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Basal endogenous losses of amino acids in protein nutrition research for swine and poultry

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ABSTRACT

In this review, the definition and terminology of amino acid (AA) digestibility and ileal endogenous losses of AA (IAA_{end}) in poultry and swine nutrition are discussed. Compared with apparent (AID) and true (TID) ileal digestibility, standardised ileal digestibility (SID) of AA is recommended for the expression of digestible AA contents of feed ingredients and for describing nutritional requirements of poultry and swine. To determine the SID of AA, total ileal flow of AA should be corrected for basal IAA_{end}. Therefore, the measurement of basal IAA_{end} is of great importance for the accuracy of the SID estimation in feed ingredients. The techniques for measuring basal IAA_{end} in poultry and swine include the use of a nitrogen-free diet (NFD), a highly digestible or enzyme hydrolyzed protein diet, and the regression method. The classic method for basal IAA_{end} determination involves the feeding of a NFD to experimental animals and measuring the ileal AA flow. This IAA_{end} output is considered as basal IAA_{end}, and it is assumed that the excretion of basal IAA_{end} depends only on DM intake, regardless of dietary composition. There are criticisms with the NFD method about the abnormal physiological state induced by severe AA deficiency. Although this AA deficiency may affect the estimate of basal IAA_{end} for dispensable AA, especially proline and glycine because of the degradation of body protein, the NFD method is still the most widely used method for basal IAA_{end} measurements. According to the definition of basal IAA_{end}, the NFD should be the preferred methodology in SID determination, because the basal IAA_{end} should be only related to dry matter intake. Additionally, the SID coefficients in feed ingredients generated by NFD method are considered to be additive in a complete diet. However, the results generated from NFD method can vary among studies due to the variance in the experimental animals and diet composition. To improve the accuracy of estimating the SID of AA in feed ingredients, it is suggested that a mandatory NFD be included in individual studies to generate basal IAA_{end} for correcting total ileal amino acid flow in determining SID of AA. In addition, research is needed to investigate the standard diet formulation of NFD.

Abbreviations: AA, amino acid; AID, apparentand ileal digestibility; AIA, acid-insoluble ash; IAA_{end}, ileal endogenous losses of AA; NFD, nitrogen-free diet; SID, standardized ileal digestibility; TID, true ileal digestibility.

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1. Introduction

Accurate determination of amino acid (AA) digestibility in feed ingredients is the cornerstone for estimation of AA requirements and formulating diets for animals. For poultry and swine, it has been well demonstrated that the small intestine is the main site for AA transport because the net absorption of AA in large intestine is negligible and the microbial activity in hind gut can modify the profile of AA in the digesta (Laplace et al., 1985; Nyachoti et al., 1997a; Ravindran et al., 1999). Therefore, accurate evaluation of AA digestibility requires collection of digesta sample from the end of ileum, rather than over the total tract. The ileal AA digestibility of AA in feed ingredients for poultry and swine has been an important research topic in the last few decades (Stein et al., 2007). Among the methods for collecting ileal digesta, collection from the ileum after animals are euthanised is the most common method in poultry, and through surgically fitted simple T-cannula in pigs (Kadim and Moughan, 1997; Stein et al., 2007). There are other methods for collecting ileal digesta from animals, such as through the use of caecectomised rooster and ileorectal anastomosis in pigs, which are well documented in previous studies and reviews (Payne et al., 1971; Sauer et al., 2001).

The basic equation for apparent ileal digestibility (AID) estimation is:

$$\text{AID, \%} = [(\text{AA intake} - \text{ileal AA output})/\text{AA intake}] \times 100. \quad (1)$$

However, for some of the ileal digesta collection methods mentioned above, the quantity of ileal AA output has to be estimated by an index marker, because in some studies, total collection of ileal digesta is not possible. Thus, the equation for AID calculation is:

$$\text{AID, \%} = [1 - (M_{diet}/M_{ileal}) \times (AA_{ileal}/AA_{diet})] \times 100. \quad (2)$$

Where M_{diet} and M_{ileal} are the concentrations of index marker (g/kg, DM basis) in diet and ileal digesta, respectively; AA_{ileal} and AA_{diet} represent AA (g/kg, DM basis) concentrations in diet and ileal digesta, respectively.

