



## Evidence for validity of ileal digestibility coefficients in monogastrics

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### Abstract

Measures of amino acid (AA) digestibility are used widely to estimate bioavailability of AA in feed and food ingredients for monogastric animals. In principle, the digestibility assay is simpler than *in vivo* assessments of AA bioavailability and allows for simultaneous estimation of the bioavailability of all AA in an experimental diet. It is generally assumed that absorption of intact AA in the hindgut of monogastrics is minimal, even though colonocytes do contain AA transporters and have been shown to absorb AA. This assumption is supported by the observation that infusion of AA into the hindgut does not improve nitrogen balance in monogastrics. In addition, growth performance of monogastrics is more highly correlated with ileal than faecal AA digestibility. Therefore, ileal digestibility coefficients provide better estimates of AA bioavailability than faecal digestibility coefficients. Measures of apparent ileal digestibility (AID) of AA are confounded with endogenous gut AA losses (EAAL). The curvilinear increase in AID of AA with increasing dietary AA level has been attributed to the relatively large contribution of EAAL to total ileal AA flows at low dietary AA levels. Subtracting basal EAAL from total ileal AA flows yields standardized ileal digestibility (SID) coefficients that appear to be more additive than AID coefficients in mixtures of feed ingredients. An implicit assumption when using SID AA coefficients in diet formulation is that the post-absorptive utilization of AA is not influenced by the dietary protein source. This assumption appears inappropriate when using feed or food ingredients that have been over-heated, induce large amounts of EAAL, or contain substantial amounts of fermentable fibre. Improved understanding of processes that contribute to the discrepancy between bioavailability and ileal digestibility will allow a more effective use of AA digestibility coefficients in diet formulation.

**Key words:** amino acids; bioavailability; ileal digestibility; monogastrics

### Introduction

Estimates of the bioavailability of amino acids (AA) in foods and feeds are required to properly meet dietary requirements for indispensable AA and nitrogen that are largely independent of dietary protein source and to assess the nutritional value of diverse dietary protein sources. Given the complexities associated with determining AA bioavailability, measures of digestibility are widely used in diet evaluation and formulation. However, the simplest measure of AA digestibility, apparent faecal digestibility, is confounded with enteric fermentation and endogenous gut protein losses from the host, which occur throughout the gastrointestinal tract. Both enteric fermentation and endogenous gut protein losses should be considered when extrapolating estimates of AA bioavailability from digestibility coefficients.

In this contribution, support is provided for the use of ileal digestibility coefficients for the routine assessment of bioavailability of AA in foods and feeds. In addition, the scientific basis is presented for using standardized ileal digestibility

coefficients (i.e. apparent ileal digestibility coefficients that are corrected for basal endogenous gut AA losses) in the evaluation of diets for monogastrics. Finally, some of the limitations of using ileal digestibility coefficients in diet evaluation are discussed.

The experimental observations that are presented in this contribution are derived primarily from growing pigs. Pigs are a valuable research model for exploring concepts that are applicable to human nutrition, largely because of similarities in digestive physiology and our ability to apply a wide range of rather invasive surgical techniques to pigs<sup>(1)</sup>. The application of digestibility coefficients that have been obtained in pigs to human nutrition has been addressed elsewhere<sup>(2,3)</sup> and is addressed in other contributions to this special journal issue.

### Amino acid bioavailability and the digestibility assay

As outlined by Fuller<sup>(4)</sup>, the bioavailability of a dietary AA can be defined as the proportion of the AA intake that is absorbed

**Abbreviations:** AA, amino acid; AID, apparent ileal digestibility; AFD, apparent faecal digestibility; EAAL, endogenous amino acid losses; SID, standardized ileal digestibility; TID, true ileal digestibility.

