

Amino Acid Availability in Feedstuffs for the Growing Pig

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Introduction

Assessing amino acid (AA) availability in dietary ingredients is integral to the optimal formulation of diets both in terms of maximising nutrient utilization and profitability. Although the concept that nutrients in feedstuffs are not fully absorbed and metabolized by the animal is now widely accepted, a distinction needs to be made between the concepts of digestibility and availability. Digestibility is often referred to as the uptake of an AA from the gut, whereas availability is usually defined as the degree of uptake and subsequent utilization of the AA for protein synthesis and other anabolic processes. Amino acid availability is a complex phenomenon affected by many interacting factors. For most AAs availability will digestibility, but there is likely to be some discrepancy between digestibility and availability, particularly for the amino acid lysine in heat-treated foods (Moughan 1989). Although the following discussion will place emphasis on aspects of AA digestibility, the availability of absorbed AA's will also be considered with reference to heat-treated feed ingredients. The application of AA digestibility data, particularly in relation to diet formulation is stressed.

Determining Amino Acid Digestibility

It is useful when considering methods for determining AA digestibility to make a distinction between processed and unprocessed feedstuffs.

Amino acid digestibility in unprocessed feedstuffs

The traditional approach to determining AA digestibility in feedstuffs for the pig is to determine faecal AA excretion. The quantitative importance, however, of the microbial metabolism of protein in the mammalian large intestine is well established (McNeil 1988). Amino acids entering the hind-gut from the small intestine may be acted upon by the micro-flora, leading usually to a net disappearance though sometimes a net appearance of AAs between the ileum and rectum (Rerat 1981). It would also appear that AAs

are not absorbed across the mammalian large intestinal mucosa to any significant extent (Wrong *et al.* 1981; Low and Zebrowska 1989; Darragh *et al.* 1994), with amino nitrogen being absorbed from the hind-gut mainly as ammonia, which under normal circumstances is of no nutritional value to the host. An indication of the significance of hindgut microflora metabolism is that around 80% of faecal nitrogen is present in microbial bodies (Mason *et al.* 1984). This means that only a very small proportion of the faecal AA excretion directly relates to the flow of undigested dietary AAs.

Given the influence of hind-gut microbial activity on the faecal excretion of AAs, measurement of AA flow and determination of digestibility at the end of the ileum (Payne *et al.* 1968) is now generally recognised as a more acceptable approach (Rerat 1981; Tanksley and Knabe 1984; Sauer and Ozimek 1986; van Weerden 1989). A significant amount of literature on the ileal digestibility of AAs in feeds for pigs is now available.

There are several routinely adopted techniques for the collection of ileal digesta from pigs, the details of which have been reviewed extensively (Fuller 1989; Low 1990; Sauer and de Lange 1992). Methods for digesta collection with large animals such as the pig primarily involve surgical implantation of gut cannulae. The potential impact of any form of cannulation on the normal physiological functioning of the animal, however, should not be underestimated. Livingstone and McWilliam (1985) reported that pigs with simple T-cannulae implanted in the ileum had similar voluntary food intakes to their non-cannulated counterparts but grew more slowly and less efficiently. Wenham and Wyburn (1980) in radiological studies with sheep found that several types of cannulation, including simple T-cannulation, caused some disruption to normal digesta flow.

An alternative to collecting digesta via intestinal cannulae, is to sample digesta from the terminal ileum of animals under anaesthesia (Moughan *et al.* 1989). The so-called 'slaughter technique' has the distinct advantage of involving minimal disruption to normal

digestive function in the animal and allows samples of digesta to be taken from several parts of the digestive tract. The main technical criticism of this method concerns the potential difficulty of obtaining representative samples of digesta. When a frequent feeding regimen is adopted in combination with the slaughter technique, digestibility data are no more variable than those found with cannulated animals (Donkoh *et al.* 1994a).

