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ORIGINAL ARTICLE

# Use of homoarginine for measuring true ileal digestibility of amino acids in food protein

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**Abstract** A useful application of homoarginine in animal nutrition is the determination of the true ileal digestibility (TID) of amino acids (AA) in swine complete diets and feed ingredients. The homoarginine method involves the conversion of dietary lysine to homoarginine in a guanidination reaction with methylisourea. Accurate determination of TID of AA, especially in heat-treated feed ingredients, is a key prerequisite for accurate diet formulation with respect to the provision of dietary AA. Thus, the aim of this review is to highlight the homoarginine methodology and its application in animal nutrition. Based on the data from published studies, the homoarginine method can be used to accurately determine the digestibility of lysine and the majority of other acid-stable AA in complete diets and feed ingredients fed to animals.

**Keywords** Homoarginine · Guanidination · Amino acids · True ileal digestibility

**Abbreviations**

| | |
|---|---|
| AA | Amino acids |
| AID | Apparent ileal AA digestibility |
| TID | True ileal digestibilities |

## Introduction

Protein and amino acids (AA) are important nutrients that must be supplied in sufficient quantities and proportions to support optimal animal performance (Wu 2013a, b, 2014). As protein is an expensive nutrient, balancing its dietary supply with requirements is critical for controlling feed cost and for minimizing nitrogen excretion in manure, which is implicated in environmental pollution (Wu et al. 2014). To this end, it is important to accurately determine the availability of dietary AA in feeds and feed ingredients so as to allow accurate diet formulation with respect to optimal protein nutrition (Yin et al. 1993, 2000).

For non-ruminant animals, it is now accepted that AA digestibility coefficients determined at the end of the small intestine provide a better estimate of the amounts of AA that are available to the animals (Yin et al. 1991, 1994, 2002; Wu et al. 2014). For a long time, apparent ileal AA digestibility (AID) coefficients were determined and widely used to evaluate the availability of dietary AA in broiler chicks (Perryman and Dozier 2012; Rochell et al. 2012), pigs (Stein et al. 2005; Xue et al. 2014), ducks (Kong and

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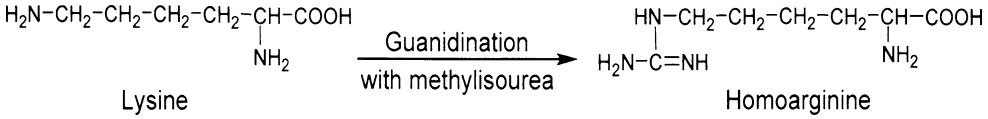


**Fig. 1** Conversion of lysine to homoarginine via guanidination reaction

Adeola 2010), and dogs (Hendriks and Sritharan 2002). However, due to the confounding effects of endogenous AA contribution to ileal digesta, which are not accounted for in the determination of AID coefficients, utilization of such coefficients in diet formulation increases the risk for inaccurate diet formulation. This is because AID values underestimate the true ileal digestibilities (TID) of AA in complete diets and feed ingredients (de Lange et al. 1990).

The endogenous AA originate from various digestive secretions, mucoproteins and desquamated epithelial cells that enter the gastrointestinal tract at various segments during the normal processes of digestion and absorption (Moughan et al. 1998; Ravindran et al. 2009). To overcome the shortcomings associated with AID coefficients, it is now recommended that TID coefficients be used in formulating poultry and swine diets with respect to dietary AA supply (NRC 2012). The TID values are obtained by correcting AID values for basal (minimum) endogenous AA (Stein et al. 2007). Determination of TID of AA in diets provides accurate data on the release of AA from dietary protein into the lumen of the small intestine for absorption (Fan and Sauer 2002; NRC 2012; Rutherfurd et al. 2006; Wu 2013b).

