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Feed-induced specific ileal endogenous amino acid losses: Measurement and significance in the protein nutrition of monogastric animals

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ABSTRACT

The endogenous ileal amino acids recovered at the terminal ileum of monogastric animals comprises of two components, namely basal and specific losses. The basal losses are fixed and associated with feed dry matter intake, whereas the specific losses are variable and induced by the presence of dietary components, such as fibre and anti-nutrients. Currently there is no methodology available to directly measure the specific endogenous losses. These losses are calculated by determining the basal and total (basal plus specific) losses and then subtracting the basal losses from total losses. Two techniques, namely the guanidination and isotope markers, can be used for the determination of total endogenous amino acid losses. The fundamental features, specific applications and shortcomings of these two methodologies are discussed. Although the specific endogenous protein losses are significant metabolic costs to the animal, published data quantifying these losses are limited owing to the complexity of available methodologies.

1. Introduction

The principal function of the gastrointestinal tract is to breakdown dietary macromolecules into simple micromolecular moieties for absorption. During this process, there is a continuous secretion of endogenous proteins, in the form of digestive enzymes, mucus and desquamated enterocytes, into the lumen of the intestine. This input of endogenous proteins is significant and some estimates indicate that ingested protein was swamped four- or five-fold by endogenous protein (Nasset and Ju, 1961). These endogenous sources mix with dietary protein and are digested, and the resulting amino acids are absorbed. The magnitude of reabsorption of endogenous proteins up to the ileal level is not known with certainty. Souffrant et al. (1993) estimated that 79% of the gross endogenous secretion in pigs is reabsorbed, but the degree of re-absorption will vary depending on the relative ratio of individual endogenous components and their point of entry into the gut, being high with digestive enzymes and lower with mucin. These losses represent the net balance between protein ingested plus endogenous proteins and absorbed dietary plus reabsorbed endogenous protein (Moughan, 2003). The unabsorbed endogenous portion is lost to the animal and referred to as inevitable losses. It is also recognised now that the endogenous amino acid losses must be measured at the ileal level, rather than in the excreta, because of the variable and modifying effects of the hindgut on protein nutrition.

Abbreviations: DMI, dry matter intake; EAA, endogenous amino acid; HA, homoarginine.

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Table 1
Methods used for the determination of endogenous amino acid losses[a].

Basal losses
• Fasting of birds for up to 48 h[b]
• Feeding of a protein-free diet
• Protein-free diet supplemented with synthetic amino acids
• Protein-free diets with intravenous amino acid infusion
• Natural proteins devoid of specific amino acids
• Enzyme hydrolysed casein and ultrafiltration
• Feeding of low levels of highly digestible protein, *e.g.* wheat gluten, soy protein isolate, casein[c]
• Linear regression, following feeding of diets containing graded levels of specific ingredient
Total losses
• Guanidinated dietary protein
• Isotope-labelled markers

[a] After Moughan et al. (1998).
[b] Used only in poultry to measure losses in the excreta.
[c] These proteins are assumed to be 100 % digestible and that excreted amino acids therefore represent basal losses.

Accurate quantification of endogenous amino acid (EAA) losses is important for several reasons. First, the correction for these inevitable losses is required for the calculation of true amino acid digestibility of feed ingredients. Second, it is necessary for the determination of protein and amino acid requirements by the factorial method. Third, these losses have significant implications in terms of the metabolic cost associated with protein synthesis and turnover in the gut. Finally, the measurement of these losses and better understanding of the factors that influence these losses provide an effective strategy to improve the efficiency of protein and amino acid utilisation.

It is recognised that EAA losses are influenced primarily by dry matter intake and secondarily by the inherent composition of the feed ingredient or diet. These two fractions are referred to as basal (also known as non-specific or diet-independent) and specific (also known as diet-dependent) EAA losses, respectively (Boisen and Moughan, 1996). Basal endogenous losses can be defined as those inevitable losses closely associated with the metabolic functions of the animal and are independent of the diet type. These losses, therefore, represent the minimum losses that can be expected under any feeding situation.

Specific losses, on the other hand, are feed-induced resulting from the presence of specific dietary components, such as sources and amounts of fibre and various anti-nutritional factors (*e.g.* phytate, trypsin inhibitor, lectins *etc.*). Currently there is no methodology available to directly measure the specific EAA losses. These losses, however, can be calculated by determining the total (basal plus specific) losses and then subtracting the basal losses from total losses.

In recent decades, the measurement of EAA losses in mammalian and avian species has received much attention and several exhaustive reviews are available on this subject (Sibbald, 1987; Boisen and Moughan, 1996; Nyachoti et al., 1997; Moughan et al., 1998; Ravindran and Bryden, 1999; Adedokun et al., 2011), which provide background information for the present paper. While the measurement of basal EAA losses is examined in detail in these reviews, discussion focusing on specific EAA losses has not been extensive due largely to the limited published data. Two approaches, namely the guanidination and isotope markers, can be employed for the determination of total EAA losses. The present paper provides an overview of these two methodologies and also of factors influencing specific EAA losses, with select examples of the effects of anti-nutrients on total EAA losses.

2. Basal endogenous amino acid losses

The measurement and relevance of basal EAA losses in protein nutrition and research are dealt elsewhere in this volume (Adeola et al., 2016). However, because the basal EAA estimates are needed to calculate specific losses, a brief discussion of the measurement of basal losses is included below. It is well recognised that endogenous protein output estimates are highly variable and are influenced by a number of animal and dietary factors (Nyachoti et al., 1997; Adedokun et al., 2011; Boisen and Moughan, 1996; Moughan et al., 1998). The important animal factors include species, protein status, age, body weight and health status.