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in a chemical form that can be utilized for metabolic processes such as protein synthesis<sup>(5)</sup>. Accurate estimates of the bioavailable AA content of feeds and foods allow for greater precision when matching AA intake with AA requirements. However, currently no direct measures of bioavailability are available. Measures of AA digestibility are used widely to estimate AA bioavailability in feed and food ingredients for monogastrics, with the terms digestibility and bioavailability sometimes being used interchangeably and incorrectly. Slope-ratio assays are still considered the benchmark for determining (relative) bioavailability of AA in feeds and foods. This method involves supplying graded dietary levels of an individual AA from a particular protein source and measuring a response variable, such as whole body protein retention or AA oxidation<sup>(1,5)</sup>. Generally, the response to the test ingredient is compared with the response to a reference protein in which the AA bioavailability is known or is assumed to be 100%, which yields relative estimates of bioavailability<sup>(5)</sup>. The slope-ratio assay is a time consuming, expensive, and laborious method that only provides a measure of bioavailability for one AA at a time. Moreover, estimates of bioavailability obtained with the slope-ratio assay may be confounded with endogenous AA losses, dietary nutrient imbalances, leading to incorrect estimates of bioavailability<sup>(6)</sup> and may lack precision and/or repeatability<sup>(7)</sup>. It should be noted that when absorbed AA are all available for metabolism, bioavailability estimates obtained with this assay more closely represent apparent digestibility than true digestibility, as it reflects the net uptake of AA from the digestive tract. To evaluate bioavailability, as defined earlier, measures of true ileal digestibility combined with chemical availability are required. In principle, the digestibility assay is simpler than *in vivo* assessments of AA bioavailability and allows for simultaneous estimation of the bioavailability of all AA in an experimental diet or an individual diet ingredient. However, measures of digestibility are based on AA disappearance from the digestive tract and do not reflect the form in which AA and AA metabolites are absorbed<sup>(5)</sup>.

### Apparent faecal digestibility coefficients

Apparent faecal, or total tract, digestibility (AFD) is the most basic *in vivo* method for estimating AA bioavailability and reflects disappearance of AA along the entire gastrointestinal tract. The faecal digestibility method has long been considered an inappropriate approach to determining AA bioavailability for monogastric animals, largely because of enteric fermentation in the hindgut<sup>(7)</sup>.

Undigested protein that enters the hindgut is susceptible to microbial degradation via deamination with carbon skeletons used as an energy source<sup>(8)</sup>. Studies have shown that protein, both of dietary and endogenous origin, is the major substrate for intestinal ammonia production<sup>(9–12)</sup>. Using an infusion of isotopic urea, it was determined that the contribution of urea hydrolysis to faecal ammonia in humans is less than 10% with the remainder derived from fermentation of undigested dietary and endogenous protein<sup>(9,10)</sup>. It has also been shown that an increase in protein flow into the hindgut results

in an increase in digesta ammonia concentration<sup>(12)</sup> providing further evidence that protein is the major substrate for gut ammonia production. Ammonia produced in the hindgut is readily absorbed by the animal, transported to the liver for ureagenesis, and excreted largely in urine. Therefore, the majority of nitrogen disappearance from the hindgut is likely due to fermentative losses and is of limited nutritional value to the animal.

Some studies, however, suggest the hindgut is not entirely incapable of AA absorption. Heine *et al.*<sup>(13)</sup> demonstrated that when labelled yeast was infused into the colon of human infants, the majority of absorbed label (i.e. not excreted in faeces) was retained in body protein (i.e. not excreted in urine). The authors used these observations to conclude that the colon is capable of absorption of intact AA that can be utilized for metabolic processes. However, the <sup>15</sup>N-protein infused in this study may have been absorbed as non-protein nitrogen (i.e. ammonia) and utilized for endogenous production of dispensable AA or utilized in the upper gut for indispensable AA synthesis. The presence of AA transporters, such as the ATBo+ and B<sup>o+</sup> transport systems, have been demonstrated in the colonic mucosa<sup>(14)</sup> indicating the capacity for AA absorption. In addition, uptake of AA through the colonic mucosa has been demonstrated<sup>(15,16)</sup>. However, it should be noted that measurement of AA uptake in these studies was done in isolated colonocytes grown in culture or on mounted sections of tissue and does not necessarily indicate what would occur *in vivo*. In addition, these studies were performed in newborn pigs in which it has been demonstrated that gut structure and function differs from older pigs. Indeed, it was shown that the ability of colonic tissue to transport AA was reduced in 4-day-old pigs and virtually eliminated in 10-day-old pigs<sup>(15)</sup>. This would suggest that the ability of the colon to absorb AA is lost quickly and would not be a significant factor in the growing pig and humans, except perhaps for young infants.

The inadequacy of using AFD coefficients to estimate AA bioavailability is further supported by a large number of studies in which protein or individual AA have been infused into the hindgut of pigs with little or no retention of the infused AA<sup>(17–22)</sup>. In several of these studies statistically insignificant improvements in whole body nitrogen retention were observed, suggesting that some of the experimental observations may not have been sufficiently sensitive to identify retention of AA that were infused into the hindgut<sup>(23)</sup>. However, in carefully controlled studies, and with AA that were limiting in the diet, infusion of AA into the hindgut of pigs did not improve whole body AA retention. For example, Darragh *et al.*<sup>(24)</sup> infused lysine or methionine into the proximal colon of young pigs fed either lysine or methionine deficient diets with the assumption that nitrogen balance would be improved if the dietary indispensable AA were absorbed intact thus correcting the diet deficiency. However, no improvement in nitrogen balance was observed; in fact nitrogen balance was somewhat reduced when compared to pigs receiving additional lysine or methionine orally. Wünsche *et al.*<sup>(22)</sup> performed a similar study examining lysine and isoleucine infusion into the colon of animals receiving lysine or

