# Amino Acid Digestibilities: Determination and Application in Poultry

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## Introduction

It is generally accepted that the provision of amino acids, either free or, much more usually, in the form of protein, accounts for approximately a quarter of the cost of practical diets for poultry. However, as is the case with a number of nutrients the economic influence of amino acids is probably much greater than this, because any dietary shortfall impairs productivity substantially. An important objective of animal nutritional science is to formulate diets to allow a predetermined rate of production to be achieved at least cost. In theory this implies that, as far as protein is concerned, the ideal diet should exactly satisfy the requirements of the target species for amino acids, given that these can be specified. In practice, however, this goal is probably unrealistic, partly because of variable demand by individual animals and partly because of constraints imposed by the amino acid profiles of the raw materials accessible to the animal feed trade. The increasing use of synthetic amino acids in diet formulation is reducing the importance of the latter of these two limitations. Nevertheless, it is unlikely that the maximum economic return from poultry production will be achieved until the amino acid concentrations in the diets are known to meet the requirements of the animals being fed.

### *Amino Acid Composition*

In this context it is universally recognised that the contribution made by dietary protein to the nutritional needs of the animal depends not only on its amino acid composition but also on how effectively these amino acids are used. The advent and development of ion-exchange and high performance liquid chromatography has made the determination of most of the amino acids present in foods a relatively routine, if somewhat exacting problem in analytical chemistry. Even today in the age of sophisticated automated equipment, high skills and careful attention to detail are required for meaningful results to be obtained. Although many techniques have been applied to separate mixtures of amino acids; the use of automated equipment centred round ion-exchange liquid chromatography is the most popular by far. However, both gas chromatography and, more recently, high performance liquid chromatography have been used to separate amino acid mixtures rapidly with high resolution and sensitivity. These latter two approaches invariably require that the amino acids be converted to appropriate derivatives before application to the column and this has meant that cation exchange chromatography is still probably the favoured method applied in amino acid analysis. Since 1958 (Spackman *et al.*,1958) when it was first introduced, there have been many modifications and improvements. These have resulted in the reduction of the analysis time from *24* h to 30 min and much increased sensitivity (nanomole to picomole).

### *Hydrolysis*

The determination of the total amino acid composition of food proteins first requires their hydrolysis into the constituent monomers. This initial step is arguably considered to be the most significant source of variation in the results that are reported. Although acids, alkalis and enzymes have all been used as hydrolytic agents it is generally accepted that the best procedure involves treatment with 6 M hydrochloric acid at 110° for 24 h. With the exception of methionine and cysteine, which undergo partial oxidation under these conditions, and tryptophan which is destroyed by acids and where alkaline hydrolysis is necessary, the use of 6 M hydrochloric acid is the preferred reagent in most laboratories. Recent developments have tended to concentrate on automating and reducing the duration of the reaction stage by carrying out the hydrolyses at higher temperatures (up to 200°C) in sealed tubes, with vapour phase hydrochloric acid or in microwave ovens. However, because most of these new techniques have been developed for use with pure proteins, care and validation will need to be taken before applying them routinely to foodstuffs.

Because of the nutritional importance of methionine and cysteine particularly for poultry, special conditions must be applied. In our laboratory controlled oxidation to methionine sulphone and cysteic acid using performic acid (Moore, 1963) is performed before acid hydrolysis, and the amino acids separated

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by ion-exchange chromatography using a short programme (30 min). Promising results have recently been obtained using 4M methanesulfonic acid which does not appear to cause the destruction of methionine, cysteine or tryptophan.

The accuracy achieved in amino acid analysis will depend on several factors. Some of these will be internal to the analyser and its operation and will largely be governed by the stability of the instrument, the effectiveness of the calibration, the reagent quality and its stability and the peak resolution and identification. Some will be external and will relate to the samples analysed and their preparation. In the analyses of foods and excreta where it is the absolute quantities of each amino acid that is required, external factors are almost invariably the source of most variation. These result from inadequate care being taken with sample selection, preparation and (perhaps) storage.