The digesta collected from the ileum may contain both dietary undigested materials and endogenous protein and AA (Laplace et al., 1985). Thus, the ileal digestibility of AA calculated without considering endogenous AA losses is defined as AID. The AID of AA is not accurate in formulating diets, because the endogenous AA flow in the ileal digesta can lead to an underestimation of AA digestibility and lack of additivity in diets containing multiple protein sources (Fan et al., 1994), especially for ingredients with low protein content (Stein et al., 2005; Xue et al., 2014). Therefore, in order to accurately measure the ileal digestibility of AA for diet formulation, accurate determination of ileal endogenous AA losses (IAA_{end}) is necessary (Stein et al., 2007).

2. Basal endogenous AA losses and standardised ileal digestibility of AA

The main sources of endogenous AA losses in animals are the proteins that are synthesised and secreted in the lumen of the digestive tract in animal but not reabsorbed (e.g., digestive enzymes, mucin protein, and serum albumin), sloughed intestinal epithelial cells, and bacterial protein from hind gut (Nyachoti et al., 1997a). The IAA_{end} can be divided into two parts, basal (non-specific, or diet-independent) and specific endogenous losses (McDonald et al., 2011). As shown in Fig. 1, the basal IAA_{end} is defined as the inevitable loss of AA in the digestive tract of animals, which is related to the amount of DM intake but otherwise unrelated to dietary composition (McDonald et al., 2011).

The specific endogenous loss is considered as the portion of endogenous AA flow, which is over and above basal IAA_{end} induced by the ingestion of diets of specific composition, such as protein level, fibre type, and anti-nutritional factors (Cowieson and Ravindran, 2007; Stein et al., 2007). The inclusion of high a concentration of protein in the diet can increase the specific endogenous loss of AA, because secretion of digestive enzymes in the digestive tract will be elevated in response to the high protein intake (Nyachoti et al., 1997b; Hodgkinson et al., 2000; Adedokun et al., 2008b). Likewise, fibre content and type can also affect the specific endogenous loss by changing the viscosity and passage rate of digesta in the small intestine, which can impact the secretion of mucin and epithelial cell turnover (Parsons et al., 1983; Mosenthin and Sauer, 1991). Anti-nutritional factors can also increase specific endogenous losses of AA. Cowieson and Ravindran (2007) reported that phytic acid can induce the secretion of specific endogenous losses of AA, and this increase of IAA_{end} can be ameliorated by the addition of phytase into the diet. This phenomenon can partly explain the effect of phytase in improving AID of AA in feed ingredients. Ileal digestibility that is adjusted for IAA_{end} can be termed either as true ileal digestibility (TID), when the total IAA_{end} is corrected for in the calculation; or standardised ileal digestibility (SID), if corrected for basal IAA_{end} (Stein et al., 2007). For common feed ingredients such as corn, SBM, DDGS, and canola meal, SID of AA is additive in complete diets (Stein et al., 2005; Xue et al., 2014). The TID and SID calculation are:

$$\text{TID, \%} = [(\text{AA intake} - (\text{ileal AA output} - IAA_{end}))/\text{AA intake}] \times 100; \quad (3)$$

$$\text{SID, \%} = [(\text{AA intake} - (\text{ileal AA output} - \text{basal } IAA_{end}))/\text{AA intake}] \times 100. \quad (4)$$

If the AID is already estimated, the equations become:

$$\text{TID, \%} = \text{AID} + (IAA_{end}/AA_{diet}) \times 100; \quad (5)$$

$$\text{SID, \%} = \text{AID} + (\text{basal } IAA_{end}/AA_{diet}) \times 100. \quad (6)$$

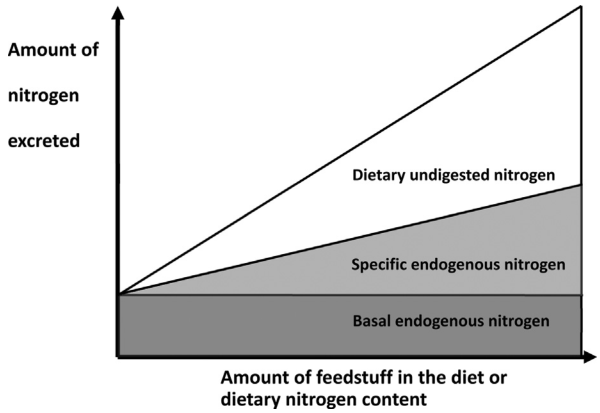

Fig. 1. Partition of ileal nitrogen flow [adapted from McDonald et al. (2011)].