There may be advantages in using smaller mammals as models for protein digestion in the pig. As such, the laboratory rat has been adopted for the routine determination of ileal AA digestibility in pig feeds. Ileal digesta samples can be obtained quickly and easily from the rat after slaughter and this species lends itself to relatively inexpensive, well-controlled experimentation with large numbers of animals being able to be studied at any one time. Several studies have shown that the rat is a good model for studying aspects of protein digestion in pigs with general agreement being found between the rat and pig for the ileal digestibility of protein in several feed ingredients (Moughan *et al.* 1984; Moughan *et al.* 1987; Donkoh *et al.* 1994b). The laboratory rat is useful for the routine assessment of ileal protein digestibility in a range of feed ingredients, possibly with the exception of some legumes and plant foods containing high levels of anti-nutritional factors (ANFs). Extending the concept of model animals beyond the sphere of pig nutrition has led to a comprehensive case being established for the pig as a model animal for studying aspects of human nutrition (Miller and Ullrey 1987; Moughan and Rowan 1989; Moughan *et al.* 1992a; Moughan *et al.* 1994). The growing pig has been used to investigate the digestibility of proteins in the diet of adult humans (Rowan *et al.* 1994), and the suckled pig has been used as a model animal for the human infant (Newport and Henschell 1989; Innis 1993; Darragh and Moughan 1995).

It is possible, therefore, to routinely determine the digestibility of AAs in a feedstuff by measuring the flow of AAs at the end of the terminal ileum in either the pig or a suitable model animal and relating this back to the dietary AA intake. A potential criticism of the ileal measure, however, is that there may be interference from a population of microorganisms present in the upper digestive tract (Horvath *et al.* 1958; Williams Smith 1965; Wiesemuller, 1983; Bergner *et al.* 1986). Amino acids may be catabolized or synthesized or incorporated into microbial protein. The *in vitro* and *in vivo* studies of Dierick *et al.* (1986a,b) indicate that there may be a small degree of net catabolism of AAs by the flora in the upper digestive tract of the pig. Nevertheless, ileal digestibility coefficients have been shown to be accurate in describing the extent of uptake of AAs from the animal's gut, at least for feedstuffs which have not sustained damage to their protein during processing (Just *et al.* 1985; Moughan and Smith 1985).

Amino acid digestibility in processed feedstuffs

For feedstuffs which have been subjected to processing and have sustained damage to the AAs, neither the ileal nor faecal digestibility assay is expected to be accurate at least for some amino acids (Moughan 1991a). Heat induced changes to lysine in particular, impose serious limitations on the methodology. This is of concern since lysine tends to be the first limiting essential AA in most pig diets. Lysine possesses an e-amino group which can react with a wide range of compounds present in feeds to produce nutritionally unavailable derivatives, for example, Maillard products. During the acid hydrolysis step of conventional AA analysis, which is used to break down the protein into its constituent AAs, a proportion of these heat-induced lysine derivatives revert back to lysine. As a consequence, conventional AA analysis leads to an over-prediction of the actual lysine present in heat-treated protein or in ileal digesta from an animal fed a heat-treated diet.

The effect of this is clearly demonstrated in a study by Batterham *et al.* (1990) where growing pigs were fed either a cottonseed meal (severely heat-treated) or a soyabean meal based diet. Both diets were formulated to contain equal and limiting amounts of ileal digestible lysine. Whole body lysine retention was significantly lower in those pigs fed cottonseed meal, compared to those fed soyabean meal which led Batterham *et al.* (1990) to conclude that the ileal digestible lysine content was overestimated in the cottonseed meal.

Chemical methods, such as the fluorodinitrobenzene (FDNB) lysine assay, for determining reactive lysine, while giving accurate assessments of reactive lysine in heated protein sources, are unsuitable for determining available lysine because they do not take into account incomplete digestion and absorption of reactive lysine (Desrosiers *et al.* 1989). In order to determine the availability of lysine in heat-treated proteins, a modification of the conventional digestibility assay is required. The limitations of the ileal digestibility assay could be overcome if the reactive rather than total lysine content in both the diet and ileal digesta were determined, resulting in a coefficient for digestible reactive lysine. The FDNB lysine assay is unsuitable for this purpose as there are free amino acids, and di- and tri-peptides present in the digesta collected at the terminal ileum and the FDNB method would lead to indiscriminate labelling of all free amino-groups including both the a- and e-amino groups in the lysine residue, resulting in an overestimation of reactive lysine.