The problems associated with hydrolysis are now better understood and in some cases corrections can be made for them. For example serine and threonine are known to decompose at rates proportional to the time and temperature of heating and these can be allowed for using standard factors (3% loss of threonine and 6% loss of serine after 24 h) or hydrolysing separate samples for different times and extrapolating recoveries to zero time of heating. The other amino acids are stable to acid hydrolysis, except for glutamine and asparagine (which are completely converted to glutamic and aspartic acids, respectively) and tryptophan. Some peptide bonds involving isoleucine and valine are known to be resistant to hydrolysis, with isoleucine-isoleucine bonds being particularly stable. Laboratories seldom report what correction factors they have applied although commendable exceptions occur (Siriwan *et al.* 1993).

Despite the care taken to carry out amino acid analyses and an increased awareness of the probable problems, results from collaborative trials designed to test the precision of the analytical procedures have been disappointing (Williams, 198 1). In protein hydrolysates the individual amino acids that give most cause for concern are cysteine, methionine and tryptophan and these clearly require to be estimated separately. Of the others the poorest precision, in general, is found for histidine, proline, phenylalanine and arginine. It is our experience that histidine consistently gives problems particularly, when droppings are being analysed. This is attributed to the imperfect separation between histidine and its 1- and 3-methyl derivatives. 3-methyl histidine is a common component of chicken urine and l-methyl histidine is also a metabolite of muscle and can appear in hydrolysates of excreta. The best precision appears to be obtained with aspartic acid, glutamic acid, glycine and leucine, all of which are generally large, well-resolved peaks.

Therefore, although automated, high performance ion-exchange chromatography of amino acids has been in practice for more than 25 years there are still doubts about the reliability and precision of the methods. Paul and Southgate (1978) were most critical of the data published on foods because details of the conditions used for hydrolysis were seldom described and hydrolytic losses ignored. They considered hydrolysis to be the most critical part in amino acid analysis and, although this is probably true, the criticism that few laboratories carry out experiments to determine the optimum hydrolysis time is unrealistic and unjustified. Tristram and Smith (1963) defined the ideal analysis as one where the protein hydrolysis had been carried out for 20, 40, 70 and 140 h with results being averaged or extrapolated to provide the best data. This is probably true for pure proteins but for proteins in feedingstuffs where large quantities of carbohydrate are often present, there is evidence that a single hydrolysis time of 24 h can be justified for most amino acids (Rudemo *et al.,* 1980). The precision within a laboratory is generally adjudged to be satisfactory (in our laboratory we achieve coefficients of variation of less than 5%) but the current tendency towards faster and faster analyses is counterproductive, because effective peak resolution is often sacrificed in favour of speed.

### *Amino acid availability*

Not all amino acids contained in dietary protein become available to the bird during digestion and metabolism. Although much emphasis has been placed on the amino acid compositions of feedingstuffs and diets, it has been recognised for many years that for almost all foods these values are only useful in predicting the potential worth of the protein. Sibbald (1987) uses the term bioavailable to define that portion of the ingested nutrient which is used for normal metabolic functions. Available amino acids are usually considered to be those actually supplied at the sites of protein synthesis. Despite many attempts to devise methods capable of measuring what proportion of the amino acids from the protein ingested reaches these sites, quantitative data which can be used in diet formulations are very limited, often restricted to one amino acid (lysine, say) and are not universally accepted. At the present time about the only acknowledgement that is made to availability in commercial diet formulation is to increase slightly the specification of some of the key nutrients by a small percentage, the precise amount depending on the nature of the ingredient, the marginal cost and the judgement of the nutritionist. In the current climate of high food costs and small profit margins in poultry production in the UK, there is considerable pressure to reduce the extent of overformulation, at least of price-sensitive nutrients. In some parts of Europe the need to reduce pollution from excretion of excess nitrogen is driving diet

formulators to match the amino acid content of the diets to the amino acid demands of the stock being fed as closely as possible.