Because of the relative ease of determining basal IAA_{end} and the additivity of SID in a complete diet, SID is accepted as the preferred measure of digestible AA requirement and content in swine diets. However, for poultry, the data of SID of AA in ingredients is not as copious as for swine. This is partly because the concept of SID was introduced after the latest version of NRC (1994) for poultry.

3. Methods for the determination of basal endogenous AA losses

The methodologies for measuring IAA_{end} were developed in the last few decades. These include use of nitrogen-free diet (NFD), fasting method (Green et al., 1987), highly digestible protein diet (Adedokun et al., 2007a), enzyme hydrolyzed protein diet (Yin et al., 2004), regression method (Eklund et al., 2015), homo-arginine diet (Nyachoti et al., 1997b), and ^{15}N isotope marked technique (Hess et al., 1998). Each of these methodologies have advantages and shortcomings. For example, some of these methods can be used for the determination of basal IAA_{end} (e.g., NFD and regression method), or total IAA_{end} (e.g., homo-arginine diet and ^{15}N isotope marked technique). However, in practical nutrition studies, it is technically difficult to completely distinguish the undigested dietary protein from the specific endogenous losses, and the procedure for separation of these fractions are both laborious and expensive. In addition, the SID of AA in feed ingredients is shown to be additive in a complete diet, which is important for diet formulation on digestible nutrient basis (Stein et al., 2005; Xue et al., 2014). Therefore, instead of TID, SID is an alternative for the expression of digestibility of AA in feed ingredients (Mosenthin et al., 2000; Jansman et al., 2002; Stein et al., 2007).

The equation for basal IAA_{end} estimation is:

$$\text{basal } IAA_{end} = AA_{ileal} \times (M_{diet}/M_{ileal}). \quad (7)$$

The main concern with NFD method is the physiological status of experimental animals (de Lange et al., 1989). Due to the deficiency of AA, the degradation of body proteins in animals fed with NFD is increased to release AA for maintaining normal biological functions, which will lead to a high level of dispensable AA, especially proline and glycine, in the ileal AA flow (Jansman et al., 2002). It has been reported that the predominant endogenous AA in IAA_{end} are glutamic acid, aspartic acid, leucine, and threonine (de Lange et al., 1989; Nyachoti et al., 1997b; Hess et al., 1998). However, when the NFD method is applied, it has been noted that the basal IAA_{end} are high in proline and glycine (Kim et al., 2009; Urriola et al., 2009a; Zhai and Adeola, 2011). This phenomenon sometimes results in SID estimates that are above 100% for those two AA.

Based on this consideration, it has been suggested that low level inclusions of highly purified and digestible proteins (e.g., casein) should be added to NFD to ameliorate the AA deficiency and the abnormal physiological status of experimental animals (Adedokun et al., 2007a). The ileal AA flow should still be of endogenous origin, because it is assumed that the added protein is 100% digestible. Thus, the digestibility of casein should be measured within each study to ensure that the determined ileal AA flow is of endogenous origin. Cervantes-Pahm and Stein (2010) reported that SID of AA in casein for growing pigs is approximately 96% for most AA, measured with direct method. However, Jezierny et al. (2011); Eklund et al. (2015) indicated the SID of AA in casein is almost 100%, estimated by the regression method. The IAA_{end} in an animal fed casein might be increased with the inclusion of casein. However, if the basal IAA_{end} is determined by NFD, the estimated IAA_{end} could be lower than it is in a casein diet, which leads to a lower value of SID when the direct method is applied.