The methodology used also has a marked influence on the measurement of basal EAA flow (Table 1). In poultry, values ranging from 6.3 g/kg dry matter intake (DMI) for the protein-free diet to 16.4 g/kg DMI for the regression method have been reported (Lemme et al., 2004).In pigs, the reported range varies from 7.0 g/kg DMI for the protein-free diet to 18.8 g/kg DMI for the regression method (Boisen and Moughan, 1996). The reliability of available methods, under a given set of dietary circumstances, had been a major constraint in the measurement of basal EAA losses and The perfect technique for measuring EAA losses is yet to be developed (Ravindran and Bryden, 1999).

A challenge for the nutrition researchers is, therefore, which method must be used to determine the basal EAA loss estimates to be considered for the calculation of specific losses. The question is whether the estimate from protein-free DMI be used as has been suggested by Stein et al. (2007) or that from protein-containing DMI as argued by some groups (Lemme

et al., 2004; Moughan et al., 2014), because diets always contain protein and the methods used for the determination of basal losses should use diets that contain protein. Protein is not an anti-nutrient and, by definition, it does not induce specific losses. If this argument is accepted, then estimates determined following the feeding enzyme-hydrolysed proteins and low levels of highly digestible proteins should be considered as representing basal EAA loss values.

3. Measurement of total endogenous protein losses

3.1. Techniques involving isotope markers

3.1.1. Infusion of isotopes

During the 80's and 90's, considerable research was undertaken to measure the total EAA using either stable (^{15}N) or radioactive (^{14}C, ^{35}S, ^{75}Se) isotopes. The ^{15}N isotope dilution technique of Souffrant et al. (1982) has been particularly popular and employed by number of researchers (de Lange et al., 1990; Roos et al., 1994; Schulze, 1994) to differentiate endogenous proteins from non-digested dietary proteins in the ileal digesta of pigs. The method involves the infusion of ^{15}N-labelled leucine into the blood of pigs consuming the test protein. The labelled amino acid is incorporated in the endogenous secretions, and the dilution of ^{15}N measured in the digesta gives an estimate of the amount of endogenous nitrogen. The data from isotope dilution demonstrated that the recovery of endogenous proteins in the ileal digesta was higher than those determined by feeding a protein-free diet.

Intravenous infusion of ^{14}C-lysine or ^{14}C-phenylalanine has also been used in pigs (Simon et al., 1983; Zebrowska et al., 1986; Nyachoti et al., 2000). The amounts of radioactive labelled amino acids are detected in the digesta and the flux rate of amino acids entering the intestine is quantified. Simon et al. (1983) suggested that the dilution of in-flowing labelled amino acids may change by mixing with unlabelled amino acids derived from intracellular protein degradation, thus affecting the specific radioactivity of amino acids.

Other isotopes used for measuring EAA secretions include ^{75}Se and ^{35}S. Ochao-Solano and Gitler (1968) simultaneously fed trace amounts of ^{35}S methionine and ^{75}Se selenomethionine labelled ovalbumin to rats and differentiated exogenous and endogenous proteins in the gastrointestinal tract. There was evidence for appreciable quantities of endogenous protein secretion and the amounts substantially exceeded those of exogenous origin in the intestine.

Though attractive, this technique suffers from several constraints, because the ^{15}N enrichment of the endogenous secretions is not easy to determine. The inability to measure the recovery of all individual amino acids in ileal digesta (de Lange et al., 1990; Lien et al., 1994) and the rapid precursor pool recycling (Souffrant et al., 1982; Leterme et al., 1996) are other drawbacks. These limitations have been elegantly discussed by Leterme et al. (1996); Moughan et al. (1998). In particular, standardisation of conditions such as feeding frequency, diet type, infusion protocol, rate of tracer infusion, sampling procedures, sample preparation and choice of precursor pool(s) is imperative if reliable comparisons of data from different laboratories are to be made (Gabert et al., 1997).

3.1.2. Labelling feed proteins with isotopes and feeding such labelled proteins

The availability of ^{15}N-labelled fertilisers has enabled the labelling of some protein sources (for example, peas and wheat) and feeding of diets containing such proteins to measure EAA losses. An aspect that favours the use of labelled dietary proteins is its simplicity; the dietary and endogenous protein and amino acids can easily be identified, separated and quantified (Moughan et al., 1998). Unfortunately, rapid recycling of the label is now recognised to be a major limitation of this approach (Leterme et al., 1996). Dietary amino acids supplied by the 15 N label is quickly absorbed and incorporated into body proteins, complicating the differentiation of unabsorbed dietary and endogenous proteins (Tamminga et al., 1995).

3.2. Homoarginine technique

A novel approach, using homoarginine (HA) as a marker, to determine EAA losses was proposed by Hagemeister and Erbersdobler (1985). In this method, ε-amino group of lysine in dietary proteins are converted into HA by the guanidination reaction, which involves treatment with O-methylisourea under alkaline conditions. After the labelled protein is fed, endogenous losses of amino acids are determined by comparing amino acid: HA ratios in the diet and ileal digesta. Homoarginine is not found in normal feedstuffs. However, it is digested and absorbed in a manner similar to other amino acids (Siriwan et al., 1994), but does not reappear in endogenous secretions into the gut and this unique feature of HA enable the calculation of total EAA losses.

The HA method is based on a number of premises, most of which have been shown to be tenable, and has been previously discussed at length (Moughan et al., 1998; Ravindran and Bryden, 1999). A brief discussion of these assumptions is presented below:

1. Homoarginine must not be recycled into the gut: An assumption implicit in the use of this technique is that the absorbed HA is not incorporated into tissue proteins and therefore is not re-cycled into the small intestine. This has been confirmed in studies with broiler chickens, where HA was not detected in intestinal contents after the intravenous infusion of HA for 3 h (Angkanaporn et al., 1997).