### *Digestibility*

As a first step to describing amino acids in terms of their availability and in order that some progress can be seen to be being made, it seems sensible to establish the extent by which the amino acids contained in the dietary protein are absorbed from the gastrointestinal tract during digestion, the so-called digestibility coefficients. Although it is possible to imagine circumstances whereby an amino acid could be digested but not be available for use by the host animal, it is obvious that undigested amino acids (those appearing in the faeces) have made no contribution to the needs of the animal. Therefore, describing the proteins in feedingstuffs in terms of their digestible amino acids, although perhaps not ideal, is almost certainly closer than total to reflecting the amount that actually becomes available for maintenance and production.

Digestible amino acids are generally calculated from the differences between measures of the amounts in the food and those in the excreta. It is common to express this difference as a proportion of the amount consumed (the digestibility coefficient):
(Equation 1)

**Equation 2**

$$\text{Amino acid digestibility} = \frac{\text{Amino acid consumed - amino acid in faeces}}{\text{Amino acid consumed}}$$

In discussions of this sort, confusion often arises over the terminology used. Strictly speaking the above term should be referred to as apparent digestibility because, of the amino acids in the faeces, only part has arisen from undigested food residues. Part has come from the animal itself and consists of gut secretions, sloughed-off gut tissue and bacteria. Sibbald (1987) distinguished between what he calls the metabolic faecal component (secretions, abraded cells, mucus, bile) and the endogenous faecal fraction (bacteria and bacterial debris) but in this paper both are grouped as endogenous faecal material. Its measurement allows true digestibility to be calculated thus: (equation 2)

To derive this term some means of measuring the amount contained in the endogenous component has to be devised.

A further source of debate in measurements of digestibility is the effect of bacteria in the hind gut, an activity that could influence the amounts of both endogenous and exogenous amino acids excreted. Defmitions of digestibility can accommodate, at least partly, the effects of the microflora in poultry, either by using caecectomised birds (the caeca are generally acknowledged to be the principal site of microfloral activity) or, arguably better, by basing values on amino acid concentrations in the terminal ileum (i.e. before the bacteria exert any effect). To relate the amino acid concentration at the ileum to that in the food requires the addition of an indigestible marker (such as chromium sesquioxide) to the food and its measurement in both food and ileal contents. Measurement of ileal contents also almost invariably require the birds to be killed. Although cannulation has been used for this type of assay (Raharjo and Farrell, 1984) it requires skilful surgery which is laborious and expensive to carry out on large numbers of birds; maintenance of the flock is also labour-intensive.

A further factor complicating the determination of faecal digestibility with poultry is the fact that birds excrete faeces and urine together and the collection of faeces requires the birds to be colostomised. It has, however, become increasingly common to overlook the effect of urine in assays designed to determine amino acid digestibility, the rationale being that the urinary contribution to the amino acids in poultry excreta is exceedingly small and barely affected by the nature of the input. However, this assumption should be tested and, to be strictly correct, balance experiments where amino acids are measured in excreta determine unmetabolised rather than undigested protein. Also if an amino acid appears in the urine as a metabolite it will result in misleading information.

In addressing the topic of amino acid digestibility in poultry and devising techniques for its measurement, all the factors outlined above have, at one time or another, been considered important enough to have been taken into account. Despite this there is still no clear indication whether data derived from excreta differs from that derived from faeces, whether the microflora affect digestibility measurements and the

**Equation 2**

$$\text{True amino acid digestibility} = \frac{\text{Amino acid consumed - (amino acid in faeces - endogenous amino acid in faeces)}}{\text{Amino acid consumed}}$$

significance of any effects or whether values should be expressed as true or apparent coefficients of digestion. In other words, no consensus exists as to a preferred system for expressing the extent to which amino acids in dietary protein are digested by birds.