An alternative method for determining digestible reactive lysine in heat-treated proteins has been developed (P.J. Moughan and S.M. Rutherford, unpublished). In a process described as guanidination, O-methylisourea reacts specifically with the e-amino group of lysine to form the acid-stable derivative homoarginine. The guanidination reaction can be

coupled with an ileal AA digestibility assay, to allow determination of the ileal digestibility of reactive lysine. Moughan and Rutherford found that in a heated skim milk powder, where approximately 40% of the original lysine in the unheated sample was degraded or chemically altered, the true ileal digestibility of almost all of the AAs was lower in the heated sample compared to the unheated sample. For a number of the AAs this difference was statistically significant (Table 1). Furthermore, conventional AA analysis underestimated the digestibility of lysine in the heated skim milk powder by approximately 25% units in comparison with the new reactive lysine digestibility method (Table 2). This underestimation is probably due in part to a higher ratio of modified to unmodified lysine residues present in the **digesta** compared to the diet, since the modified lysine residues will probably be digested and absorbed to a lesser degree than the unmodified lysine residues. The reduced digestibility of the modified residues is most likely due to the inability of proteolytic enzymes to cleave **peptide** bonds neighbouring these heat modified lysine residues. Variable decreases in digestibility were also found for almost all the AAs, ranging from 0.4% units for leucine to 13% units for histidine. The digestibility of reactive lysine was approximately 2.5%

units lower in the heated skim milk powder compared to the unheated skim milk powder. This decrease was in accord with the decreases in digestibility seen for the other AAs, and was in contrast to the significantly greater decrease in lysine digestibility (28%) observed using conventional AA analysis. The extent of the decrease in digestibility for individual AAs in the heat processed feed compared to the non-heat processed feed may depend on their proximity to modified lysine residues.

It has been claimed that digestible lysine calculated using lysine digestibility coefficients and lysine concentrations determined using conventional methods is equal to the digestible lysine calculated as the product of dietary reactive lysine and reactive lysine digestibility determined using guanidination techniques. Although it is possible that this may occur for some protein sources, for most this is not the case. Certainly, with heated skim milk powder Moughan and Rutherford (unpublished data) found large differences (up to 50% units) between these two values. Further, for these two values to be equal, the diet and **digesta** must contain the same amount of lysine that has reverted back during analysis, from heat-induced lysine derivatives. Given that the percentage reversion in the diet and **digesta** are likely to be similar while the

Table 1 Ileal digestibility^a (%) of essential amino acids in unheated and heated skim milk powder in the rat determined using an ileal digestibility assay coupled with conventional amino acid analysis (P.J. Moughan and S.M. Rutherford, unpublished).

Amino Acid	Unheated skim milk powder	Heated skim milk powder	Overall SE	Significance
Threonine	92.8	90.4	1.08	NS
Valine	93.9	92.1	0.81	NS
Isoleucine	90.9	86.3	1.22	*
Leucine	98.2	97.8	0.39	NS
Phenylalanine	98.9	99.3	0.75	NS
Histidine	96.0	83.5	1.02	***
Arginine	98.8	95.5	0.81	*

Table 2 Lysine digestibility (%) in an unheated skim milk powder (SMP) determined using a rat ileal digestibility^a assay coupled with conventional amino acid analysis, and in a heated SMP determined using the rat ileal digestibility^a assay coupled with either conventional amino acid analysis or the guanidination method (P.J. Moughan and S.M. Rutherford, unpublished).

^a Corrected for endogenous amino acid excretion (Moughan *et al.* 1990; Butts *et al.* 1993)

	Unheated SMP		Heated SMP		Overall SE	Significance
	Total Lysine ^b	Total Lysine ^b	Reactive Lysine ^c	Reactive Lysine ^c		
Lysine Digestibility	96.6	69.1	94.1	94.1	0.77	***

^a Corrected for flows of endogenous amino acids at the terminal ileum of the rat.

