DIGESTIBLE AMINO ACID VALUES: VARIATION AND APPLICATION

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Summary

Formulation of poultry diets on a digestible amino acid basis has been shown to be superior to formulation on a total amino acid basis when using ingredients with low amino acid digestibility. However, few commercial nutritionists currently formulate diets based solely on digestible amino acids because of 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. There is also insufficient knowledge of the batch-to-batch variation of amino acid digestibility values for locally grown feedstuffs.

I. INTRODUCTION

The matching of feed quality to amino acid requirements of poultry will depend on a description of the response of a defined genotype (laying hen or broiler chicken strain) to amino acids supplied under defined conditions and of an understanding of the relationship between measured feed quality and amino acid supply from that feed to the bird. As the title of this paper implies it is the second part, namely the quality of dietary protein, that is the focus of this presentation. An important feature of protein quality for the feed industry is knowledge of the availability of amino acids in feedstuffs. Reliable values will permit more efficient formulation of diets. Many attempts have been made to determine amino acid availability (defined as that proportion of dietary amino acids that is in a form suitable for digestion, absorption and utilisation) using in vitro (enzymatic and chemical assays), indirect (microbiological or plasma amino acids) or direct (growth and digestibility assays) methods (reviewed by Ravindran and Bryden, 1999a). Discussion in this paper will be confined to digestibility assays.

II. DIGESTIBILITY ASSAYS

The digestibility assay has become the most favoured technique for estimating availability, largely because the values apply directly to the bird and all amino acids can be measured in the one assay. Digestibility assays are applied assuming that the difference between input and output is a valid indicator of bioavailability and that digestibility is likely to be the rate limiting step in amino acid availability. Digestibility assays may be divided into faecal and ileal procedures.

(a) Excreta digestibility

Excreta digestibility has been used by many workers because of its simplicity. Estimates of amino acid absorption made by using excreta of intact birds are in error because avian urine contains some amino acids (Sibbald, 1987). However, the very low concentrations of amino acids in urine mean that the error is likely to be small. Determination of amino acid digestibility in excreta has been widely criticised because intestinal microflora in the hindgut have a substantial affect on the amount of individual amino acids excreted in faeces. Some estimates put this as high as 25% of excreta protein (Parsons et al., 1982). Caecetomised
birds were developed to overcome the problem of microbial modification of dietary protein and microbial protein synthesis in the hindgut. However, the influence of caecotomy on apparent amino acid digestibility appears from the literature to be quite variable (Ravindran and Bryden, 1999a). Nevertheless, the excreta method using precision-fed roosters has been widely adopted in Canada, the United States and France and in the latter two countries the birds are caecetomised. In this procedure true amino acid digestibility is determined after correction for endogenous amino acid secretion into the gut.

(b) Ileal digestibility

Since microbial activity is concentrated in the hindgut and the main sites of absorption of amino acids are the jejunum and ileum, Payne et al. (1968) suggested that the analysis of ileal contents rather than excreta might be a reliable method for assessing protein and amino acid digestibility. Ileal digestibility can be determined in two ways depending on the technique of sample collection. The simplest method for the collection of ileal digesta is to kill the bird and the alternative is to use an ileal cannula. Ileal cannulation has been developed for adult cockerels (Rajaho and Farrell, 1984; Gurnsey and James, 1985). Although ileal cannulation seems to provide some theoretical advantages over the other method it is a sophisticated technique for practical application. Some questions may arise such as the rejection of the cannula, the type and placing of the cannula, the free flow of digesta through the cannula or the use of an appropriate marker (see Sauer et al., 1989). Moreover, for the cannulation technique to be cost effective, it must be undertaken with adult birds and there is always the question that digestibility measured with adults may not reflect digestibility in the rapidly growing broiler chicken. It is for these reasons that at Camden we have developed an ileal digestibility assay with five week old broiler chickens and have published a monograph that contains the digestibility of 92 samples representing 23 feedstuffs (see Ravindran et al., 1998a).

III. VARIATION IN DIGESTIBILITY VALUES

A number of factors influence amino acid digestibility. The nature and digestion of dietary protein will reflect breeding programs, agronomic conditions, presence of antiinhibitory factors and processing. Variation in digestibility values will also arise from difficulties associated with the conduct of assay procedures and the measurement of endogenous amino acid losses. Surprisingly, there are few instances in the literature where the significance of these sources of variation have been evaluated.

(a) Dietary protein digestion

All dietary sources of protein are heterogenous mixtures of different proteins. It would be anticipated, therefore, that different proteins would be digested at different rates and this in turn would cause a variation in the rate at which different amino acids were taken up from the gut. However, the situation is more complex than this as proteins, although different in their chemical compositions, are not isolated entities but have various linkages with carbohydrate, lipids and other proteins so that these interactions and the composition of the diet may affect the digestibility of dietary protein (Hughes and Choc, 1999). In addition, digestion and absorption may be inhibited by the presence of anti-nutritive factors in the diet. Protease inhibitors, lectins, polyphenolic compounds, saponins and non-starch polysaccharides are examples of anti-nutritive factors that depress protein digestion and utilisation (Bryden, 1996; Hughes and Choc, 1999). Ironically, those feedstuffs (grain
legumes, oil seed meals) which are used extensively as sources of dietary protein also contain the highest concentrations of anti-nutritional factors. For example, soyabean meal contains a range of anti-nutritional factors, many of which are heat labile and destroyed during feedstuff manufacture (Dale, 1996). Heat treatment, essential for inactivation of many anti-nutrients, may reduce protein quality in the presence of carbohydrates by Maillard type reactions.