2. The dietary protein must be homogenously labelled with HA: An essential criterion for the application of HA technique is that the transformation of lysine to HA in guanidinated proteins occurs randomly. This assumption has been validated by sequential proteolysis *in vitro* of guanidinated materials where the ratios of HA to other amino acids were found to remain unchanged for casein and soybean protein, though somewhat aberrant values were noted for some amino acids in cereals and other protein supplements (Siriwan et al., 1994).
3. Homoarginine must behave within the digestive tract like other amino acids: Homoarginine incorporated into dietary proteins is released during digestion and then absorbed at rates similar to those of other amino acids. Studies have shown that guanidination has only minor effects on the structure of protein and, in particular, on its susceptibility to proteolysis. It has also been shown that guanidination does not cause significant modification in the concentrations of any of the acid-stable amino acids, except lysine, when O-methylisourea is used as the guanidinating agent. In most feed proteins, the recoveries of amino acids were close to 100%. Ravindran et al. (1998) reported that the guanidination has no influence on the *in vivo* digestibility of amino acids (except lysine) for broiler chickens, confirming that that protein racemisation is not an issue.
4. Homoarginine *per se* must not influence endogenous amino acid losses: While direct evidence of absence of HA influence on endogenous protein losses has not been obtained, the fact that HA behaves in the small intestine like a typical amino acid suggests that HA *per se* has no effect on endogenous protein output.
5. Homoarginine must not be preferentially metabolised by gut microflora: The conversion of HA to lysine and urea within the digestive tract, catalysed by microbial arginase, must be negligible. As shown by Siriwan et al. (1994), HA is no more susceptible to microbial action than other amino acid.
6. Homoarginine must be easily and accurately determined: Homoarginine can be determined by ion-exchange chromatography as a typical amino acid during routine amino acid analysis without the need for specific hydrolysis or separation procedures, an important analytical advantage compared to isotope labelling.

Guanidination has been investigated with a diverse group of feedstuffs, with widely varying lysine concentrations. The degree of conversion of lysine to HA varies greatly depending on the protein source (Table 4). The important factors affecting the degree of conversion are the pH of the reaction mixture, the duration and temperature of incubation and the ratio of lysine and O-methylisourea in the reaction mixture. The optimal guanidination conditions vary depending on the protein source. For example, optimum pH can vary from 9.3 to 12.0 for different proteins (Maga, 1981) and within the same protein – 10.3–10.8 for soy protein isolate and 11.5 for soybean meal. Further research is needed to identify feedstuff-specific conditions to optimise the degree of HA conversion.

Based on the degree of guanidination, endogenous protein losses are determined in two ways:

3.2.1. Direct method (complete guanidination)

Purified forms of protein can be completely guanidinated. Since all dietary lysine is converted to HA, any lysine detected in the digesta must be of endogenous origin.

3.2.2. Indirect method (partial guanidination)

In native protein sources, guanidination is only partial; thus both lysine and HA will be present in the ileal digesta and endogenous lysine is calculated based on relative absorption rates. *i.e.* by comparing amino acid: HA ratios in the diet and ileal digesta. Any deviation from diet amino acid: HA ratio in the ileal digesta is used as an estimate of the total endogenous loss of each amino acid. The endogenous lysine is estimated by the difference between true and apparent coefficients of HA and lysine absorption, respectively.

The special attribute of the HA method is that it can be used to determine the EAA secretions in animals given protein sources which contain fibre, anti-nutritional factors or both. This approach has been employed to measure EAA losses in poultry (Angkanaporn et al., 1994; Siriwan et al., 1994; Angkanaporn et al, 1996; Ravindran et al., 2004) and pigs (Barth et al., 1993; Caine et al., 2008). All studies confirm that ileal EAA losses determined by HA method is substantially higher than those determined by the protein-free diet. For example. Siriwan et al. (1994); Ravindran et al. (2004) reported that values for EAA losses in broilers obtained by the use of guanidinated casein were 2–3 times greater than those estimated either by feeding a nitrogen-free diet or by extrapolation to zero nitrogen intake. In studies with pigs, the values obtained by HA method have been reported to be of similar magnitude to those measured using the ^{15}N-dilution technique (Roos et al., 1994).

3.3. Summary

Overall, these two techniques are attractive to determine total endogenous flows in animals that are in a physiologically normal state, following the feeding of wide range of protein sources. Both, however, suffer from practical limitations. Both are tedious, time-consuming and costly. Isotope dilution techniques, in particular, require highly specialised personnel, equipment and facilities. However, estimates from the isotope marker approach provide direct information only of the endogenous flow of one amino acid – leucine in most cases of isotopes; the flow of other amino acids are then calculated assuming a constant composition of endogenous protein, but this assumption may not be valid for specific losses since different anti-nutrients and fibre sources have different effects on the individual components of endogenous protein. On the

Table 2
Amino acid composition of basal ileal endogenous protein (g/100 g crude protein) in different classes of poultry, in comparison to those reported in pigs (all measured using the enzyme hydrolysed casein method).