^b Determined after conventional AA analyses.

^c Determined using the new method based on the guanidination reaction

total amount of lysine derivatives in diet and digesta are likely to be different, since a portion of lysine derivatives are absorbed from the small intestine of the pig, it would seem likely that if these two values were similar then it would only be by chance.

There are many methods for determining reactive lysine in feeds, all with their unique advantages and disadvantages, but currently there is no ideal method that allows the routine determination of the digestibility of reactive lysine in heat processed feeds. The new ileal reactive lysine digestibility method described here may be a means by which this can be achieved. Work is now underway at our institute, using pig growth studies, to establish the accuracy of the reactive lysine digestibility coefficients.

True Ileal Amino Acid Digestibility

Ileal digesta contain appreciable quantities of non-dietary protein from sources such as digestive secretions, mucus and cells, hair and bacteria, and to obtain a "true" estimate of digestibility, correction should be made for this non-dietary (mainly endogenous) component. "True" as opposed to "apparent" estimates of digestibility should more clearly describe the AAs absorbed from a diet.

True digestibility is a fundamental property of a feed ingredient, and is a measure unaffected by the dietary conditions under which that ingredient is fed to an animal. This is not so for apparent digestibility which is greatly influenced by conditions of the assay used in its determination. With removal of the effect of the confounding variable of endogenous excretion, true digestibility values should be more accurate in detecting differences in the digestibility of various protein sources. Apparent estimates of digestibility, on the other hand, are variable and are open to errors

based on the assay methodology. Also, there is an increasing trend, with the use in practice of pig growth simulation models, towards expressing daily AA requirements in units of grams of absorbed AA. The requirement value is likely then to include that part of the maintenance AA cost associated with endogenous AA loss from the gut, and in this case it is appropriate to use true AA digestibility coefficients in dietary formulation. The application in practice of true digestibility coefficients dictates the need to determine endogenous AA loss at the terminal ileum. There are problems, however, in determining endogenous AA loss.

It has been clearly demonstrated, using a variety of different experimental techniques (de Lange *et al.* 1990; Darragh *et al.* 1990; Moughan and Rutherford 1990; Butts *et al.* 1993) that the traditional protein-free method for determining endogenous AA loss in monogastric animals leads to considerable underestimation of ileal endogenous AA excretion. Further, and given that the regression technique generates similar values to those obtained after feeding pigs a protein-free diet (Leibholz and Mollah 1988), use of this method also appears to be inappropriate. The measurement of endogenous ileal AA flow has been the subject of a recent review (Moughan 1991 b).

An alternative approach which allows measurement of endogenous AA excretion under physiological conditions has recently been developed (Moughan *et al.* 1990; Butts *et al.* 1993). An animal is fed an enzyme hydrolysed casein based diet and the digesta collected from the terminal ileum are ultrafiltered to separate dietary and endogenous AAs. Application of this method demonstrates that the presence of dietary peptides in the gut supports a much higher ileal endogenous AA flow in comparison to the unphysiological protein-free feeding (Table 3).

Table 3 Mean endogenous flows of essential amino acids^a at the terminal ileum for growing pigs fed an hydrolysed casein based diet or a protein-free diet (Moughan *et al.* 1992b).

Amino acid	Endogenous Flow		Overall SE	Significance
	Hydrolysed Casein ^b	Protein-free		
Lysine	461	312	36	*
Histidine	319	231	25	NS
Arginine	373	480	42	NS
Threonine	909	572	84	*
Valine	593	321	52	**
Isoleucine	504	230	49	***
Leucine	528	400	34	*
Phenylalanine	278	238	16	NS

^a mg/kg dry matter intake; (n=5)

^b Digesta were centrifuged and ultrafiltered; flows were based on amino acids in the precipitate plus retentate (MW > 10,000 Da)

Estimates of ileal endogenous AA flow can also be obtained indirectly from endogenous lysine flow determined using the homoarginine technique (Hagemeister and Erbersdobler 1985; Moughan and Rutherford 1990). These latter estimates can be used in conjunction with those determined using the enzymically hydrolysed casein method to routinely generate true ileal digestibility coefficients. Further research into methods for determining ileal endogenous AA excretion is required, but true ileal AA digestibility coefficients should ultimately provide more meaningful data on AA absorption in the pig.