An alternative to supplementation of the NFD with a highly digestible protein source such as casein is the use of enzyme hydrolyzed protein (Golian et al., 2008). As the only source of protein in the diet is peptides of a (known) low molecular mass, endogenous protein can be separated from the undigested hydrolyzed protein by the size separation. However, considering the potential stimulation of specific endogenous losses by the additional peptide, this method may overestimate the basal

IAA_{end}. Another alternative method is the regression method (Fan and Sauer, 1997; Eklund et al., 2015) where a series of diets with increasing protein concentration can be used. The regression equation, which is a variation of Eq. (6) is:

$$AID_C = (SID_C/100) \times AA_{diet} - \text{basal } IAA_{end}. \quad (8)$$

Where AID_C and SID_C are the content of digestible AA in experimental diet. AID_C and AA_{diet} are the dependent and independent variables, respectively. The slope provides an estimate of standardised ileal digestible AA in diet, and the intercept stands for basal IAA_{end}.

Jansman et al. (2002) reviewed previous data on IAA_{end} determined by the NFD, casein diet, and regression methods and summarised that the value of IAA_{end} generated from NFD was slightly lower than casein diet, except for proline and glycine, but similar to those of the regression method. Eklund et al. (2015) published data in cannulated pigs to determine the SID of AA in rapeseed meal using regression method and casein to measure the basal IAA_{end}. Three levels of casein diet were used to estimate the SID of casein and the basal IAA_{end}. The results showed that the SID of AA in casein was close to 100% and the estimated basal IAA_{end}, which represents the intercept of the linear regression, was similar to the results generated from NFD method except for lower proline and glycine (Table 1). Similar results were reported in previous studies from the same group (Eklund et al., 2008; Jezierny et al., 2011; Eklund et al., 2014). Thus, the regression method with casein may be a useful method for determining basal IAA_{end} without the high level of proline and glycine in the ileal AA flow. However, the regression method is more laborious and requires more dietary treatments than the NFD method. Meanwhile, the intercept of the linear regression is obtained by extrapolation, which may not be statistically reliable due to the interval of regressor.

The average values of basal IAA_{end} of AA reported in pigs during past decade are summarised in Table 2. As shown in the table, values from casein diet are generally higher than values from the NFD or regression method. Excessive secretion of proline and glycine is observed in NFD and casein diet, but not in regression method. Similar to previous observations of Jansman et al. (2002), the variation in determined basal IAA_{end} of dispensable AA is greater than that in indispensable AA. Arginine, as a conditionally indispensable AA, varied more in the results of basal IAA_{end} between studies.

In poultry studies, the fasted caecectomised rooster model is also one of the methods for estimating IAA_{end} (Aldrich et al., 1997). However, it has been reported that the fasted caecectomised rooster model underestimates IAA_{end} (Parsons, 1986; Adedokun et al., 2008a; Adedokun et al., 2009) because excretion of basal IAA_{end} depends on DM intake. In addition, the unit IAA_{end} estimated from fasted caecectomised rooster is g/d, rather than based on DM intake. Thus, fasted animals cannot provide comparable estimates of basal IAA_{end} for determination of SID of AA in feed ingredients.

4. Factors in the determination of basal endogenous AA losses

As shown in Table 1, the amount and profile of basal IAA_{end} determined with NFD may vary between labs due to several factors. Even within the same lab, the determined value of basal IAA_{end} can vary among studies. Therefore, it is necessary to include NFD in every AA digestibility experiment, and to use the determined value of basal IAA_{end} for the calculation of SID. The recommended NFD formulation is shown in Table 3.

4.1. *Experimental animal*

Across poultry species (Table 2), the basal IAA_{end} (g/kg DM intake) in turkeys and ducks is higher than broiler chickens (Adedokun et al., 2007b, 2007c; Kong and Adeola, 2013b). Within the same species, the age of experimental animal can also impact the determination of basal IAA_{end} in digestibility studies. Adedokun et al. (2007b, 2007c, 2007d) reported an age-related decrease in basal IAA_{end} in broiler chickens and turkeys. The basal IAA_{end} (g/kg DM intake) is higher in broiler chicks and turkeys on day 5 after hatch than on day 15, and is then maintained at similar level to d 21. It has also been found that the basal IAA_{end} was similar between 6-wk old broilers and 70-wk old layers and roosters (Ravindran and Hendriks, 2004). Thus, the use of birds that are at least 15 days old is preferred for determining AA digestibility in feed ingredients, especially when only AID of AA is investigated. The higher excretion of IAA_{end} may explain the lower digestibility of AA that is commonly observed in younger animals. The trend is similar in pigs (Hess and Sève, 1999; Hodgkinson et al., 2000; Leterme and Théwis, 2004). Weanling pigs secrete more endogenous AA compared with growing pigs. For growing pigs, the basal IAA_{end} (g/kg DM intake) tends to decrease with increasing BW (Pahm et al., 2008).