Amino acid	Broilers[a]	Layers[a]	Roosters[a]	Growing pigs[b]
Aspartic acid	7.2	7.4	7.9	7.7
Threonine	6.3	7.5	7.0	5.1
Serine	6.0	6.2	8.3	6.7
Glutamic acid	13.7	10.5	15.1	16.5
Proline	7.8	6.4	6.6	11.8
Glycine	5.2	5.8	6.2	7.8
Alanine	3.4	5.2	3.1	3.7
Valine	4.6	4.9	5.5	4.0
Isoleucine	3.4	3.3	4.7	3.4
Leucine	4.7	4.9	4.7	4.1
Tyrosine	2.6	2.7	2.3	2.2
Phenylalanine	3.4	3.4	2.9	2.4
Histidine	2.3	2.4	2.2	2.0
Lysine	3.0	3.2	3.6	3.0
Arginine	3.0	3.0	2.5	2.7
Methionine	1.3	1.2	1.1	–
Cysteine	2.0	2.3	2.1	–

[a] Ravindran and Hendriks (2004).
[b] Jansman et al. (2002).

other hand, in the case of guanidination method, the endogenous flow of all amino acids can be estimated; the deviation of the ratio of amino acid to HA in the ileal digesta from that in the diet for each amino acid is used to generate endogenous flow for each amino acid.

4. Sources of endogenous secretions

Endogenous proteins predominantly originate from various digestive secretions (saliva, bile, pancreatic secretions, gastric secretions and intestinal secretions), mucoproteins and desquamated intestinal epithelial cells (Zebrowksa et al., 1983). As noted earlier, endogenous protein flow determined at the terminal ileum is the net result of the overall dynamics of endogenous protein sources along the digestive tract and represents the algebraic difference between that secreted and reabsorbed. The source of endogenous proteins and the entry point into the digestive tract appears to be critical in determining this dynamics. When digestive secretions predominate the endogenous flow, the proteins pass through the duodenum and jejunum where there is more opportunity for digestion and absorption. In contrast, if mucus secretion or desquamation is significant, particularly if they occur distal from the jejunum, then the opportunity for digestion is lower and relatively higher endogenous losses in the form of these sources will be inevitable at the ileal level. However, owing to this complicated nature of entry points along the digestive tract, it is difficult to assess the true contribution of each endogenous protein source.

Studies with pigs have shown that the amino acid composition of ileal endogenous protein is generally constant, independent of diet and method of determination, but subject to individual variation (Boisen and Moughan, 1996). The amino acid composition of endogenous proteins measured at the terminal ileum of various classes of chickens, in comparison with pigs, is shown in Table 2. Interestingly class of chicken has little influence on the EAA profile and, the profiles of chickens and pigs were similar. As can be seen, the most abundant amino acids in the ileal endogenous protein of both species were glutamic acid, aspartic acid, threonine, proline, serine and glycine. These amino acids are found in high concentrations in intestinal and pancreatic secretions, and mucoproteins (Table 3), confirming that these are major components of the endogenous protein. Glycine, the abundant amino acid in biliary secretions in pigs, escapes reabsorption as deconjugated glycine. Threonine, proline, serine and glutamic acid predominate intestinal mucus glycoprotein (Lien et al., 2001), indicating high presence in ileal protein flow of mucins, which are thought to be resistant to enzymatic hydrolysis (Montagne et al., 2004).

Evidence suggests that, depending on the amount and type of fibre and anti-nutrient, the effect on components of endogenous secretion may be different. For example, the effect of trypsin inhibitor appears to be largely on pancreatic secretions, (Schneeman et al., 1997), whereas that of insoluble fibre may be mainly on mucin and of soluble, viscous fibre may possibly have a major effect of bacterial component (Nyachoti et al., 1997). The latter component though not generally considered as endogenous, it is not dietary either.

5. Regression method

The regression method has been promoted in some studies for the direct measurement of true amino acid digestibility of feed ingredients (Furuja and Kaji, 1989; Fan et al., 1995; Short et al., 1999; Rodehutscord et al., 2004). It has also been suggested that the total (basal plus specific) ileal EAA losses can be determined under normal protein alimentation conditions using this method (Furuja and Kaji, 1989). In this method, diets containing graded levels of AA from an ingredient are fed

Table 3
Amino acid composition (g/100 g amino acids) of various endogenous secretions.

Amino acid	Pancreatic secretions[a]	Bile, pigs[b]	Bile, broilers[c]	Mucin[d]
Aspartic acid	12.5	0.4	2.1	7.8
Threonine	5.2	0.3	1.5	16.4
Serine	6.9	0.3	1.6	10.9
Glutamic acid	10.3	1.1	3.2	10.1
Proline	5.0	0.3	1.4	12.0
Glycine	6.2	95.0	1.9	5.5
Alanine	5.4	0.0	1.1	7.4
Valine	7.2	0.3	1.7	5.9
Isoleucine	5.9	0.2	1.0	3.0
Leucine	8.3	0.4	1.8	5.7
Tyrosine	5.7	0.2	1.1	3.2
Phenylalanine	4.4	0.2	1.0	3.5
Histidine	2.6	0.2	0.8	1.7
Lysine	5.1	0.3	1.2	2.8
Arginine	5.1	0.3	2.5	3.5
Taurine	–	–	73.7	–
Methionine	1.1	0.1	0.7	0.8
Cysteine	1.7	0.6	1.7	10.0

[a] Pancreatic secretions, pigs; Corring and Jung (1972).
[b] Juste (1982).
[c] Tancharoenrat, P., Zaefarian, F. Ravindran, V., Massey University, Palmerston North, New Zealand (unpublished data).
[d] Mucin from the small intestine, pigs; Lien et al. (1997).

Table 4
Reported degrees of guanidination (conversion of lysine to homoarginine) in different protein sources[a].