For determining TID of AA, the undigested dietary AA present in the ileal digesta and the endogenous losses of AA should be accurately evaluated. Currently, there are several methods to differentiate between the undigested dietary AA from endogenous AA losses in animals. These methods include feeding N-free diets (Adedokun et al. 2007), regression analysis (Fan and Sauer 2002), as well as the use of enzyme-hydrolysed proteins (e.g., casein) coupled with ultrafiltration, the homoarginine technique, and $^{15}$N-labeled proteins (Kluess et al. 2010). In the homoarginine method, the test feed ingredient is subjected to a guanidination treatment in which dietary lysine is converted to homoarginine (Fig. 1). As the absorbed guanidinated protein cannot reappear in the digestive tract, homoarginine concentrations in chime have been used to distinguish between exogenous AA and ileal endogenous AA in animal nutrition (Huang et al. 2003; Nyachoti et al. 1997a, b; Pomar et al. 2008; Stein et al. 2007). This review focuses mainly on the use of the homoarginine method to determine endogenous AA flow and the TID of AA in complete diets and feed ingredients fed to animals.

## The homoarginine technique

The basic principle underlying the homoarginine technique is that, upon treatment with methylisourea, dietary lysine is converted to homoarginine, which is normally absent from diets fed to animals (Schmitz et al. 1991). It is assumed that, compared with diet-derived homoarginine, there is little or no secretion of endogenous homoarginine into the lumen of the small intestine (Nyachoti). Thus, the apparent digestibility of homoarginine represents the TID of lysine (Nyachoti et al. 1997a). The process of producing homoarginine involves primarily two steps: preparation of methylisourea solution and the guanidination reaction. Although detailed procedures for each one of these steps have been modified by various research groups (e.g. Nyachoti et al. 2002; Boucher et al. 2009), the basic procedures are shown in Fig. 2.

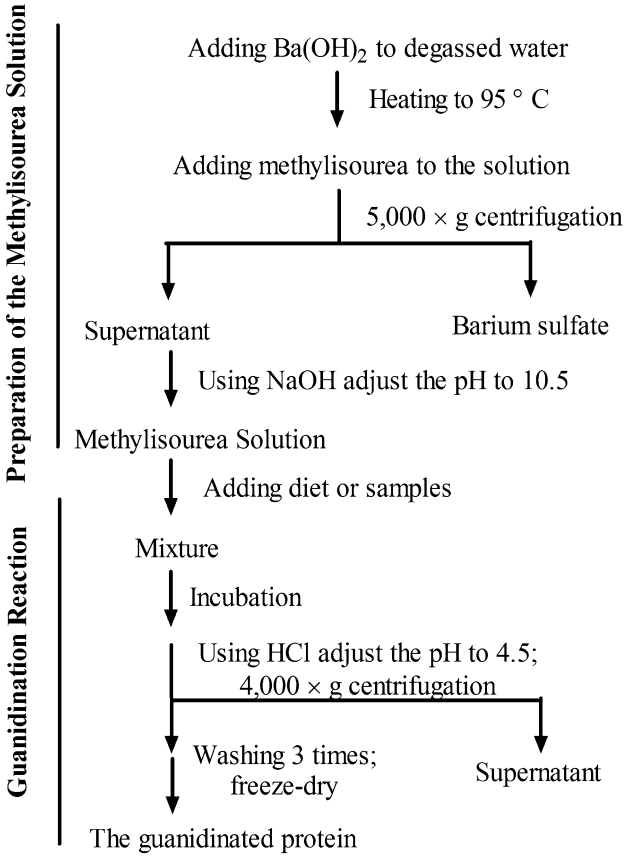


**Fig. 2** Preparation of the *O*-methylisourea solution and guanidination reaction

### Preparation of the methylisourea solution

The methylisourea solution is prepared by reacting methylisourea with barium hydroxide [$Ba(OH)_2$] followed by

centrifugation at 5000×*g* to remove the precipitated barium sulfate. In general, BaOH is mixed with degassed water (distilled water boiled for 30 min) and then the solution is heated to 95 °C before adding a known amount of methylisourea (0.3–0.6 M) to the solution. The resulting mixture is centrifuged at 5000×*g* for 15 min, and the supernatant fluid is recovered and its pH is adjusted to pH 11–12 with 1 M HCl before use (Nyachoti et al. 1997a, b, 2002; Pahm et al. 2008a, b).