4.2. *Feed intake*

Although by definition, basal IAA_{end} should be constant on a g/kg DM intake basis, estimates from basal IAA_{end} determination can be influenced by feed intake level (Furuya and Kaji, 1992). Moter and Stein (2004) suggested that the level of basal IAA_{end} (g/kg DM intake) decreased with an increase from energy intake at a maintenance level to ad libitum energy intake. The severe nutrient and energy deficiency may interfere with basic metabolism in the animal and result in a higher ileal basal IAA_{end}. Thus, the feed intake level should be maintained ad libitum or at least equivalent to 3× maintenance energy level for an accurate estimation of basal IAA_{end} (Moter and Stein, 2004). As long as the feed intake level is maintained, numbers of meal per day does not appear to influence the basal IAA_{end} and SID determination in pigs (Chastanet et al., 2007).

Table 1
Estimated basal endogenous loss of AA and the ratio of each AA to Lys in pigs from different studies.

	Stein et al. (2005)		Zhai and Adeola (2011)		Xue et al. (2014)		Eklund et al. (2015)	
Method	Nitrogen-free diet		Nitrogen-free diet		Nitrogen-free diet		Regression method with casein diets	
Initial BW	92.1 ± 3.19 kg		47.1 ± 1.0 kg		61.3 ± 5.5 kg		22 ± 1 kg	
Item	Endogenous loss, g/kg DMI	Ratio to Lys[a]	Endogenous loss, g/kg DMI	Ratio to Lys	Endogenous loss, g/kg DMI	Ratio to Lys	Endogenous loss, g/kg DMI	Ratio to Lys
Nitrogen	2.26	–	–	–	2.32	–	1.92	–
Indispensable AA								
Arginine	0.45	92	0.44	104	0.54	111	0.4	80
Histidine	0.19	39	0.17	39	0.19	38	0.2	40
Isoleucine	0.42	86	0.27	63	0.33	68	0.4	80
Leucine	0.66	135	0.46	108	0.56	114	0.6	120
Lysine	0.49	100	0.43	100	0.49	100	0.5	100
Methionine	0.12	24	0.07	15	0.09	19	0.1	20
Phenylalanine	0.39	80	0.27	63	0.32	66	0.4	80
Threonine	0.55	112	0.42	100	0.49	101	0.7	140
Tryptophan	0.10	20	0.10	24	0.11	23	0.2	40
Valine	0.53	108	0.39	91	0.47	97	0.5	100
Dispensable AA								
Alanine	0.67	67	0.45	108	0.51	105	0.5	100
Aspartic Acid	0.93	93	0.64	152	0.83	169	0.9	180
Cysteine	0.28	28	0.14	32	0.19	38	0.2	40
Glutamic Acid	1.21	121	0.74	173	1.02	209	1.0	200
Glycine	1.06	106	1.05	260	1.16	237	0.6	120
Proline	2.47	247	3.10	745	3.17	651	0.7	140
Serine	0.49	49	0.35	82	0.45	91	0.6	120
Tyrosine	0.35	35	0.21	51	0.25	51	–	–
Total AA	11.36	–	9.67	–	11.39	–	8.50	–

[a] Ratios are calculated by dividing endogenous loss of each AA by the endogenous loss of lysine and multiplying by 100.

Table 2
Proposed dietary composition of nitrogen-free diet for pigs and broilers, g/kg as-fed basis.

	Pigs[a]		Broilers[b]
Ingredients, g/kg	Nursery	Growing-finishing	
Maize starch	544.0	790.0	200.5
Dextrose	150.0	100.0	640.0
Lactose	200.0	–	–
Vegetable oil	30.0	30.0	50.0
Cellulose	30.0	40.0	50.0
Limestone	5.0	5.0	13.0
Monocalcium phosphate	24.0	19.0	19.0
Indigestible marker	5.0	5.0	5.0
Salt	5.0	4.0	–
Vitamin-trace mineral premix[c]	2.0	2.0	5.0
Potassium carbonate[d]	4.0	4.0	2.6
Magnesium oxide	1.0	1.0	2.0
Sodium bicarbonate	–	–	7.5
Choline chloride	–	–	2.5
Potassium chloride	–	–	2.9
Total	1000.0	1000.0	1000.0

[a] Adapted and revised from Stein et al. (2007).
[b] Adapted and revised from Adedokun et al. (2011).
[c] Vitamin and trace minerals should meet the requirement values.
[d] $(Na^+ + K^+) - Cl^-$ milliequivalency value is 108. Sodium requirement for 0- to 3-wk old broilers is 0.2% in NRC (1994).