Protein source	% guanidination[b]
Casein	94–100
Gelatin	83–95
Soy protein isolate	83
Soybean meal	76–84
Cottonseed meal	40–64
Rapeseed meal	75–87
Sunflower meal	40–49
Field peas	88
Fish meal	69
Meat and bone meal	53–61
Sunflower meal	40–49
Maize	57
Sorghum	61
Wheat	63

[a] From Maga (1981); Rutherfurd and Moughan (1990); Siriwan et al. (1994); Imbeah et al. (1996); Ravindran et al. (1996); Caine et al. (2008).
[b] Influenced by lysine: O-methylisourea ratio, pH, temperature and incubation duration.

and a linear relationship between dietary nutrient input and their output in ileal digesta is established. This relationship permits the determination of theoretical total (basal plus specific) EAA losses and simultaneous measurement of true amino acid digestibility of the feed ingredient, as the intercept and slope of the regression, respectively.

Although, in theory, the true amino acid digestibility of the particular feed ingredient is directly determined, the laborious nature of the method limits its wider acceptance in nutritional research. Moreover, in a number of studies, regression method has yielded EAA estimates similar to that of protein-free diet (Furuja and Kaji, 1989) disputing its potential value. Another concern is that negative estimates that are reported for EAA losses in some studies (for example, Rodehutscord et al., 2004). The notion that EAA losses can be negative is physiologically untenable and suggestive of the inherent limitation of the regression method (Moughan et al., 1998). Similar negative estimates for endogenous losses of phosphorus, with the regression method, have also been reported (Shastak et al., 2012; Mutucumarana et al., 2014a,b). Overall, the negative estimates call the validity of this method to measure EAA losses into question. However, it is possible that this methodology may be improved by the use of appropriate range of graded levels of amino acids in the design (Fan et al., 1995) and further studies are needed before definite conclusions are made.

6. Published data on specific ileal endogenous amino acid losses

The measurement of specific feed-induced EAA losses is both of practical as well as scientific interest, because of its implication in intestinal dynamics, protein nutrition and energy costs (Cowieson et al., 2009; Ravindran et al., 2009). A perusal of published literature shows the existence of very limited data (Tables 5 and 6), due largely to the complexity of methods available to measure total EAA losses. The limited data from both poultry and pigs clearly indicate that the specific EAA

Table 5
Specific feed-induced ileal endogenous protein losses (g/kg dry matter intake) in the growing pig[a].

Feedstuff	Total losses[b]	Basal losses[c]	Specific losses[d]
Soybean meal	26	20	6
Wheat	27	20	7
Barley	28	20	8
Rapeseed meal	31	20	11
Field peas	34	20	14
Common bean	103	20	83

[a] From Boisen and Moughan (1996).
[b] ^{15}N dilution method.
[c] Following feeding of enzyme-hydrolysed casein.

Table 6
Specific feed-induced ileal endogenous protein losses (g/kg dry matter intake) in broiler chickens[a].

Feedstuff	Total losses[b]	Basal losses[c]	Specific losses[d]
Soybean meal, 480 g/kg crude protein	14	12	2
Soybean meal, 440 g/kg crude protein	21	12	9
Canola meal	28	12	16
Cottonseed meal	39	12	27

[a] Ravindran, V., Hew, L.I., Ravindran, G., Bryden, W.L., University of Sydney, Camden, NSW, Australia (unpublished data).
[b] Guanidination method.
[c] Following feeding of enzyme-hydrolysed casein.

Table 7
Studies evaluating the effects of purified forms of fibre or anti-nutrients on ileal endogenous amino acid losses—select examples[a].

Reference	Background	Factor examined
Growing pigs		
de Lange et al. (1989)	Protein-free diet	Cellulose, pectin
Barth et al. (1993)	Guanidinated casein	Soybean trypsin inhibitor
Schulze et al. (1994)	Highly digestible protein	Level of neutral detergent fibre
Schulze et al. (1995)	Highly digestible protein	Source and level of neutrall detergent fibre level
Morel et al. (2003)	Enzyme-hydrolysed casein	Non-starch polysaccharides
Steendam et al. (2004)	Isotope dilution and enzyme-hydrolysed casein	Condensed tannins
Woyengo et al. (2009)	Guanidinated casein	Phytic acid
Broiler chickens		
Siriwan et al. (1989)	Guanidinated casein	Fibre level
Angkanaporn et al. (1994)	Guanidinated casein	Non-starch polysaccharide
Cowieson and Ravindran (2007)	Enzyme-hydrolysed casein	Phytic acid
Cowieson et al. (2008)	Enzyme-hydrolysed casein	Phytic acid
Kluth and Rodehutscord (2009)	Regression method	Cellulose
Onyango et al. (2009)	Glucose	Phytic acid

[a] More examples can be found in Nyachoti et al. (1997).

losses vary between feedstuffs, reflecting the effects of fibre and antinutrients on endogenous protein secretion. However, comparing results from different studies is difficult, because of the compounding effects of methodological differences, diet formulation and animal factors

7. Influence of anti-nutritional factors

While studies on total EAA losses induced by native ingredients are limited, research to quantify the effects of specific dietary components has attracted considerable attention (Nyachoti et al., 1997; Boisen and Moughan, 1996; Moughan, 2003). Large number of studies have examined the influence of purified forms of fibre and individual anti-nutrients on EAA losses, by the addition of these compounds on top of diets designed to measure basal losses (*e.g.* protein-free diet) or total losses (*e.g.* guanidinated casein). Select examples are summarised in Table 7 and the results of these studies, in general, demonstrate the magnitude of EAA loss responses associated with a range of dietary factors.

8. Digestible amino acid systems

Before concluding, a note on the use of AA digestibility terminology in relation to different estimates of endogenous losses is relevant. Today, there is general consensus that ileal digestibility coefficients determined with growing animals are preferable over those determined at the total tract level (Tanksley Jr. et al., 1981; Sauer and Ozimek, 1986; Ravindran et al., 1999). Considerable confusion, however, exists regarding the ileal digestibility terminologies used in the literature – appar-

ent, true, standardised and real. In particular, the terms true *vs*. standardised digestibility are being used interchangeably in recent times and different definitions are being used by different groups, creating confusion among end users.