### Guanidination reaction

After preparation of the methylisourea solution, an amount of the test material calculated to contain 200 g of crude protein is thoroughly mixed with 1 L of the solution and adjusted to pH 10.5 using 1 M NaOH. The mixture is then incubated in a refrigerator at 4 °C for 4–6 days (Nyachoti et al. 1997a). Incubation at the low temperature of 4 °C is critical to minimize AA damage due to the alkaline conditions. Of note, some investigators incubated the mixture for the guanidination reaction at room temperature (20–25 °C) for 1–3 days (Fontaine et al. 2007). During the incubation, the pH is monitored daily and adjusted accordingly to ensure uniform conditions in the mixture. At the end of the incubation period, the guanidination reaction is stopped by lowering the pH of the mixture to the isoelectric point of the test protein (e.g., casein, 4.6; barley, 5.6; canola meal, 4.6) using 1 M HCl so as to maximize the precipitation and, therefore, the recovery of the guanidinated protein. The mixture is then centrifuged at 4000×*g* and 4 °C, and the supernatant fluid is discarded. The guanidinated protein is washed three times with distilled water whose pH is adjusted to the isoelectric point of the test protein, and the protein sample is freeze-dried before use for diet preparation. Finally, the contents of lysine and homoarginine in the guanidinated protein are measured to calculate the extent of lysine conversion to homoarginine. This is generally calculated according to the following equation:

$$\text{Lysine conversion (\%)} = \left[\text{MC}_{\text{homoarginine}} / \left(\text{MC}_{\text{homoarginine}} + \text{MC}_{\text{lysine}}\right)\right] \times 100$$

where $\text{MC}_{\text{homoarginine}}$ and $\text{MC}_{\text{lysine}}$ are the molar contents (mol/kg of DM) of homoarginine and lysine in the guanidinated protein, respectively.

## Evaluation of the guanidination reaction

### Rates of lysine conversion into homoarginine

The extent of lysine conversion into homoarginine varies with the type of materials. As shown in Table 1, for ingredients in which the lysine residues are easily accessible to the methylisourea solution (e.g., casein), the extent of lysine guanidination ranges from 83.0 to 99.6 % (Nyachoti et al. 2002). Similar lysine conversion rates have been observed for canola meal, soybean meal, wheat shorts, seed meal, lupin and fish meal. However, the conversion of lysine in cottonseed protein to homoarginine is very low (36.1 %) according to Ravindran et al. (1996). These researchers investigated different incubation times (24–144 h), lysine:methylisourea ratios (1:8–1:32), and pH (9.5–13.0) on the guanidination of lysine in cottonseed protein, and recommended that the optimum conditions for the maximum guanidination of cottonseed protein are lysine:methylisourea ratio, 1:12; pH, 12.5; and incubation time, 72 h (Ravindran et al. 1996). Meanwhile, the data from Ravindran's group also indicated that different reaction conditions (i.e., chemical reagents, reaction period, pH, etc.) can lead to differences in lysine guanidination in the same type of feed material. Nyachoti et al. (2002) further demonstrated that there are significant interactive effects of methylisourea concentration and incubation time on the extent of lysine conversion into homoarginine in barley and canola meal (Fig. 3) (Nyachoti et al. 2002). An increase in the extent of lysine conversion in both the barley and canola meal samples was observed when the concentration of methylisourea was increased from 0.4 to 0.5 M, but no further increase was observed when the methylisourea concentration was increased beyond 0.5 M irrespective of incubation period (Nyachoti et al. 2002). Meanwhile, incubation for 6 days has a higher rate of conversion of lysine into homoarginine in both the barley and canola meal samples, compared with incubation for 4 days. Based on these findings, it was recommended that a methylisourea concentration of 0.5 M and a 6-day incubation period can be used to convert lysine to homoarginine for measuring endogenous AA flow and digestibility of barley and canola meal, and perhaps other plant protein sources in the small intestine (Nyachoti et al. 2002). Indeed, in the study of Friesen et al. (2006) based on the method of Nyachoti et al. (2002), the rate of the conversion of lysine into homoarginine in different pea cultivars was 96 %.