### *Methods for determining amino acid digestibilities*

Digestibility is frequently considered to be a property of a diet or feedingstuff, but it is really a characteristic of an animal to which the food is given. It is, for example, a matter for debate whether the digestibility of a particular food is the same across all monogastric species or all ages. Digestibility measurements relate to the complete diet consumed and values for ingredients must, in most cases, be obtained by comparing results from two or more appropriate diets (substitution methods). The assumption that digestibility coefficients are additive amongst feedingstuffs is essential and little progress can be made if this assumption is not upheld. It should be noted, however, that results from at least one laboratory suggest that amino acid digestibility may be influenced by interactions between dietary ingredients (Wallis *et al.,* 1985).

Three observations are required from a bioassay designed to determine digestibility of amino acids. - 1. The amount of the amino acids consumed, 2. the amount excreted and 3. a measure of the endogenous amino acid losses - and most assays have been devised with the objective of gathering this information. There are 3 general types of balance experiments which have been developed to derive amino acid digestibility coefficients (*in vitro* tests have also been used, but these will not be pursued here) :

1. Traditional assays which almost always involve preliminary feeding periods to establish equilibrium conditions within the digestive tract of the bird. Differences in carry over at the beginning and end of the period of the assay (“end effects”) are controlled by trying to ensure they are the same. In most cases complete diets must be fed and substitution methods used for ingredients.

2. Rapid assays which use starvation before and after giving a known aliquot of test diet to control the “end effects”. The bird is allowed free access to the diet and, again, in most cases complete diets must be fed and substitution methods used for ingredients.

3. Rapid assays which rely on tube-feeding to put the test material into the birds’ crops. The need to substitute ingredients into a basal diet is almost always avoided.

Although many variations within these three general types of assay are found this is a convenient framework to keep in mind when evaluating the quality of the data.

### *Droppings vs faecal collection*

The difficulty involved in separating faeces from urine in poultry has meant that almost all published values are based on the amino acid recovery in droppings rather than the more technically correct faeces. It is generally assumed that the amino acid concentration of urine is low and can be ignored. An experiment by Bragg *et al.,* (1969) compared results from normal and colostomised birds (Table 1). These data suggested that digestibility values derived from normal birds were slightly but significantly different from those derived from colostomised birds, differences which were caused by the colostomised birds excreting greater quantities of endogenous amino acids (Table 2). Because these larger concentrations occasionally led to digestibility values greater than 100%, it was suggested that they were artefacts of the modification and that normal birds gave more realistic digestibility coefficients. Although this experiment suggests that there is little practical difference in using the technically less correct droppings in equations to derive digestibility coefficients, from a scientific standpoint verification of the findings is required.

**Table 1** True digestibility coefficients (%) of some amino acids in grain sorghum by colostomised and normal birds

| | Colostomised | Normal |
|---|---|---|
| Ala | 93.1 | 90.9 |
| Arg | 92.2 | 91.0 |
| Asp | 98.4 | 95.8 |
| Glu | 93.6 | 92.8 |
| Gly | 87.5 | 81.3 |
| His | 87.8 | 84.1 |
| iso-Leu | 92.3 | 90.5 |
| Leu | 93.5 | 92.3 |
| Lys | 91.7 | 87.9 |
| Met | 93.7 | 93.2 |
| Phe | 93.7 | 91.7 |
| Pro | 88.7 | 86.4 |
| Ser | 91.2 | 88.6 |
| Thr | 88.9 | 86.1 |
| Tyr | 94.0 | 92.3 |
| Val | 90.8 | 89.6 |

### *Effect of Fermentation*

The effect of fermentation is another largely unresolved issue. It has been argued that undigested amino acids which reach the hind gut can be deaminated by the microflora into products of no

nutritional value. Yet, because the deaminated but undigested amino acids do not appear in the faeces, they are judged to have been absorbed. Evidence to support this hypothesis is contradictory. While Johns *et al.* (1986) and Parsons (1985;1988) report important effects caused by the presence of caecal microflora (Table 3), results from our laboratory (Table 4) agree with those of Picard *et al.*( 1983) and Green *et al.* (1987) who found only small and non-significant effects. Recent studies in our laboratory (Longstaff *et al.,* 1991) with field beans continue to support the view that activity in the caeca of adult birds has little effect on the extent to which protein is digested. Furthermore, Bielori and Iosif (1987) have shown that only small differences exist between the digestibilities of the amino acids from soya bean meal in the ileum and excreta (Table 5). The lack of effect was attributed to the very rapid passage rate of digesta and the relatively small volume of the hindgut in poultry.