In the pig industry, it has long been recognised that apparent digestibility must be corrected for EAA losses and that the use of apparent values in feed formulations must be disbanded. The industry has now adopted that a constant basal estimate following the feeding of protein-free diets as being the valid value to correct for basal endogenous EAA losses (Stein et al., 2007) and the digestibility term standardised has been put forward to replace the original term true (NRC, 2012). This approach has its drawbacks and could be criticised (Moughan et al., 2014), but represents a way forward to overcome the limitations of apparent digestibility values.

In the poultry industry, though it is generally agreed that apparent digestibility coefficients suffer from number of limitations (Lemme et al., 2004), there is less agreement on what should constitute basal EAA losses in correction for true (as originally used) or standardised digestibility estimates. Consequently apparent digestibility coefficients, and not those corrected for EAA losses, continue to be widely used in feed formulations.

A further confusion arises from the use of the term real digestibility, which includes the correction for the total endogenous losses and is related to the feeding of a specific ingredient (Boisen and Moughan, 1996; Gabert et al., 1997). Real digestibility may be more reliable than true digestibility (Batterham, 1992), but (as noted above) at the current time insufficient data are available on total EAA losses to consider this system. It is suggested that the issue is probably of more theoretical rather than practical relevance and the merit of real *versus* true digestibilities in applied nutrition remains to be elucidated (Gabert et al., 1997). For an excellent discussion on the appropriate digestible amino acid system, readers are referred to Moughan et al. (2014)

9. Conclusions

Specific endogenous protein losses represent significant maintenance protein and energy costs to the animal. Quantification of these losses is clearly relevant to improve the efficiency of protein digestion and for the better understanding of the factors influencing these losses. Only limited published data, however, are available on feed ingredient-induced EAA losses due largely to the highly specialised requirements of the available methodologies (isotope marker and guanidination). Future research is warranted to further explore this important subject area.

Conflict of interest

There is no conflict of interest.

References

Adedokun, S.A., Adeola, O., Parsons, C.M., Lilburn, M.S., Applegate, T.J., 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90, 1737–1748.

Adeola, O., Xue, P.C., Cowieson, A.J., Ajuwon, K.M., 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221, 274–283.

Angkanaporn, K., Choct, M., Bryden, W.L., Annison, E.F., Annison, G., 1994. Effect of wheat pentosans on endogenous amino acid losses in chickens. J. Sci. Food Agric. 66, 399–404.

Angkanaporn, K., Ravindran, V., Mollah, Y., Bryden, W.L., 1996. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal and meat and bone meal for broilers. Poult. Sci. 75, 1098–1103.

Angkanaporn, K., Ravindran, V., Mollah, Y., Bryden, W.L., 1997. Secretion of homoarginine into the gut of chickens. Vet. Res. Commun. 21, 161–167.

Barth, C.A., Lunding, B., Schmitz, M., Hagemeister, H., 1993. Soybean trypsin inhibitor(s) reduce absorption of exogenous and increase loss of endogenous protein in miniature pigs. J. Nutr. 123, 2195–2200.

Batterham, E.S., 1992. Availability and utilisation of amino acids for growing pigs. Nutr. Res. Rev. 5, 1–18.

Boisen, S., Moughan, P.J., 1996. Dietary influences on endogenous ileal protein and amino acid losses in the pig—a review. Acta Agric. Scand. Sec. A 46, 154–164.

Caine, W.R., Sauer, W.C., Huang, G.S., Diebold, G., Schollenberger, M., Mosenthin, R., 2008. Influence of guanidination on apparent ileal digestibility of amino acids in pigs fed diets with soybean meal, rapeseed meal or peas as a protein source. Livest. Sci. 116, 300–308.

Corring, T., Jung, J., 1972. The amino acid composition of pig pancreatic juice. Nutr. Reprod. Int. 6, 187–190.

Cowieson, A.J., Ravindran, V., 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98, 745–752.

Cowieson, A.J., Selle, P.H., Ravindran, V., 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poult. Sci. 87, 2287–2299.

Cowieson, A.J., Bedford, M.R., Selle, P.H., Ravindran, V., 2009. Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. World's Poult. Sci. J. 65, 401–418.

Fan, M.Z., Sauer, W.C., McBurney, M.I., 1995. Estimation by regression analysis of endogenous amino acid levels in digesta collected from the distal ileum of pigs. Can. J. Anim. Sci. 73, 2319–2328.

Furuja, S., Kaji, Y., 1989. Estimation of the true ileal digestibility of amino acids and nitrogen from their apparent values for growing pigs. Anim. Feed Sci. Technol. 26, 271–285.

Gabert, V.M., Canibe, N., Jorgensen, H., Eggum, B.O., Sauer, W.C., 1997. Use of N-amino acid isotope dilution techniques to determine endogenous amino acids in ileal digesta in growing pigs. Acta Agric. Scand. Sect. A: Anim. Sci. 47, 168–177.

Hagemeister, H., Erbersdobler, H., 1985. Chemical labelling of dietary protein by transformation of lysine to homoarginine: a new technique to follow intestinal digestion and absorption. Proc. Nutr. Soc. 44, 133a.

Imbeah, M., Angkanaporn, K., Ravindran, V., Bryden, W.L., 1996. Investigations on the guanidination of protein. J. Sci. Food Agric. 72, 213–218.

Jansman, A.J.M., Smink, W., Van Leeuwen, P., Rademacher, M., 2002. eevaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Anim. Feed Sci. Technol. 98, 49–60.