Processing, especially heat treatment, may contribute to the variability of ingredients such as protein meals and cotton seed meal (Dale, 1996). Lysine is heat sensitive and the low digestibility of lysine in cotton seed meal may reflect heat processing of the meal. The variations in digestibilities of amino acids in meat meals are likely to be due to differences in raw ingredients, time between slaughter and rendering and the duration and temperature of the rendering process (Skurray, 1974). Obviously, optimum processing conditions for all protein meals that do not reduce amino acid digestibilities need to be established. Another aspect of processing, grinding, modifies particle size and shape without causing chemical changes in feedstuffs. It has been shown that grinding improves nutrient digestibility in birds (Hamilton, 1995). This may reflect the increased surface area available for enzyme attack during digestion.

It has been known for some time that the major influence of anti-nutritional factors on protein nutrition has been a reduction in apparent protein digestibility. It is only recently that the actual cause of the reduction on apparent digestibility has been determined with any certainty. The application of new techniques for the measurement of endogenous amino acid excretion has allowed researchers to separate the effects of reduced digestion of both exogenous and endogenous protein and increased endogenous secretion (Angkanaporn et al., 1994). Both factors would reduce apparent digestibility. The relative importance of these two avenues of amino acid loss by the bird will vary with different anti-nutritive factors (Bryden, 1996).

The application of feed enzymes to poultry diets has also demonstrated the impact of anti-nutritive factors on apparent amino acid digestibility. In a series of studies (see Ravindran and Bryden, 1999b) we have shown that the application of xylanases and phytase alone and in combination improves amino acid digestibility by amounts which can be quite significant in terms of overall feed formulation. The positive effect of enzymes on amino acid digestibility again demonstrates the impact of anti-nutritive factors on either reducing protein digestion or increasing endogenous amino acid loss. The net result is a decrease in apparent amino acid digestibility.

(b) Assay procedures

There are now a number of reference sources (see Table 1) of known digestibility values for a range of feedstuffs. However, there is great confusion when one examines these compilations to know how to compare the values obtained by different procedures. It is apparent from Table 1 that there are a number of different assay procedures that vary in terms of the age of the birds used, the collection site of digesta, feeding procedures, basal diet, dietary inclusion level of test ingredients, etc. (see Ravindran and Bryden, 1999a) which all add to the uncertainty of the values obtained. Difficulties associated with amino acid analysis can be a major source of variation which is often overlooked (Ravindran and Bryden, 1999a). Moreover, the application of rapid techniques such as NIR is dependent on the reliability of chemical analysis of amino acids.

Two major areas of contention in digestibility assays are the use of ileal versus excreta collection procedures and correcting digestibility values for endogenous secretions. There have been few direct comparisons of ileal versus excreta digestibility methods and in a
Table 1. Terminology used to describe amino acid digestibility in feedstuffs for poultry - selected samples

<table>
<thead>
<tr>
<th>Reference</th>
<th>Terminology</th>
<th>Type of bird</th>
<th>Method of feeding</th>
<th>Site of sampling</th>
<th>Endogenous output measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raharjo &amp; Farrell (1984)</td>
<td>Apparent ileal digestibility</td>
<td>10-week-old cockerels</td>
<td>Allowance feeding, 100 g</td>
<td>Ileal digesta</td>
<td>-</td>
</tr>
<tr>
<td>Raharjo &amp; Farrell (1984)</td>
<td>Apparent ileal digestibility</td>
<td>Adult cockerels, ileal-cannulated</td>
<td>Allowance feeding, 100 g</td>
<td>Ileal digesta</td>
<td>-</td>
</tr>
<tr>
<td>Sibbald (1986)</td>
<td>True digestibility</td>
<td>Adult cockerels, intact</td>
<td>Precision-feeding, 25-40 g</td>
<td>Excreta</td>
<td>Fasting</td>
</tr>
<tr>
<td>Green (1987)*</td>
<td>True digestibility</td>
<td>Adult cockerels, caecectomised</td>
<td>Precision-feeding, 50 g</td>
<td>Excreta</td>
<td>Protein-free diet</td>
</tr>
<tr>
<td>Parsons (1991)*</td>
<td>True digestibility</td>
<td>Adult cockerels, caecectomised/intact</td>
<td>Precision-feeding, 25-50 g</td>
<td>Excreta</td>
<td>Fasting/protein-free diet</td>
</tr>
<tr>
<td>Rhone-Poulenc (1993)*</td>
<td>True digestibility</td>
<td>Adult cockerels, caecectomised</td>
<td>Precision-feeding, 50 g</td>
<td>Excreta</td>
<td>Protein-free diet</td>
</tr>
<tr>
<td>Rhone-Poulenc (1995)*</td>
<td>True ileal digestibility</td>
<td>Adult cockerels, caecectomised</td>
<td>Precision-feeding, 50 g</td>
<td>Excreta</td>
<td>Protein-free diet</td>
</tr>
<tr>
<td>Angkanaporn et al. (1996b)</td>
<td>True ileal digestibility</td>
<td>Adult cockerels, caecectomised</td>
<td>Precision-feeding, 30 g</td>
<td>Ileal digesta</td>
<td>Homoarginine labelling</td>
</tr>
<tr>
<td>Ravindran et al. (1998a)*</td>
<td>Apparent ileal digestibility</td>
<td>5-week old broilers</td>
<td><em>Ad libitum</em></td>
<td>Ileal digesta</td>
<td>-</td>
</tr>
</tbody>
</table>