4.3. Dietary fibre and mucin secretion

Mosenthin et al. (1994) indicated that the inclusion of pectin could decrease the AID of AA in SBM. This could be attributed to the effect of fibre on IAA_{end}. The type of dietary fibre may influence the pattern of IAA_{end} (Leterme and Théwis, 2004). The capacity of fibre to absorb water is critical for determining the viscosity of the digesta and the secretion of mucin in the small intestine. Therefore, the content and type of fibre could change the viscosity and rate of passage of digesta, as well as the dynamics and excretion of mucin (Nyachoti et al., 1997a; Piel et al., 2005). Usually, cellulose (solka-floc) will be added in the NFD to increase the bulkiness and maintain the physical texture of digesta, because the other ingredients in an NFD such as starch and dextrose are highly soluble components. Cellulose is added to NFD at between 4 and 5% of the diet among studies. The inclusion of cellulose should be maintained for consistency between studies. However, there is a dearth of studies investigating the effect of the level of cellulose on the determination of basal IAA_{end} using NFD in poultry and pigs.

4.4. Ingredient composition of the nitrogen-free diet

The basal IAA_{end} using the NFD method can be influenced by the ingredient composition of the NFD. It was observed in broiler chickens that the basal IAA_{end} was higher when maize starch was completely replaced by dextrose in the NFD, compared with partial or no replacement of maize starch (Kong and Adeola, 2013c). However, this effect is not observed in pigs (Kong et al., 2014b). Cervantes-Pahm and Stein (2008) observed different SID of AA in SBM in semi-purified diets with or without added soy oil. This result may suggest that oil content might have an effect on basal IAA_{end}. However, there is a lack of information on the effect of NFD oil content on basal IAA_{end}. Favero et al. (2014) indicated that the type of index marker can also affect the result of basal IAA_{end}. In this particular study, acid-insoluble ash (AIA), chromic oxide, and titanium dioxide were added in NFD. The results showed that basal IAA_{end} calculated based on chromic oxide index was lower than those from AIA or titanium dioxide.

5. Implications

SID of AA is widely accepted as a method for expressing AA digestibility in diet formulation and AA requirement. The accurate measurement of basal IAA_{end} is critical to the determination of SID. Because the estimation of basal IAA_{end} can be affected by experimental animals, diets, and methods, we suggest a mandatory NFD be included in individual studies to generate basal IAA_{end} for correcting total ileal amino acid flow in determining SID of AA. Using NFD is the preferred method because of its simplicity and the definition of basal IAA_{end}. Although the basal IAA_{end} estimate for a dispensable AA is influenced by the physiological state of the experimental animal, the SID of most AA in feed ingredients based on NFD method is additive in a complete diet. Due to the influence of ingredient composition on the determination of basal IAA_{end}, standard NFD formulation should be applied for consistency among studies. Besides the NFD method, the regression method using casein is an alternative to estimate basal IAA_{end} for calculating SID of AA.

Additional studies are needed to understand the factors that affect the determination of basal IAA_{end}. Dietary composition of NFD, such as fibre and oil content, need to be investigated for standardising the composition of NFD used for the

Table 3
Mean of determined basal endogenous losses of AA (g/kg DM intake basis) with different methods in some previous studies in past decade (2005–2015).