Juste, C., 1982. Apports endogenes par les secretions digestives chez le porc. In: Laplace, J.P., Corring, T., Rerat, A. (Eds.), Physiologie Digestive Chez Le Porc. Institut National de la Recherche Agronomique, Paris, pp. 155–173.
Kluth, H., Rodehutscord, M., 2009. Effect of inclusion of cellulose in the diet on the inevitable endogenous amino acid losses in the ileum of broiler chicken. Poult. Sci. 88, 1199–1205.
Lemme, A., Ravindran, V., Bryden, W.L., 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. J 60, 421–435.
Leterme, P., Thewis, A., Francois, E., van Leeuwen, P., Wathelet, B., Huisman, J., 1996. The use of ^{15}N-labelled dietary proteins for determining true ileal amino acid digestibilities is limited by their rapid recycling in the endogenous secretions of pigs. J. Nutr. 126, 2188–2198.
Lien, K., Sauer, W., Mosenthin, R., Souffrant, W., 1994. Determination of ileal amino acid digestibilities with N-isotope dilution techniques. In: Proceedings of the VIth International Symposium on Digestive Physiology in Pigs, Volume I, (Eds. Souffrant, W.B., W.B., Hagemeister H.,) EAAP Publication No. 80, pp. 31–34. (Bad Doberan: Germany).
Lien, K.A., Sauer, W.C., Fenton, M., 1997. Mucin output in ileal digesta of pigs fed a protein free diets. Z. Ernahrungswiss 36, 182–190.
Lien, K.A., Sauer, W.C., He, J.M., 2001. Dietary influences on the secretion into and degradation of mucin in the digestive tract of monogastric animals and humans. J. Anim. Feed Sci. 10, 223–245.
Maga, J.A., 1981. Measurement of available lysine using the guanidination reaction. J. Food Sci. 46, 132–134.
Montagne, L., Piel, C., Lalles, J.P., 2004. Effect of diet on mucin kinetics and composition: nutrition and health implications. Nutr. Rev. 62, 105–114.
Morel, P.C.H., Padilla, R.S., Ravindran, G., 2003. Effect of non-starch polysaccharides in mucin secretion and endogenous amino acid losses. Asian Aust. J. Anim. Sci. 16, 1332–1338.
Moughan, P.J., Souffrant, W.B., Hodgkinson, S.M., 1998. Physiological approaches to determining gut endogenous amino acid flows in the mammal. Arch. Anim. Nutr. 51, 237–252.
Moughan, P.J., Ravindran, V., Sorbara, J.O.B., 2014. Dietary protein and amino acids –consideration of the undigestible fraction. Poult. Sci. 93, 2013–2410.
Moughan, P.J., 2003. Amino acid digestibility and availability in foods and feedstuffs. In: Proceedings IXth International Symposium on Digestive Physiology in Pigs, Banff, Canada, pp. 199–221.
Mutucumarana, R., Ravindran, V., Ravindran, G., Cowieson, A.J., 2014a. Measurement of true ileal digestibility and total tract retention of phosphorus in corn and canola meal for broiler chickens. Poult. Sci. 93, 412–419.
Mutucumarana, R., Ravindran, V., Ravindran, G., Cowieson, A.J., 2014b. Measurement of true ileal digestibility of phosphorus in some feed ingredients for broiler chickens. J. Anim. Sci. 92, 5520–5529.
NRC, 2012. Nutrient Requirements of Swine. Animal Nutrition Series. National Research Council of the National Academies, The National Academies Press, Washington, D.C.
Nasset, E.S., Ju, J.S., 1961. Mixture of endogenous and exogenous protein in the alimentary tract. J. Nutr. 74, 461–465.
Nyachoti, C.M., De Lange, C.F.M., McBride, B.W., Schulze, H., 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. Can. J. Anim. Sci. 77, 149–163.
Nyachoti, C.M., De Lange, C.F.M., McBride, B.W., Leeson, S., Gabert, V.M., 2000. Endogenous gut nitrogen losses in growing pigs are not caused by increased protein synthesis rates in the small intestine. J. Nutr. 130, 566–572.
Ochao-Solano, A., Gitler, C., 1968. Digestion and absorption of ingested and secreted protein labelled with ^{75}Se-selenomethionine and ^{35}S-methionine in the gastrointestinal tract of rat. J. Nutr. 94, 249–254.
Onyango, E.M., Asem, E.K., Adeola, O., 2009. Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. Br. J. Nutr. 101, 836–842.
Ravindran, V., Bryden, W.L., 1999. Amino acid availability in poultry—in vitro and in vivo measurements. Aust. J. Agric. Res. 50, 889–908.
Ravindran, V., Hendriks, W.H., 2004. Endogenous amino acid flows at the terminal ileum of broilers layers and adult roosters. Anim. Sci. 79, 265–271.
Ravindran, V., Imbeah, M., Angkanaporn, K., Bryden, W.L., 1996. Guanidination of lysine in cottonseed protein. J. Agric. Food Chem. 44, 1812–1815.
Ravindran, V., Hew, L.I., Bryden, W.L., 1998. Influence of guanidination on apparent ileal amino acid digestibility in some protein sources for broilers. Poult. Sci. 77, 873–877.
Ravindran, V., Hew, L.I., Ravindran, G., Bryden, W.L., 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in feed ingredients for poultry. Br. Poult. Sci. 40, 266–274.
Ravindran, V., Hew, L.I., Ravindran, G., Bryden, W.L., 2004. Endogenous amino acid flow in the avian ileum: quantification using three techniques. Br. J. Nutr. 92, 217–223.
Ravindran, V., Morel, P.C.H., Rutherfurd, S.M., Thomas, D.V., 2009. endogenous flow of amino acids in the avian ileum is increased by increasing dietary peptide concentrations. Br. J. Nutr. 101, 822–828.
Rodehutscord, M., Kapocius, M., Timmler, R., Dieckmann, A., 2004. Linear regression to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45, 85–92.
Roos, N., Pfeuffer, M., Hagemeister, H., 1994. Labelling with ^{15}N as compared with homoarginine suggests a lower prececal digestibility of casein in pigs. J. Nutr. 124, 2404–2409.
Rutherfurd, S.M., Moughan, P.J., 1990. Guanidination of lysine in selected dietary proteins. J. Agric. Food Chem. 38, 209–211.
Sauer, W.C., Ozimek, L., 1986. Digestibility of amino acids in swine –results and their application a review. Livest. Sci. 15, 367–388.
Schneeman, B.O., Chang, I., Smith, L., Lyman, L.R., 1997. Effect of dietary amino acids, casein and soybean trypsin inhibitors on pancreatic protein secretion in rats. J. Nutr. 107, 281–288.
Schulze, H., 1994. Endogenous Ileal Nitrogen Losses in Pigs—Dietary Factors. PhD. Thesis. Wageningen Agricultural University, Wageningen, the Netherlands.
Schulze, H., van Leeuwen, P., Verstegen, M.W.A., Huisman, J., Souffrant, W.B., Ahrens, F., 1994. Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. J. Anim. Sci. 72, 2362–2368.
Schulze, H., van Leeuwen, P., Verstegen, M.W.A., van den Berg, J.W.O., 1995. Dietary level and source of neutral detergent fiber and ileal nitrogen losses in pigs. J. Anim. Sci. 73, 441–448.
Shastak, Y., Witzig, M., Hartung, K., Rodehutscord, M., 2012. Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers. Poult. Sci. 91, 2201–2209.
Short, F.J., Wiseman, J., Boorman, K.N., 1999. Application of a method to determine ileal digestibility in broilers of amino acids in wheat. Anim. Feed Sci. Technol. 79, 195–209.
Sibbald, I.R., 1987. Estimation of bioavailable amino acids in feedstuffs for poultry and pigs: a review with emphasis on balance experiments. Can. J. Anim. Sci. 67, 221–230.
Simon, O., Zebrowska, T., Bergner, H., Munchmeyer, R., 1983. Investigations on the pancreatic and stomach secretion in pigs by means of continuous infusion of ^{14}C-amino acids. Arch. Anim. Nutr. 33, 9–12.
Siriwan, P., Bryden, W.L., Annison, E.F., 1989. Effects of dietary fibre and protein levels on endogenous protein secretions in chickens. Proc. Nutr. Soc. Aust. 14, 143.
Siriwan, P., Bryden, W.L., Annison, E.F., 1994. Use of guanidinated dietary protein to measure losses of endogenous amino acid in poultry. Br. J. Nutr. 71, 515–529.
Souffrant, W.B., Kohler, R., Gebhardt, G., 1982. Determination de l'azote endogene dans les contenus digestifs par la technique isotopique (^{15}N). In: Physiologie Digestive chez le Porc., 2e Seminaire International. (Eds. Laplace, J.P., Corring, T., Rerat, A.) pp 175-87. (Institute National de la Recherche Agronomique: Paris.).
Souffrant, W.B., Rerat, A., Laplace, J.B., Darcy, B.V., Kohler, R., Corring, T., Gebhardt, G., 1993. Exogenous and endogenous contributions to nitrogen fluxes in the digestive tract of pigs fed a casein diet iii. refluxing of endogenous nitrogen. Reprod. Nutr. Dev. 33, 373–382.