isoleucine deficient diets. Again it was found that while faecal digestibility was not affected by the infusion, nitrogen balance was decreased and urinary ammonia increased in these animals<sup>(22)</sup>. In another study, nitrogen digestibility and retention was compared in pigs in which a casein supplement was given orally or infused into the terminal ileum. Total tract nitrogen digestibility from the hindgut was decreased only slightly when casein was administered ileally, suggesting that nitrogen was being absorbed from the hindgut, but there was a significant reduction in nitrogen retention in these pigs [Zebrowska *et al.* (1978) as referenced in Buraczewski<sup>(25)</sup>], which indicates that nitrogen absorption from the hindgut was of no nutritional significance to the animal. In a review of studies in which casein was infused into the caecum of pigs, Fuller & Reeds<sup>(26)</sup> found that even though faecal digestibility was not different with casein infusion, indicating that nitrogen was being absorbed from the hindgut, there was little or no improvement in whole body nitrogen balance. This is consistent with the observation that nitrogen that is absorbed from the hindgut contributes primarily to urinary nitrogen excretion. Indeed, while it was shown that approximately 18% of total nitrogen absorption occurred in the colon of pigs<sup>(27,28)</sup>, the absorption of labelled nitrogen from the colon was entirely accounted for by label excretion in urine<sup>(28)</sup>.

The AA profile of faecal protein, and therefore AA digestibility, is extensively modified by enteric microbes, which can be attributed to both fermentative AA catabolism and *de novo* synthesis of microbial protein. As a result the AA profile of faecal protein bears little resemblance to the dietary AA profile and even to the AA present in ileal digesta. When comparing ileal and faecal digestibility values for lysine in different feed ingredients, Rademacher *et al.*<sup>(29)</sup> demonstrated that there can be significant differences between the two measures and that faecal digestibility is not always the greater value. This demonstrates the combined effects of protein fermentation and microbial synthesis of AA in the hindgut of pigs and their effects on estimates of AA digestibility. The faecal digestibility method is therefore considered inappropriate with respect to determining AA bioavailability.

Only in two scenarios can ammonia absorbed from the hindgut provide a benefit in supplying nitrogen to the host<sup>(30,31)</sup>. Firstly, when the amount of absorbed AA is insufficient to satisfy the host's demand for dispensable AA, ammonia may be used in transamination reactions yielding dispensable AA<sup>(30)</sup>. Secondly, urea that is generated from absorbed ammonia may be secreted, or recycled, into the gut and be used by microbes in the upper gut for the microbial production of indispensable and dispensable AA. Like dietary AA, microbially produced AA may be absorbed from the upper gut and can contribute to the supply of AA to the host. The latter has been shown to occur in pigs<sup>(32-34)</sup>, humans<sup>(35-38)</sup>, and rats<sup>(39)</sup>. It has been estimated that about 30% of non-protein nitrogen that is generated in the pig's body is recycled into the gut and, therefore that only 70% of the whole body non-protein nitrogen flux is excreted in urine<sup>(26)</sup>. It can thus be speculated that recycling of non-protein nitrogen is an important contributor to nitrogen

economy and impacts the host's utilization of indispensable AA and total nitrogen. It follows then that nitrogen disappearance from the hindgut due to protein fermentation, and thus AFD, may in some scenarios be important for meeting the total nitrogen needs of the animal. However, for both of these scenarios the efficiency of capturing ammonia that is generated in the hindgut, and therefore the net contribution of protein fermentation in the hindgut to the host's supply of dispensable and indispensable AA, remains to be quantified<sup>(26)</sup>.

### Apparent ileal digestibility coefficients

Amino acid disappearance from the gut proximal to the terminal ileum, reflected in apparent ileal digestibility (AID), is considered a better estimate of AA bioavailability than AFD<sup>(7,23)</sup>. The AID of AA largely represents enzymatic protein digestion and absorption of intact AA with some influence from microbial fermentation in the upper gut<sup>(40)</sup>. In order to determine AID coefficients digesta samples must be obtained from the distal ileum. The latter requires rather invasive sampling methods, involving the use of surgically modified subjects and digestibility markers. Practical aspects of sampling ileal digesta from animals and humans are addressed by Fuller<sup>(4)</sup>. Caution must be used when using protein or nitrogen digestibility as an indicator of AA digestibility since there can be considerable differences between the digestibility values for individual AA<sup>(41,42)</sup>.