Real Ileal Amino Acid Digestibility

There will be instances where the feed ingredient will induce an endogenous AA loss (EAAL) greater than that determined using techniques such as the enzyme hydrolysed casein method (basal excretion). For example, it has been shown that protein sources containing fibre or ANFs will stimulate increased endogenous losses. Using a constant (constant for a given dietary dry matter intake, ie. basal) EAAL will, therefore, lead to an underestimation of the digestibility of the protein. To determine the “real” digestibility of the protein in ingredients containing fibre or ANFs a direct measure of the EAAL associated with each specific type of dietary ingredient is required.

By using isotope or tracer techniques it is possible to distinguish between dietary and endogenous nitrogen in the ileal digesta after feeding a pig a particular ingredient, thus allowing determination of the real digestibility of the protein in the ingredient (de Lange 1990). Techniques such as the ^{15}N method can only directly determine endogenous nitrogen in the digesta, however, not the endogenous AAs. An endogenous AA composition, usually based on protein-free feeding and which is assumed to be constant, is subsequently used to determine real ileal AA digestibility values. Several of the assumptions and techniques required in the application of tracer methodology, however, are questionable (Moughan *et al.* 1992c). The cost of tracer-labelled substances also detracts from the routine use of this type of methodology in the determination of real digestibility.

Alternatively, *in vitro* digestibility techniques such as those described in a recent review (Boisen and Eggum 1991) can be used to determine the real digestibility of a dietary ingredient. The *in vitro* approach provides data on AA digestibility rapidly, cheaply and with precision. It is very difficult, however, to simulate adequately the complex processes occurring in the mammalian gut, which calls into question the accuracy of such *in vitro* assays. In the past, numerous methods have been proposed and sometimes high correlations with *in vivo* data have been found, but only too often a strong *in vitro*, *in vivo* association has not been confirmed in subsequent studies. Most correlations between *in vivo* and *in vitro*

digestibility have been performed with apparent digestibility values, however, which may not give a satisfactory general relationship. Ileal digestibility values determined *in vivo* would be more appropriate as a base-line for validation of *in vitro* digestibility values. Alternatively, comparison of *in vivo* apparent digestibility values with apparent digestibility predicted from *in vitro* real digestibility values has been suggested (Boisen and Fernández 1995).

Application of Digestibility Values

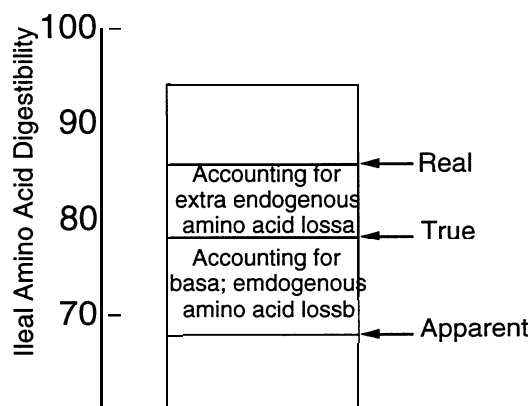
It is possible to determine either apparent, true or real ileal AA digestibility values. Apparent digestibility values will always underestimate AA availability in feedstuffs and are influenced by the digestibility assay conditions (eg. protein level of the test diet). In feed ingredients that do not contain fibre and/or ANFs, true and real digestibility are numerically the same and both will provide an accurate assessment of AA absorption. In ingredients that do contain fibre or ANFs, however, only real digestibility will provide an accurate measure of AA absorption, with true digestibility underestimating dietary AA absorption.

These different aspects of digestibility are depicted in Figure 1.

Figure 1 The influence of endogenous protein losses on the determination of digestibility.

^a Determined on a dry matter basis using techniques such as the ^{15}N , or homoarginine methods.