Fontaine et al. (2007) analyzed the effects of the guanidination reaction on total lysine and homoarginine in soy products and in corn distillers dried grain with solubles. These ingredients were subjected to deliberate heat damage for up to 30 min in an autoclave with 135 °C hot steam. In this case of heat treatment, both total lysine and homoarginine were decreased in a time-dependent manner, but homoarginine became a more sensitive indicator of lysine damage than was total lysine. Rutherfurd and Moughan (1997) treated skim milk powder and peas with various temperatures (110, 121, 135, 150, and 165 °C) and found that total lysine decreased by 33 % after 10 min of heating,

**Table 1** The extent of lysine conversion to homoarginine in different materials

| Materials | Rates of conversion (%) | L:M | pH | Incubation time | References |
|---|---|---|---|---|---|
| Casein sample | 83.0–99.6 | 0.5 M | 10.5 | 4 days at 4 °C | Libao-Mercado et al. (2006), Nyachoti et al. (1997b, 2002) |
| Barley | 72.5–88.0 | 1:14 | 10.5 | 6 days at 4 °C | Nyachoti et al. (1997b, 2002) |
| Canola meal | 72.3–86.6 | 1:4 | 10.5 | 6 days at 4 °C | Nyachoti et al. (1997b, 2002) |
| Soybean meal | 68.0–74.5 | 0.5 M | 10.5 | 1 day at room temperature or 4 days at 4 °C | Pomar et al. (2008), Ravindran et al. (1996), Siriwan et al. (1994) |
| Wheat shorts | 62.0–62.2 | 0.5 M | 10.5 | 6 days at 4 °C | Libao-Mercado et al. (2006), Siriwan et al. (1994) |
| Oilseed meal | 68.8–79.8 | 0.5 M | 10.5 | – | Nyachoti et al. (2002), Siriwan et al. (1994) |
| Lupin and fish meal | 69.0–74.5 | – | 10.5 | 1 day at room temperature | Ravindran et al. (1996) |
| Maize | 57.3 | 0.6 M | 10.5 | 4 days at 4 °C | Siriwan et al. (1994) |
| Meat meal | 50 | 0.6 M | 10.5 | 4 days at 4 °C | Siriwan et al. (1994) |
| Sunflower meal | 49.3 | 0.6 M | 10.5 | 4 days at 4 °C | Siriwan et al. (1994) |
| Cottonseed protein | 36.1–36.7 | 1:12 | 12.5 | 3–4 days at 4 °C | Ravindran et al. (1996), Siriwan et al. (1994) |
| Soy products, CP = 36.6, 42.6, or 46.8 % | 60–82 | 0.6 M | 11.5 | 2 days at room temperature | Fontaine et al. (2007) |
| DDGS, CP = 23.8 or 27.0 % | 38.1–78 | 0.6 M | 12 | 2.5 days at room temperature | Fontaine et al. (2007) |
| Milk-based products | 81.1–100 | 0.6 M | 10.6–11.0 | 1 or 7 days at 21 °C | Rutherfurd and Moughan (2005) |

*L:M* lysine:methylisourea or methylisourea concentration, *DDGS* distillers dried grain with solubles

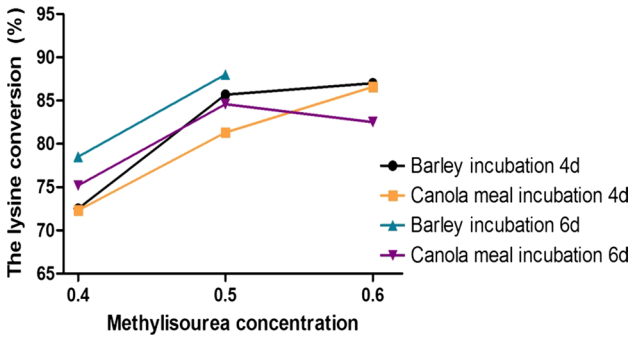


**Fig. 3** Effects of incubation days and methylisourea concentration on the conversion of lysine into homoarginine in barley and canola (Nyachoti et al. 2002)

whereas homoarginine decreased by 82 %. Although heating of feed ingredients at high temperatures may not affect the guanidination reaction, this processing condition results in damage to lysine and homoarginine. Thus, heat processing should be consistent for feed ingredients when the homoarginine method is used for measuring TID of lysine.

## Guanidination on chemical composition

The guanidination process is associated with incubation, freeze-drying, and washing the test material. Thus, it has been speculated to influence the chemical composition of the guanidinated material or diet. Nyachoti et al. (2002) have investigated the effects of guanidination treatment on dry matter, crude protein, crude fiber, crude fat, ash, and AA in the barley and canola meal (Nyachoti et al. 2002). The results showed that a 6-day incubation period in 0.5 M methylisourea solution and subsequent washings reduced dry matter and ash content in the guanidinated samples, but increased crude protein and crude fiber contents.

