This emphasises the need to clarify the modes of action of both exogenous enzymes in increasing the apparent ileal digestibility of amino acids, which may also involve the reduction of endogenous protein losses. Such clarification should result in a more effective use of dietary enzymes to increase the utilisation of protein by poultry.

### Table 1. The effects of exogenous enzymes on apparent ileal digestibility of essential amino acids in wheat based broiler diets

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Basal (B)</th>
<th>B + Phytase</th>
<th>B + Xylanase</th>
<th>B + Phytase + xylanase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>0.840&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.836&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.837&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.904&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.840&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.865&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.871&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.910&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.802</td>
<td>0.840</td>
<td>0.837</td>
<td>0.841</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.888&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.904&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.901&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.932&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.850&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.888&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.880&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.931&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.877&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.891&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.904&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.923&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenylalanin</td>
<td>0.878&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.904&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.900&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.932&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>e</td>
<td>0.718&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.800&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.790&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.801&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.845&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.877&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.866&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.904&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Tyrosine</td>
<td>0.806&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.847&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.840&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.871&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Valine</td>
<td>0.834&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.865&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.863&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.895&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Mean</td>
<td>0.834&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.865&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.863&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.895&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
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% Increase

|           | 3.7 | 3.5 | 7.3 |

<sup>abc</sup> Means in a row with the same superscript are not significantly different (P >0.05).

### DIET FORMULATIONS BASED ON DIGESTIBLE AMINO ACIDS

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The 'bioavailable' form of amino acids may be defined as amino acids which can be released by digestion, absorbed and utilised by animals. The two methods available for the direct estimation of available amino acids are growth assays and digestibility assays (Ravindran and Bryden, 1999a). Of these, the assay of digestibility has become the most favoured technique for estimating availability, largely because the values apply directly to the animal and all amino acids can be measured in one assay. Digestibility assays may be based on excreta or ileal digesta analysis. Most published values currently available on digestible amino acids for poultry are based on excreta analysis because of its simplicity. In particular, research in the area of amino acid digestibility has increased greatly since the development of a rapid assay (Sibbald, 1979) which has proved popular in North America and Europe. A major concern with excreta digestibility assays is the effect of the hindgut microflora on faecal amino acid output, but limited published data is available on these effects.

### Excreta versus ileal digestibility

An earlier study at Camden (Wallis and Balnave, 1984) found that the amino acid digestibility in soyabean meal was underestimated by excreta analysis. Ileal and excreta amino acid digestibility determinations have been compared recently at Camden (Ravindran et al., 1999c) using three cereals (maize, sorghum and wheat), four plant protein sources (soyabean meal, canola meal, cottonseed meal and sunflower meal) and five animal protein sources (meat meal, meat and bone meal, feather meal, blood meal and fish meal). The influence of site of measurement, in terms of magnitude and direction, was inconsistent varying among feed ingredients, among samples within an ingredient and among different amino acids within...
an ingredient. Ileal amino acid digestibility values in some feed ingredients were similar to the corresponding excreta values, but significantly lower or higher in some others. Average ileal and excreta digestibilities in sorghum and maize were similar, although significant differences were noted for some individual amino acids. In contrast, the ileal amino acid digestibility values were 10 - 25 percentage units higher than the corresponding excreta amino acid digestibility values in wheat. The average ileal and excreta digestibilities of amino acids in the three soyabean meal samples were similar, although small, but significant, differences were noted for individual amino acids. Site of measurement had no effect on the digestibility of amino acids in canola meal. In the case of sunflower meal and cottonseed meal, excreta digestibilities of some individual amino acids were underestimated by excreta analysis. Amino acid digestibilities in animal protein meals, except for highly digestible blood meal and fish meal, were consistently overestimated by excreta analysis. Ileal-excreta differences in individual amino acids digestibilities were more evident in feather meal, meat meal and meat and bone meal. Among the indispensable amino acids, threonine and valine were more frequently modified by microbial fermentation in the hindgut. Differences observed between ileal and excreta digestibilities in these studies clearly demonstrated that amino acid metabolism by hindgut microflora in chickens may be substantial and that digestibilities determined at the terminal ileum are more accurate measures of amino acid availability than those determined at the excreta level.