In pigs several studies have shown that AID values provide more accurate estimates of AA bioavailability than AFD<sup>(29,41,43-45)</sup> and that AID is a more sensitive assay than AFD for identifying differences in nutritional value among protein sources<sup>(46-48)</sup>. For example, Low *et al.*<sup>(44)</sup> and Just *et al.*<sup>(45)</sup> showed that growth performance and body protein gain of pigs is more highly correlated with ileal rather than faecal protein digestibility. Just *et al.*<sup>(45)</sup> determined AID and AFD of AA and protein in 24 diets and found that nitrogen retention was slightly more correlated with ileal rather than faecal digestible daily crude protein intake (0.81 vs. 0.75). However, AID and AFD intake of AA and nitrogen are highly confounded across diets in this study and the dietary AA balances vary among the diets and, therefore, these findings should be interpreted with caution. In contrast, Dierick *et al.*<sup>(49)</sup> compared feed efficiency in 8 diets formulated to contain similar levels of faecal digestible protein, total dietary lysine, and ratios among key indispensable AA but to differ in ileal digestible nitrogen content. It was determined that there was a higher correlation between feed efficiency (0.87 vs. 0.65) or growth rate (0.76 vs. 0.34) and ileal nitrogen digestibility vs. faecal nitrogen digestibility. Moreover, it was shown that pig performance suffered as ileal digestibility of protein decreased<sup>(49)</sup>. Moughan & Smith<sup>(41)</sup> and Rademacher *et al.*<sup>(29)</sup> showed that AID values accurately predicted AA bioavailability. Rademacher *et al.*<sup>(29)</sup> formulated diets with decreasing amounts of AID lysine by replacing soyabean meal with canola meal, meat meal, alfalfa meal, beet pulp, and wheat bran in which the AID of lysine was previously determined. It was shown that as the amount of AID lysine

was decreased in the diets the growth performance of the pigs decreased confirming the validity of using AID values for estimating lysine bioavailability<sup>(29)</sup>. Likewise, Moughan & Smith<sup>(41)</sup> determined the AID of lysine in barley, meat and bone meal, fish meal, and pea meal and then compared the growth performance and carcass measures in pigs fed a diet based on all the protein sources to a casein based diet formulated to have the same level of AID lysine as the barley diet. Mough & Smith<sup>(41)</sup> found no difference between any of the measured responses between the two diets and concluded that the AID lysine in each feedstuff could be used to accurately formulate and predict the AID lysine content of a complete diet.

The benefit of using AID coefficients is also demonstrated when comparing the differences in protein quality among and within foods, feeds, and dietary protein sources. For example, Sauer *et al.*<sup>(47)</sup> showed that unlike AFD, AID coefficients differed among four sources of barley. In a study by Holmes *et al.*<sup>(50)</sup> comparing AID and AFD of nitrogen and AA between soyabean meal and rapeseed meal in pigs, it was found that while AID values for soyabean meal correlated well with AFD values, this was not the case for rapeseed meal. Moreover, the difference between nitrogen digestibilities at the faecal level for these two protein sources was much less than observed at the ileal level. On average, Austic<sup>(51)</sup> reported that AFD is 6.5 percentage units higher than AID and that this discrepancy becomes greater (ileal digestibility lower and/or faecal digestibility higher) for low digestibility protein sources. This is likely a combination of decreased digestibility and increased excretion of microbial protein when high-fibre feedstuffs are included in diets. Sauer *et al.*<sup>(47)</sup> showed that measures of ileal digestibility are more sensitive than faecal digestibility and can be used to identify the impact of diet processing on the nutritional value of protein sources.

It should be noted that in some instances AID coefficients overestimate AA bioavailability, especially for lysine in heat-processed protein sources, for threonine in diets containing large amounts of fermentable fibre, and when large endogenous gut protein losses are incurred. This will be addressed in further detail in a subsequent section of this review.