Species	Pigs			Broilers			Turkeys			Ducks
Method	Nitrogen-free diet[a]	Casein diet[b]	Regression[c]	Nitrogen-free diet[d]	Casein diet[e]	Regression[f]	Nitrogen-free diet[g]	Casein diet[h]	Regression[i]	Nitrogen-free diet[j]
N	33	4	3	10	5	6	2	2	2	2
CP	17.28	19.14	12.47	11.29	–	–	–	–	–	36.30
Indispensable AA										
Arginine	0.59	0.73	0.43	0.39	0.22	0.18	0.27	0.31	0.27	1.49
Histidine	0.17	0.37	0.23	0.18	0.13	0.07	0.15	0.17	0.14	0.51
Isoleucine	0.30	0.46	0.43	0.37	0.38	0.20	0.25	0.39	0.26	1.26
Leucine	0.50	0.61	0.63	0.56	0.37	0.27	0.41	0.49	0.40	2.15
Lysine	0.40	0.47	0.50	0.39	0.30	0.15	0.27	0.35	0.24	1.18
Methionine	0.11	0.35	0.13	0.11	0.10	0.06	0.09	0.12	0.10	2.23
Phenylalanine	0.32	0.32	0.43	0.37	0.33	0.28	0.26	0.28	0.24	1.21
Threonine	0.52	0.73	0.70	0.60	0.45	0.34	0.44	0.47	0.38	1.43
Tryptophan	0.13	–	0.17	0.09	0.09	0.08	0.07	0.07	–	0.24
Valine	0.46	0.59	0.57	0.51	0.39	0.24	0.40	0.49	0.37	1.76
Dispensable AA										
Alanine	0.57	0.68	0.57	0.39	0.28	0.19	0.31	0.37	0.29	1.33
Aspartic Acid	0.75	1.01	0.93	0.73	0.56	0.36	0.56	0.68	0.53	2.34
Cysteine	0.17	0.67	0.25	0.41	0.16	0.14	0.21	0.23	0.21	1.04
Glutamic Acid	0.94	1.70	1.33	0.98	1.11	0.41	0.70	1.14	0.52	3.03
Glycine	1.46	1.45	0.70	0.47	0.28	0.22	0.33	0.36	0.51	1.64
Proline	4.95	6.20	0.67	0.50	0.38	0.24	0.40	0.46	0.35	1.87
Serine	0.65	0.90	0.77	0.56	0.61	0.33	0.39	0.57	0.38	1.64
Tyrosine	0.35	0.25	–	0.30	0.17	0.11	0.19	0.22	0.18	0.70
Total AA	13.30	17.42	9.37	8.40	6.61	4.04	6.64	7.75	5.95	31.05

[a] Means of previous studies (Bohlke et al., 2005; Stein et al., 2005; Gottlob et al., 2006; Stein et al., 2006; Mateo and Stein, 2007; Pedersen et al., 2007; Widmer et al., 2007; Pahm et al., 2008; Yin et al., 2008; Baker and Stein, 2009; Kim et al., 2009; Urriola et al., 2009b; Cervantes-Pahm and Stein, 2010; Cozannet et al., 2010; Jacela et al., 2010; Almeida et al., 2011; González-Vega et al., 2011; Jacela et al., 2011; Ren et al., 2011; Zhai and Adeola, 2011; González-Vega and Stein, 2012; Ji et al., 2012; Sulabo et al., 2013; Favero et al., 2014; Kong et al., 2014a; Xue et al., 2014).

[b] Means of previous studies (Opapeju et al., 2006; Lan et al., 2008; Yin et al., 2008; Woyengo et al., 2010a; Yang et al., 2010).

[c] Means of previous studies (Eklund et al., 2008; Jezierny et al., 2011; Eklund et al., 2015).

[d] Means of previous studies (Adedokun et al., 2007a,b,c,d; Golian et al., 2008; Woyengo et al., 2010b; Cozannet et al., 2011; Kong and Adeola, 2013c; Toghyani et al., 2015).

[e] Means of previous studies (Adedokun et al., 2007a,d; Golian et al., 2008).

[f] Means of previous studies (Adedokun et al., 2007b; Golian et al., 2008).

[g] Means of previous studies (Adedokun et al., 2007b).

[h] Means of previous studies (Adedokun et al., 2007b).

[i] Means of previous studies (Adedokun et al., 2007b).

[j] Means of previous studies (Kong and Adeola, 2013a, 2013b).

determination of basal IAA_{end}. A mandatory NFD be included in individual studies to generate basal IAA_{end} for correcting total ileal amino acid flow in determining SID of AA.

Conflict of interest

The authors declare no conflict of interest.

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