**Digestible amino acid systems**

Interest in amino acid digestibility of poultry feed ingredients has increased greatly in recent years. A large volume of published values including several compilations on amino acid digestibility coefficients of ingredients is now available. However, confusion about the terminology used to describe the amino acid digestibility estimates will soon become clear to anyone perusing the available digestibility data for poultry. For each amino acid in an ingredient, a range of terms have been used to present the digestibility values: apparent or true; excreta or ileal; adult cockerel (intact or caecectomised) or broilers. Almost all amino acid digestibility values reported in the literature have been determined using excreta analysis with adult cockerels employing the rapid assay procedure of Sibbald (1979) or modifications thereof. However, a compilation of apparent ileal amino acid digestibility of 92 samples representing 23 feed ingredients for 5-week old broilers is also now available (Ravindran et al., 1998a).

A question often posed by commercial nutritionists concerns which digestible amino acid system is most appropriate for use in the formulation of poultry diets - apparent or true digestibility values. Apparent digestibility measures the digestibility of amino acids of both dietary and endogenous origins. True digestibility, on the other hand, includes a correction for endogenous amino acid secretions. The relative merits of these two systems have been discussed in detail by Ravindran and Bryden (1999a). It would appear that the choice of the appropriate system of digestible amino acids may depend on the method of formulating diets. If diets are being formulated to least-cost using linear programming, then apparent ileal digestibility values are the most appropriate as they take into account the endogenous cost of digestion. On the other hand, if diets are being formulated in computer simulation models, then true digestibility values will be relevant as the model should correct for the endogenous cost of digestion. It should be appreciated, however, that both digestible amino acids systems are superior to the total amino acid system currently employed in practical feed formulations and that all current methods of amino acid evaluation have specific applications and shortcomings.

**Use of digestible amino acids in diet formulation**

With the accumulation of amino acid digestibility data, there is also growing interest in how to use these values in feed formulation. Clearly, digestible amino acid levels more closely approximate the amino acid requirements of chickens than the current practice of using total amino acid concentrations. However, currently few commercial nutritionists formulate diets based solely on digestible amino acids. The major reasons include: (1) wide variations in published digestible amino acid values from different sources, arising from differences in sample variation, type of birds, assay diets and assay methodology, (2) insufficient knowledge of the batch-to-batch variation of amino acid digestibility values, and (3) limited published information on broiler responses to diets formulated on the basis of digestible amino acids. The major advantage of using digestible amino acids in formulation lies in increasing the inclusion levels of alternate ingredients (in particular, low quality protein sources) in poultry diets. In effect, it will increase the range of ingredients that can be incorporated, improve the precision of formulation and ensure more predictable bird performance. In a series of studies evaluating canola meal (Ravindran et al., 1998b), cottonseed meal (Ravindran and Bryden, 1999b) and meat and bone meal (Ravindran and Bryden, 1999c), the beneficial effects of using apparent ileal digestible amino acids in broiler diet formulations to increase the inclusion levels of poorly digestible ingredients were demonstrated. In these studies, as expected, increasing dietary levels of canola meal, cottonseed meal and meat and bone meal on a total amino acid basis significantly lowered weight gains and feed efficiency of broilers. The observed depressions were, however, largely overcome when the diets were balanced on a digestible amino acid basis. These results confirm that the inclusion levels of poor quality protein sources in broiler diet formulae can be increased as long as they are based on amino acid digestibility values.
CONCLUSIONS

The data reported in these three review papers clearly indicate that further refinements are necessary if maximum utilization of amino acids from poultry diets is to be achieved. Refinements include a more accurate knowledge of amino acid requirements in differing environments and the definition of correct formulation strategies to meet these requirements.

It is apparent that the optimal dietary amino acid balance varies with temperature, most notably as a result of differences in digestibility and intestinal uptake. This is especially important now that a high proportion of the world's poultry production occurs in tropical and semi-tropical countries in the absence of controlled-environment housing. The studies relating to dietary arginine:lysine ratio have shown that not only is the optimum ratio for broilers increased at heat-stress temperatures but also that it is influenced by dietary electrolytes and by other amino acid sources.

The relatively recent introduction of feed enzymes has, and will continue to have, important ramifications for feed formulation. While the first generation of enzymes targeted energy utilization from feeds it is apparent that they also impacted positively on amino acid utilization. Phytase, which is of ecological importance because of its value in improving dietary phytate-phosphorus availability, has also been shown to improve amino acid utilization. These advantages for dietary amino acid utilization can only improve further with the development of more specific feed enzymes and enzyme combinations with a resulting reduction in the variability of "available" amino acids.

With this reduction in dietary amino acid variability comes the opportunity to optimise feed formulation strategies. This requires the acceptance of digestible amino acids as the basis of dietary formulations although it is apparent that considerable research will be required before a standardised digestibility procedure will prove acceptable to the poultry industry.

REFERENCES


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