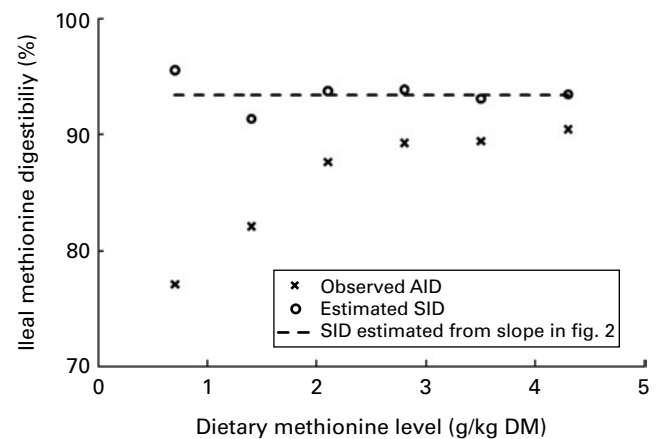
A critical issue with AID coefficients is the curvilinear increase in AID of AA with increasing dietary AA content, which occurs even when the dietary AA content is altered by varying the inclusion level of a particular protein source in the diet (Fig. 1)<sup>(52)</sup>. This variability in AID coefficients for individual protein sources raises concerns about the impact of protein levels used in experimental diets to establish AID coefficients and about the lack of additivity of AID coefficients among food or feed ingredients. By considering the impact of endogenous gut protein losses on AID coefficients these concerns can largely be overcome.

### Endogenous gut amino acid losses and ileal digestibility coefficients

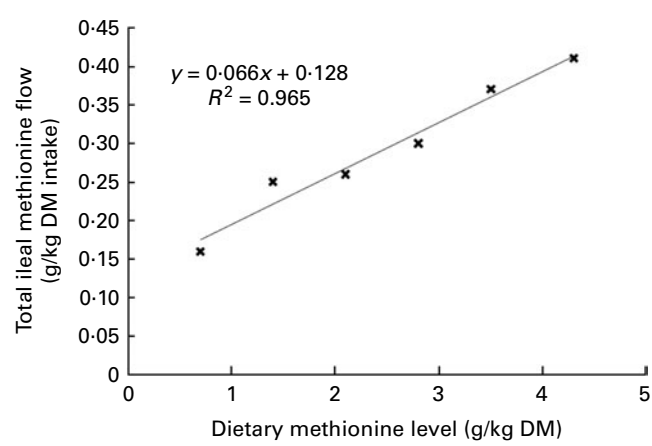
Measures of AID of AA are confounded with endogenous gut AA losses (**EAAL**). The EAAL represent AA and synthesized proteins that are secreted into the intestine and are not reabsorbed prior to the terminal ileum, and originate from saliva,

gastric secretions, bile, pancreatic juice, digestive enzymes, sloughed epithelial cells, and mucins<sup>(53–55)</sup>. The amount of protein from endogenous origin may exceed the amount of ingested dietary protein and is, just like dietary protein, partly digested and absorbed. Since endogenous secretions cannot be distinguished from dietary proteins in conventional digestibility studies, ileal digestibility coefficients that are not corrected for EAAL should be referred to as AID coefficients<sup>(5)</sup>.

The curvilinear increase in AID of AA when increasing the dietary AA content (Fig. 1) can largely be attributed to the relatively large contribution of EAAL to total ileal AA flows at low dietary AA levels<sup>(52)</sup>. This is further illustrated in Fig. 2, in which the AID coefficients for methionine that are provided in Fig. 1 are used to calculate total ileal methionine flow per unit of feed DM intake. Figure 2 shows a highly linear relationship between total ileal methionine flow and the dietary methionine level. Similar highly linear relationships between ileal AA flow and dietary AA level have been shown for other indispensable AA and in studies in which five or more dietary protein levels have been evaluated<sup>(52,56)</sup>. The impact of dietary AA level on AID of AA (Fig. 1) can thus be attributed fully to the intercept of the relationship between total ileal AA flow and dietary AA level (Fig. 2). Furthermore, the slope of the linear relationship between total ileal AA flow and dietary AA level represents an important measure of digestibility. Historically, it was assumed that the increase in total ileal AA flow with increasing dietary AA level could be attributed entirely to an increased flow of undigested dietary AA. Hence this slope was used to derive estimates of 'true' ileal AA digestibility. However, by using more advanced techniques, which allow for separate identification of endogenous and dietary AA in ileal digesta, it has been shown that increases in total ileal AA flows can generally be attributed to increase in flow of both EAAL and dietary AA<sup>(54)</sup>. Therefore, at least two components of EAAL can be identified: (1) the basal EAAL, represented by the intercept in Fig. 2, and



**Fig. 1.** Impact of dietary methionine level on observed apparent ileal digestibility (AID) and estimated standardized ileal digestibility (SID) of methionine in growing pigs [adjusted from Fan *et al.*<sup>(52)</sup>; variable dietary methionine levels are the result of varying the level of soyabean meal across diets; SID coefficients were established by subtracting basal endogenous ileal methionine loss from total ileal methionine flows for each measurement of AID of methionine (Fig. 2)].