Digestibility based on dropping samples from unaltered birds has the decided advantage of simplicity over either the use of caecectomised birds or values based on ileal concentrations. It encourages assays to be carried out on larger numbers of birds and this increases the precision of the data. The majority of values published on amino acid digestibilities are derived from measurements made on droppings from intact birds.

**Table** 2 Endogenous amino acids excreted (mg/4h) by colostomised or normal 4-week-old chicks (Bragg *et al.,* 1969) or by adult cockerels (McNab, unpublished)

| | Colostomised | Normal | Adult |
|---|---|---|---|
| Ala | 4.4 | 5.0 | 3.0 |
| Arg | 2.5 | 1.8 | 0.7 |
| Asp | 5.8 | 4.2 | 4.1 |
| Cys | 1.5 | 1.9 | 1.7 |
| Glu | 6.9 | 5.4 | 5.7 |
| Gly | 3.5 | 4.5 | N.D. |
| His | 1.1 | 0.5 | 4.4 |
| iso-Leu | 2.2 | 1.5 | 1.6 |
| Leu | 3.8 | 2.4 | 2.5 |
| Lys | 2.1 | 0.6 | 1.7 |
| Met | 0.8 | 0.4 | 0.8 |
| Phe | 1.9 | 1.9 | 1.2 |
| Pro | 3.6 | 3.0 | 2.3 |
| Ser | 4.0 | 3.2 | 3.2 |
| Thr | 4.1 | 3.4 | 2.8 |
| Tyr | 2.2 | 1.4 | 1.5 |
| Val | 3.7 | 1.9 | 2.2 |
| Total | 54.1 | 43.0 | 39.4(43.9) |

**Table** 3 Effect of caecectomy on true amino acid digestibility coefficients (%)

| | | Normal | Caecectomised |
|---|---|---|---|
| Parsons (1988) | | | |
| Feather meal | Lys | 73.8 | 67.9 |
| | Cys | 72.3 | 59.3 |
| | Met | 78.4 | 74.6 |
| Meat meal | Lys | 86.9 | 81.6 |
| | Cys | 85.6 | 79.9 |
| | Met | 90.1 | 87.4 |
| Poultry offal | Lys | 86.0 | 80.0 |
| | Cys | 87.4 | 80.8 |
| | Met | 90.5 | 88.2 |
| Johns et al. (1986) | | | |
| Meat and bone | Thr | 78.9 | 75.4 |
| | Ser | 85.1 | 81.9 |
| | Val | 88.6 | 84.7 |
| | Met | 90.2 | 90.7 |
| | iso-Leu | 87.6 | 87.7 |
| | Leu | 88.6 | 87.2 |
| | Tyr | 82.0 | 66.5 |
| | Phe | 85.4 | 76.4 |
| | His | 86.2 | 81.6 |
| | Lys | 88.1 | 82.0 |
| | Arg | 88.9 | 88.3 |

### *True or apparent digestibility coefficients*

Because the apparent digestibilities of amino acids in a feedingstuff depend on the food intake (Fig. 2), care must be taken to ensure that comparisons of values across foodstuffs is made at constant intakes, otherwise a systematic bias may inadvertently be incurred. Probably, for this reason it is preferable to express values in terms of true digestibility coefficients which are independent of food intake (Sibbald, 1979), although, as has already been said, how the endogenous amino acid contributions are determined is still a matter for debate. However, because the endogenous amino acid excretion in birds is a relatively small percentage of the total amino acids excreted after feeding most feedingstuffs, the uncertainty associated with these values has less impact than endogenous energy losses have on true metabolisable energy values. The observation that true amino acid digestibilities established in chickens can be applied to muscovy ducklings, whereas apparent digestibility coefficients differed (Mohamed *et al.,* 1986) between the two species (Table 6) is proof that great care is required to ensure that valid comparisons are being made. More comparisons of this sort are required.