The loss of dry matter and ash content may result from the homogenization of the sample between multiple washings. In this process, the sample is solubilized in the washing solution and the supernatant fluid is discarded after centrifugation. The reason for the increased crude protein and crude fiber contents may be a selective removal of non-protein material during the guanidination process or to an incomplete removal of excess methylisourea solution during the washing procedure, which also removes soluble proteins and carbohydrates (Caine et al. 1998; Nyachoti et al. 2002).

## Nutrient digestibility after guanidination

A previous study has indicated that feeding a diet with guanidinated protein may have detrimental effects on feed intake and weight gain in chicks (Aoyagi and Baker 1994). However, Nyachoti et al. (2002) have compared the apparent ileal digestibilities of dry matter, crude protein, and AA

in the unguanidinated and guanidinated diets and found that there is no difference in the ileal digestibilities of these nutrients after the guanidination procedure (Nyachoti et al. 2002). In addition, Pomar et al. (2008) also demonstrated that feeding a guanidinated diet has no effect on feed intake, while the apparent ileal digestibility of N in diets containing guanidinated soybean meal protein is reduced by 4.5 %. In that study, feeding guanidinated soybean meal protein also decreased the digestibilities of several AA, including alanine, lysine, and isoleucine in pigs (Pomar et al. 2008). However, after a careful analysis of the data from the study by Pomar et al. (2008), it is apparent that the main reason for the decreased digestibilities of AA may be associated with the losses of material during the guanidination process as the contents of these AA are largely reduced by the guanidination procedure. Of note, this observation is not universal (e.g., Nyachoti et al. 2002). Thus, these investigations support the use of the homoarginine method for determining TID of AA in commercial diets fed to animals.

### Effects of guanidination on AA racemization

Compelling evidence has indicated that various conditions (e.g., alkaline and high temperature) can potentially cause L-amino acids racemization into D-amino acids (Chang et al. 1999; Steen et al. 2013). Thus, a particular worry during guanidination procedure is that guanidinated proteins at alkaline pH may cause formation of D-amino acids, which would underestimate AA digestibilities when the homoarginine labeling is used for investigation of the endogenous AA flow as the TID of AA. de Vrese et al. (1994) evaluated protein racemization during guanidination of casein at pH values between pH 9 and 11 and temperatures between 4 and 65 °C (de Vrese et al. 1994). In this study, the optimal conditions for the guanidination reaction were determined to be 4 °C and pH 10.5–11.0 or 22 °C and pH 10. Higher pH values, and a temperature of 22 °C or temperatures above 22 °C at each pH, lead to the formation of appreciable amounts of D-amino acids.

## Application of the homoarginine technique in animal nutrition

### Selection of digestibility markers

The homoarginine technique relies on the use of digestibility markers to make the necessary calculations. Because of the high cost of the methylisourea, it is too costly to feed test diets in which all the protein is guanidinated for an extended period of time, especially when working with large animals such as pigs. Consequently, animals are adapted to the experimental diets containing the test protein that has not been guanidinated and followed by a meal or two of the diet containing guanidinated protein. Thus, different digestibility markers (e.g. chromic oxide, dysprosium chloride, and titanium dioxide) are used to allow calculations of apparent and true ileal AA digestibilities based on the digesta from the non-guanidinated and guanidinated proteins, respectively.

### TID of lysine

As stated previously, the apparent digestibility of homoarginine is taken to represent the TID of lysine. The flow of homoarginine at the distal ileum can be calculated based on its concentration and the concentration of the marker associated with the diet containing guanidinated protein in the diet and digesta (Marty et al. 1994). Calculation of TID of lysine is calculated as follows (Pomar et al. 2008):

$$\mathrm{TID}_{\mathrm{lysine}}(\%) = \left[\left(\mathrm{homoarginine}_{\mathrm{diet}} - \mathrm{homoarginine}_{\mathrm{flow}}\right)/\mathrm{homoarginine}_{\mathrm{diet}}\right] \times 100,$$

where $\mathrm{homoarginine}_{\mathrm{diet}}$ and $\mathrm{homoarginine}_{\mathrm{flow}}$ are dietary and digesta homoarginine concentrations, respectively.