**Fig. 2.** Impact of dietary methionine level on total ileal flow of methionine in growing pigs [adjusted from *Fan et al.*<sup>(52)</sup>; variable dietary methionine levels are the result of varying the level of soyabean meal across diets; the basal endogenous ileal methionine loss (0.1289 g/kg DM intake) was estimated based on linear regression analyses and extrapolation to feeding a methionine-free diet; the slope of the line of best fit (0.066) yields an estimate of SID of methionine, which is equivalent to the horizontal line representing estimated SID of methionine in Fig. 1: SID = 100 × (1 – slope)].

(2) ingredient specific EAAL. Basal EAAL may be related to DM intake, which is the approach that has been adopted widely in animal nutrition<sup>(57)</sup>, or to the host's metabolic state (e.g. some measure of metabolic body weight), including the host's (gut) health status. The latter may be more appropriate for mature individuals or when levels of food intake are low. This is the case in humans where food intake can be considered low relative to the levels of feed intake observed in highly productive farm animals. The specific EAAL are induced when increasing amounts of different protein sources, fibre, or other EAAL inducing compounds (e.g. anti-nutritional factors such as tannins, lectins, trypsin inhibitors) are included in the diet<sup>(54,58)</sup>.

To differentiate between ileal digestibility coefficients that are corrected for total EAAL (true ileal digestibility; **TID**; sometimes also referred to as real ileal AA digestibility) and ileal digestibility coefficients that are corrected for basal EAAL only, a new term was introduced: standardized ileal AA digestibility (**SID**)<sup>(5,54)</sup>. The concept of SID coefficients has been adopted around the world for the formulation of pig diets and extensive databases summarizing SID coefficients for AA in a wide range of feed ingredients have been published<sup>(59–61)</sup>. The main advantages of using SID coefficients in feed formulation is that they are independent of dietary AA level, reduce variability in measures of ileal digestibility within food and feed ingredients, are more additive than AID coefficients in mixtures of feed or food ingredients, and can be derived experimentally without too much difficulty by correcting AID for basal EAAL<sup>(5,54,62)</sup>. Many studies have been conducted to quantify basal EAAL in pigs. In a review of the literature, *Jansman et al.*<sup>(57)</sup>, suggested that the mean estimate of basal endogenous ileal lysine losses observed in studies where pigs were fed protein-free diets (0.36 g per kg DM intake; SD 0.10; 16 studies) was similar to estimates obtained based on regression analyses, as outlined in Fig. 2 (0.38; SD 0.09; 3 studies) and only slightly lower than

estimates obtained from pigs fed purified proteins with an assumed TID of 100% (0.44; SD 0.17; 11 studies). Relative differences were similar for other indispensable AA. It should be noted that estimates of basal EAAL vary between laboratories, even when using identical methodology, and that it is appropriate to obtain local estimates of EAAL when determining AID coefficients for calculating TID coefficients for AA<sup>(5,63)</sup>. Until a methodology is in place to routinely measure total EAAL, both basal and diet specific, and these losses have been quantified for a wide range of feed ingredients, SID coefficients will provide the basis for estimated bioavailability of AA in feeds and foods.

When advanced methods have been used to estimate total EAAL, and thus specific EAAL, it has been shown that the specific EAAL varies among protein sources and feed ingredients. For example, when *de Lange et al.*<sup>(64)</sup> used an <sup>15</sup>N isotope dilution technique, they estimated total ileal endogenous lysine losses to be 1.02, 1.22, 1.10 and 1.11 g per kg feed DM intake (SE 0.10) in growing pigs fed diets that contained soyabean meal, canola meal, wheat, or barley as the only protein source, respectively. These values were all higher than values obtained in pigs that were managed under similar conditions and that were fed either a protein-free diet (0.63) or protein-free diets with a simultaneous intravenous infusion of a mixture of indispensable and dispensable AA (0.56 g per kg DM intake; SE 0.07)<sup>(65)</sup>. These observations also illustrate that endogenous gut AA are a major contributor to dietary AA requirements and that care should be taken in how EAAL are considered when estimating AA requirements. When diets are formulated based on SID coefficients, the specific EAAL are already accounted for in the nutritional value of the diet and only the basal EAAL, not the specific EAAL, should be considered explicitly when characterizing host or animal factors that contribute to AA requirements. When foods or feeds are evaluated based on TID coefficients, it is critical that both the basal and specific EAAL are considered as part of the animal or host factors contributing to AA requirements.