* Sources of compilation of amino acid digestibility data
series of studies (Ravindran et al., 1999) we have shown that there is greater variation in excreta values than there is in ileal values. 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 estimates of amino acid availability than those determined in excreta. If feed intake is low, as it is in precision-fed assays, endogenous amino acids become a greater proportion of the amino acids measured in digesta and excreta. Apparent digestibility is depressed accordingly. The problems associated with the quantification of endogenous amino acids are discussed below.

(c) Endogenous Amino Acid Losses

As shown in Table 1, most excreta digestibility assays and some ileal digestibility procedures include a correction for endogenous amino acids in an endeavour to provide a more accurate value for comparing different diets or protein sources. Approaches to the estimation of endogenous amino acids in poultry (see Angkanaporn et al., 1996a) have included the measurement of amino acids in excreta either during starvation, when fed a protein free diet, or by determining endogenous output at zero intake by regression analysis. However, the use of these practices, especially the first two, is intrinsically unsound because starvation or the absence of a nutrient, such as protein, profoundly alters metabolism and the bird can no longer be regarded as physiologically normal. Starvation or feeding a protein free diet are the methods used for endogenous correction in the precision-fed rooster excreta digestibility assay which has been adopted in many laboratories. We have used both the protein free diet and the regression analysis method to measure the entry of endogenous amino acids into the lower ileum of broilers and roosters and have shown that the two methods give different results that vary with the maturity of the bird. We have compared these techniques to the homoarginine method and have shown that both techniques significantly underestimate endogenous amino acid secretion when compared with the latter technique (Siriwan et al., 1994). Bryden et al. (1996) and Ravindran and Bryden (1999a) have discussed in detail the assumptions that are used when applying the homoarginine technique and these assumptions have been shown to be valid when tested. Interestingly, the values obtained by the homoarginine technique have been reported to be of similar magnitude to those measured using isotope dilution (Roos et al., 1994) and also the peptide elimination ultrafiltration technique (Ravindran and Bryden, 2000). All three techniques have the advantage that they measure endogenous amino acids in birds that can be considered physiologically normal.

IV. APPLICATION OF DIGESTIBILITY VALUES

The major advantage of using digestible amino acids in diet formulation is that it makes it possible to increase 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., 1999b), cottonseed meal (Ravindran and Bryden, 1999c) and meat and bone meal (Ravindran and Bryden, 1999d), 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. This is in accord with previous studies on cottonseed meal (Fernandez et al., 1995) and several byproduct ingredients (Rostagno et al., 1995; Douglas and Parsons, 1999). These results confirm that the inclusion levels of poor quality protein sources in broiler diets can be increased as long as they are based on amino acid digestibility values.

Additivity of digestible amino acids, determined in single feedstuffs, is a crucial consideration in the formulation of complete diets. Studies in our laboratory (Angkanaporn et al., 1996c) found that digestible amino acid supply in a complete diet can be predicted, with reasonable accuracy, based on apparent amino acid digestibilities determined for individual feed ingredients (soybean meal, sunflower meal, meat and bone meal). Investigations with a wider variety of ingredients may be warranted to determine the possibility of associative effects between other feedstuffs.

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 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.

V. CONCLUDING COMMENTS

As growth rates of birds improve, protein requirements increase and as enhanced feed intake is unlikely to be achieved, improved utilisation of dietary protein is required. In this regard, information on the availability of amino acids is therefore becoming increasingly important in poultry feed formulation. Not only will it allow a better match of dietary amino acids with the birds' requirements, but it will also allow a higher inclusion of less digestible and less expensive feed ingredients. However, for industry to adopt the digestible amino acid approach to feed formulation it must be confident in the reliability of the digestible amino acid values. This can only be achieved through continued research. For every ingredient sufficient samples must be assayed to estimate the variance in digestible amino acids, and identify the sources of variation (e.g. variety, location, season, agronomic, processing etc). Allowing for ingredient variability will improve the overall quality of diets. Improving quality ultimately depends on the ability of nutritionists to identify avenues for enhancing the nutritive value of raw ingredients (Dale, 1996). Opportunity to improve utilisation may occur, for example, through plant breeding, the addition of enzymes or changes in processing.

REFERENCES