Steendam, C.A., Tamminga, S., Boer, H., de Jong, E.-J., Visser, G.H., Verstegen, M.W.A., 2004. Ileal endogenous nitrogen recovery is increased and its amino acid pattern is altered in pigs fed quebracho extract. J. Nutr. 134, 3076–3082.
Stein, H.H., Seve, B., Fuller, M.F., Moughan, P.J., De Lange, C.F.M., 2007. Amino acid bioavailability and digestibility in pig feed ingredients. J. Anim. Sci. 85, 172–180.
Tamminga, S., Schulze, H., van Bruchem, J., Huisman, J., 1995. The nutritional significance of endogenous N-losses along the gastrointestinal tract of farm animals. Arch. Anim. Nutr 48, 9–22.
Tanksley Jr., T.D., Knabe, D.A., Purser, K., Zabrowska, T., Corley, J.R., 1981. Apparent digestibility of amino acids and nitrogen in three cottonseed meals and one soybean meal. J. Anim. Sci. 52, 769–777.
Woyengo, T.A., Cowieson, A.J., Adeola, O., Nyachoti, C.M., 2009. Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. Br. J. Nutr. 102, 428–433.
Zebrowksa, T., Low, A.G., Zebrowksa, H., 1983. Studies on gastric digestion of protein and carbohydrate: gastric secretion and exocrine pancreatic secretion in the growing pig. Br. J. Nutr. 49, 401–410.
Zebrowska, T., Munchmeyer, R., Bergner, H., Simon, O., 1986. Studies on the secretion of amino acids and of urea into the gastrointestinal tract of pigs: 2. Net secretion of leucine into the small and large intestines. Arch. Anim. Nutr. 36, 17–24.
de Lange, C.F.M., Sauer, W.C., Mosenthin, R., Souffrant, W.B., 1989. The effect of feeding different protein diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. 67, 745–754.
de Lange, C.F.M., Souffrant, W.B., Sauer, W.C., 1990. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. J. Anim. Sci. 68, 409–418.