### Refinements to ileal digestibility coefficients for estimating amino acid bioavailability

As mentioned above, in some instances SID, or AID, coefficients do not yield proper estimates of AA bioavailability and the confounding of EAAL and true digestible AA intake should be considered carefully when interpreting estimates of AA bioavailability, especially when these are obtained with the slope-ratio assay. As we move towards a better understanding of the discrepancy between ileal digestibility and bioavailability, refinements may be made to SID coefficients to routinely estimate AA bioavailability in feeds and foods and to better predict the host's response to varying AA intake from different protein sources. Here three specific examples of such refinements are provided.

#### Lysine bioavailability in heat treated foods and feeds

In a comparison of diets formulated to have the same level of AID lysine from expeller-extracted oil seed meal from either

cottonseed or soyabeans, Prawirodigdo *et al.*<sup>(66)</sup> found that nitrogen retention in grower pigs was lower with the cottonseed meal-based diet (17.3 vs. 23.3 g/d). In another study and when field peas were fed raw or heated to varying temperatures between 110 and 165°C it was demonstrated that heating had little impact on AID of lysine (e.g. 92% for raw peas vs. 84% for peas heated to 165°C), while relative lysine bioavailability as determined in a slope-ratio assay was gradually reduced from 96% in raw peas to 18% for peas heated to 165°C<sup>(67)</sup>. In general, with heat-processed protein sources, the AID coefficients may overestimate lysine bioavailability<sup>(68)</sup>. However by integrating the chemical form of lysine in feed ingredients, based on the reactive lysine or equivalent assays, with AID or SID coefficients the impact of heat-processing on lysine bioavailability can be accommodated<sup>(69,70)</sup>. This concept may also apply to other AA and is addressed in more detail by Rutherford<sup>(71)</sup>.

#### *Impact of soluble fibre intake on threonine utilization and enteric fermentation*

The inclusion of fibre in the diet can result in decreases in both apparent ileal and apparent faecal AA digestibility<sup>(72,73)</sup>. It is thought that this reduction in digestibility is due to a number of factors including increased EAAL<sup>(74)</sup>, impairment of nutrient absorption<sup>(72)</sup>, and an increase in nitrogen incorporation into microbial protein and thus increased microbial protein flow at the distal ileum or in faeces<sup>(75)</sup>. In addition, fibre can impact aspects of AA utilization that are not reflected in measures of digestibility. Libao-Mercado *et al.*<sup>(76)</sup> found that the efficiency of utilization of SID threonine intake for whole body protein deposition was reduced when pigs were fed diets in which casein had been replaced by wheat shorts, a co-product from the wheat starch industry and with properties that are similar to those of wheat bran. It was suggested that this was due to the high fibre content of wheat shorts. This was confirmed in a follow-up study in which graded levels of pectin, a highly soluble and fermentable source of fibre, were fed to pigs. In this study it was shown that there was an increase in the fermentative loss of threonine in pigs fed increasing dietary pectin levels, reflected in an increase in urinary excretion of nitrogen<sup>(77)</sup>. In that study there was also an increase in faecal excretion of total nitrogen and microbial protein, which was associated with an increase in mucin and colonic mucosal protein synthesis<sup>(78)</sup> and, by extrapolation, increased endogenous gut protein losses. The latter is supported by Hedemann *et al.*<sup>(79)</sup> who found that the intestinal mucous layer was thicker in the colon of rats fed high-fibre diets. The high threonine content of mucins combined with the susceptibility of mucins to microbial degradation<sup>(80)</sup> would explain the increase in threonine catabolism and the decrease in utilization efficiency of threonine.

A decrease in threonine utilization efficiency was not observed when purified cellulose, an insoluble and poorly fermentable source of fibre, was included in threonine limiting diets<sup>(77)</sup>, highlighting that the impact of dietary fibre on AA utilization is mediated primarily by its impact on enteric fermentation, likely in both the small intestine and hindgut.

A meaningful impact of additional pectin intake on utilization of other amino acids (lysine, methionine, tryptophan) for body protein deposition could not be detected<sup>(77,81,82)</sup>, which is consistent with the large contribution of threonine to EAAL, the use of endogenous gut protein as the substrate for enteric fermentation, and the stimulating effect of some fibre sources on enteric fermentation. Energy availability, and not nitrogen, from fermentable substrates has been shown to be a limiting factor for microbial protein production<sup>(83)</sup> and therefore the increase in available energy from fibre may result in an increase in microbial population and activity<sup>(84)</sup>.