**Table 4** Effect of caecectomy on the digestibility of amino acids (%) in distiller's dried grain

| | | Normal | Caecectomised |
|---|---|---|---|
| Parsons (1988) | | | |
| Feather meal | Lys | 73.8 | 67.9 |
| | Cys | 72.3 | 59.3 |
| | Met | 78.4 | 74.6 |
| Meat meal | Lys | 86.9 | 81.6 |
| | Cys | 85.6 | 79.9 |
| | Met | 90.1 | 87.4 |
| Poultry offal | Lys | 86.0 | 80.0 |
| | Cys | 87.4 | 80.8 |
| | Met | 90.5 | 88.2 |
| Johns *et al.* *(1986)* | | | |
| Meat and bone | Thr | 78.9 | 75.4 |
| | Ser | 85.1 | 81.9 |
| | Val | 88.6 | 84.7 |
| | Met | 90.2 | 90.7 |
| | iso-Leu | 87.6 | 87.7 |
| | Leu | 88.6 | 87.2 |
| | Tyr | 82.0 | 66.5 |
| | Phe | 85.4 | 76.4 |
| | His | 86.2 | 81.6 |
| | Lys | 88.1 | 82.0 |
| | Arg | 88.9 | 88.3 |

**Table** 5 True digestibility of soya bean meal amino acids (%) at the ileum and in the excreta of chicks (Bielori and Iosif, 1989)

| | Ileum | Excreta |
|---|---|---|
| Ala | 83.5 | 84.4 |
| Arg | 86.3 | 89.7 |
| Asp | 81.1 | 85.6 |
| Cys | 81.6 | 95.0 |
| Glu | 87.9 | 90.3 |
| His | 85.0 | 88.5 |
| iso-Leu | 84.3 | 86.6 |
| Leu | 84.0 | 86.5 |
| Lys | 86.7 | 88.5 |
| Met | 88.9 | 89.5 |
| Phe | 85.2 | 87.3 |
| Pro | 83.7 | 87.4 |
| Ser | 82.8 | 84.6 |
| Thr | 82.2 | 82.8 |
| Tyr | 85.6 | 88.5 |
| Val | 85.2 | 85.9 |

**Table** 6 Apparent and true digestibilities (%) of 3 amino acids in a soya bean meal based diet by chicks and muscovy ducklings (Mohamed *et al.,* 1986)

| | Apparent | | True | |
|---|---|---|---|---|
| | Chicks | Ducks | Chicks | Ducks |
| Arg | 92.9 | 87.5 | 94.5 | 96.9 |
| Lys | 80.7 | 85.5 | 96.9 | 99.0 |
| Thr | 76.3 | 76.6 | 93.3 | 91.8 |

## Conclusions

Although many questions still remain to be resolved on the most valid techniques to measure amino acid digestibility coefficients there are good grounds for optimism. More work is required to determine what factors affect endogenous losses and whether the use of caecectomised birds results in the derivation of significantly different and more meaningful digestibility coefficients. The age of the bird is another factor which may require to be taken into consideration and whether digestibility coefficients derived with adult cockerels can be used with turkeys and ducks.

For poultry nutrition, generally, the prospects are undoubtedly exciting. The introduction and development of rapid assays allows raw materials to be studied directly and values are to longer subject to either the vagaries of food intake or the uncertainties of extrapolation. Their cheapness and speed have allowed many more of them to be carried out with a consequent increase in the amount and detail of nutritional information.

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