^b Determined on a dry matter basis using such methods as the enzymically hydrolysed casein method.



When deciding upon which type of digestibility measure to use it is important to establish the objective for acquiring such information. For example, if the objective is to obtain an accurate estimate of the uptake of AAs from the digestive tract of an animal, and the animal is fed a diet that does not contain either fibre or ANFs, then either true or real digestibility, as described in the current context, would be appropriate. If the diet contains fibre or ANFs, however, real digestibility would need to be used.

However, digestibility coefficients are usually determined to aid in the formulation of diets and an integral part of dietary formulation is the inclusion of estimates of nutrient requirements. It is the methodology used in the estimation of nutrient requirements which, in the end, should determine which type of digestibility value is used. Most estimates of the nutrient requirements for pigs are based on empirical methods such as dose-response or factorial estimation (ARC 198 1). Alternatively, pig growth models can be used to estimate AA requirements (Moughan 1995).

Estimates of the AA requirements of pigs, be they empirically or model-simulation based, have usually taken into consideration the endogenous loss of AAs by the animal. In most instances, however, this EAAL will pertain to the basal level, rather than the increased EAAL associated with specific ingredients. This latter fact has implications for the choice of digestibility value used. For example, if apparent digestibility values are chosen then EAAL in the pig is costed against the feed. The subsequent use of apparent digestibility values in conjunction with estimates of a pig's AA requirement which have already accounted for EAAL will result in a double penalty against the feed. Obviously, this is unacceptable. Using true digestibility values, however, would result in a fair representation of the protein source, particularly if it does not contain fibre or ANFs, as the basal EAAL used to obtain the true values will in most cases be similar to the basal EAAL pertaining to estimation of the pig's AA requirements.

The issue becomes more complicated when considering the effect of ANFs and fibre on EAAL. As mentioned previously such factors lead to an increase (above basal) in EAAL. Most estimates of a pig's AA requirements will not include a factor to accommodate for these elevated EAALs. If real digestibility values are used the pig's increased AA requirements (due to increased EAALs) would not be taken into account. Consequently, the "real" requirement would not be met and a drop in productivity or efficiency of utilisation would result.

The cost of any extra EAAL can be readily costed against the feed, but the AA requirement estimates are more difficult to alter. When formulating diets with ingredients containing fibre or ANFs it would seem more appropriate, therefore, to determine true ileal AA digestibility values. Thus the cost of the additional EAAL would be borne by the diet through an undervaluing of the absorbable AAs in the ingredient. In this way the increased requirement for AA by pigs fed such diets would be offset by the underestimation of digestibility due to using the true digestibility assay.

In summary, therefore, it would seem that the most useful digestibility value to determine is true ileal AA digestibility, as this measure is independent of assay conditions, relatively easy to determine and appears to be most consistent with the way in which nutrient requirements are commonly given. Using the enzymically hydrolysed casein method, it is possible to

determine, under the physiologically normal condition of peptide alimentation, a basal EAAL relative to dry matter intake. This basal EAAL can be used in the routine determination of true ileal AA digestibility coefficients for all feedstuffs.

In practical feed formulation, tabulated values of true ileal AA digestibility determined by *in vivo* assays can be used. In most feedstuffs, however, the digestibility may vary considerably between different batches. It would be useful if these variations could be monitored so as to increase the accuracy of diet formulation. The costly nature of *in vivo* digestibility assays both financially and ethically may limit their use for routine analysis. For rapid, and relatively inexpensive monitoring of the availability of AAs in pig feeds, the *in vitro* digestibility assay seems at present to be the most promising method. Accepting the previous discussion, the 'real' digestibility values generated using *in vitro* techniques, however, would need to be converted back to 'true' values before they could be used effectively in dietary formulation.

Boisen and Fernández (1995) have developed a method for predicting apparent ileal digestibility values in feedstuffs from *in vitro* analyses. Using similar principles it should also be possible to make a prediction of true digestibility. However, the methodology developed by Boisen and Fernández (1995) will require further evaluation before it can be routinely applied in the prediction of the true AA digestibility of feedstuffs.

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