These observations also highlight the potential impact of enteric fermentation in the upper gut of monogastrics on AA utilization. As described by Fuller<sup>(4)</sup>, enteric fermentation occurs in the upper gut of monogastrics and contributes to *de novo* microbial AA synthesis<sup>(32,33)</sup> as well as (fermentative) catabolism of AA<sup>(11)</sup>. Libao-Mercado *et al.*<sup>(11)</sup> determined that greater than 70% of ammonia production at the terminal ileum was due to fermentation of dietary and endogenous protein and not due to urea hydrolysis. Based on measured ammonia flow at the ileum it was estimated that 2.1 g of protein per kg DM intake is catabolized by gut microbes<sup>(85)</sup>. Libao-Mercado<sup>(85)</sup> stated that this was likely an underestimation of ammonia generation due to the absorption and utilization of ammonia prior to the ileum. In his review, Wu<sup>(86)</sup> suggested that the reported 30% catabolism of the AA histidine, lysine, methionine, phenylalanine, threonine, and tryptophan by enterocytes *in vivo* and the lack of *in vitro* catabolism and activity of AA degrading enzymes for these AA in enterocytes suggests that the catabolism of AA is due to fermentation by intestinal microbes. The extent of fermentation will also depend on the individual AA, with lysine and arginine being rapidly fermented and methionine, cysteine, and branched-chain AA, being slowly or incompletely fermented<sup>(87)</sup> resulting in overestimates for bioavailability of some AA and not others. Clearly, AA losses in the upper gut due to microbial fermentation may be extensive. Further investigation is required to quantify the impact of enteric fermentation in the upper gut on AID and SID of AA and on the net supply of AA to the host.

#### *Metabolic inefficiencies associated with endogenous gut protein losses*

As mentioned earlier, EAAL contribute to dietary AA requirements and care should be taken in how to account for basal and specific EAAL when evaluating the bioavailable AA content of foods and feeds. In addition to EAAL themselves, the metabolic costs associated with EAAL should be considered, simply because there is a metabolic inefficiency associated with synthesis and recycling (i.e. secretion and re-absorption) of endogenous gut proteins. The latter becomes even more relevant when one realizes that EAAL represent roughly only 25% of endogenous protein that is secreted into the gut and that the remainder is recycled<sup>(54)</sup>. The metabolic inefficiency can be attributed largely to AA catabolising enzymes that are present primarily in the liver and some of the other digestive organs<sup>(86)</sup>, to which AA are

exposed during absorption and passage through the liver. Fermentative AA catabolism by enteric microbes can contribute to this metabolic inefficiency as well.

Until recently, very few well controlled studies have been conducted to quantify this metabolic inefficiency<sup>(88)</sup>. Lahaye *et al.*<sup>(89)</sup> fed diets that induced varying endogenous ileal nitrogen losses to growing pigs, caused by differential processing, and established a relationship between total endogenous ileal nitrogen losses, measured based on isotope dilution, and whole body nitrogen retention. These researchers concluded that the metabolic costs associated with an increase in endogenous ileal nitrogen losses were in fact larger than the actual increase in endogenous ileal nitrogen losses themselves. Even when corrections were made for treatment effects on SID nitrogen intake, reductions in whole body nitrogen retention (12.6%) appeared larger than increases in endogenous gut nitrogen losses (4.0%). These observations suggest that metabolic costs associated with EAAL can be substantial and provide an important incentive to accurately quantify EAAL and its associated metabolic costs.

### Conclusions and implications

There is a large body of evidence to suggest that in many instances measures of ileal amino acid (AA) digestibility yield reasonable estimates of bioavailability of AA in feeds and foods. A key limitation of using apparent ileal digestibility (AID) coefficients for diet evaluation and estimating AA requirements is that they are confounded with dietary AA levels. The latter can be attributed to the varying contribution of endogenous gut AA losses (EAAL) to total ileal AA flows. The EAAL can be separated into two main components, basal and ingredient specific EAAL. When correcting AID coefficients for basal EAAL only, standardized ileal digestibility (SID) coefficients are derived that are independent of diet AA level, less variable for a particular dietary ingredient, and more additive in mixtures in feed or food ingredients. The concept of SID is used extensively when formulating diets for monogastric farm animals. When using SID coefficients in diet evaluation, only the basal EAAL should be considered part of the animal or host effects on AA requirements. In some instances SID (as well as AID and TID) coefficients will provide inaccurate estimates of AA bioavailability, in particular bioavailability of lysine in heat treated ingredients, bioavailability of threonine in diets that contain large amounts of fermentable fibre, and that of AA in diets that induce large amounts of specific EAAL. The concept of SID coefficients may be refined for specific feeds and foods to account for instances where SID coefficients do not yield proper estimates of AA bioavailability.

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