### Endogenous lysine loss

Endogenous lysine loss ($\mathrm{Endo}_{\mathrm{lysine}}$) at the terminal ileum (g/kg of DMI) can be calculated using the formula (Libao-Mercado et al. 2006):

$$\mathrm{Endo}_{\mathrm{lysine}}(\%) = \mathrm{diet}_{\mathrm{lysine}} \times \left(\mathrm{TID}_{\mathrm{lysine}} - \mathrm{AID}_{\mathrm{lysine}}\right)/100,$$

where $\mathrm{diet}_{\mathrm{lysine}}$ and $\mathrm{AID}_{\mathrm{lysine}}$ are lysine content (g/kg of DM) and the apparent ileal digestibility (%) of lysine in the diet, respectively. $\mathrm{AID}_{\mathrm{lysine}}$ can be calculated based on concentration of lysine in the diet and digesta, as well as the digestibility marker added to the diet containing unguanidinated protein (Stein et al. 2007).

### TID of AA other than lysine

As pointed out by Friesen et al. (2006), in the homoarginine technique, only the endogenous losses of lysine and, therefore, the TID of lysine are directly determined. The endogenous gut losses of other AA are derived based on the ratio of homoarginine to the contents of other AA in the guanidinated diet and ileal digesta (de Lange et al. 1990; Siriwan et al. 1994). Once the endogenous flow of each AA has been established, the TID ($\mathrm{TID}_{\mathrm{AA}}$) values for AA other than lysine are calculated according to Nyachoti et al. (1997b):

$$\mathrm{TID}_{\mathrm{AA}}(\%) = \mathrm{AID}\,(\%) + (100 \times [\mathrm{Endo}_{\mathrm{AA}}/\mathrm{AA}_{\mathrm{diet}}]),$$

where AID, $\mathrm{Endo}_{\mathrm{AA}}$, and $\mathrm{AA}_{\mathrm{diet}}$ are the apparent ileal digestibility (%) of AA, endogenous ileal AA loss (g of

**Table 2** Application of homoarginine method in animal nutrition

| Materials | Investigation | Animal | References |
|---|---|---|---|
| Dietary phytic acid | Ileal digestibility and endogenous flow of amino acids | Piglets | Woyengo et al. (2009) |
| Corn distillers dried grains with solubles | Lysine digestibility | Growing pigs | Pahm et al. (2008b) |
| Milk-based products | Lysine digestibility | Growing rats | Rutherfurd and Moughan (2005) |
| Microbial phytase | True and apparent ileal amino acid digestibilities | Growing-finishing pigs | Pomar et al. (2008) |
| Wheat shorts | True ileal amino acid digestibility | Growing pigs | Libao-Mercado et al. (2006) |
| Soybean | Endogenous amino acids | Growing pigs | Caine et al. (1998) |
| Barley and canola meal | True ileal amino acid digestibilities | Growing pigs | Nyachoti et al. (2002) |
| Soybean meal, rapeseed meal or peas | True ileal amino acid digestibility | Growing pigs | Huang et al. (2003) |

DMI/kg), and AA content in the diet (g of DM/kg), respectively. Alternatively, the $TID_{AA}$ value also can be calculated as follows:

$$TID_{AA}(\%) = \left\{\left[AA_{diet} - (AA_{flow} - EndoAA_{flow})\right]/AA_{diet}\right\} \times 100,$$

where $AA_{flow}$ and $EndoAA_{flow}$ are the flow and the endogenous flow of AA at the distal ileum, respectively (Pomar et al. 2008). Again, the $EndoAA_{flow}$ of AA rather than lysine is calculated based on the observed endogenous lysine flow and the composition of AA in endogenous secretions relative to lysine (de Lange et al. 1990; Pomar et al. 2008).

The homoarginine method has been used to study the digestibility of AA in feed ingredients for non-ruminant animals (especially pigs) and in investigating the effects of various factors that influence dietary AA utilization for protein deposition in animals. In this review, we have highlighted several investigations on the application of the homoarginine technique to quantify endogenous AA flow at the distal ileum to allow for the determination of the TID of AA in feed and feed ingredients (Table 2). However, some investigators have criticized the suitability of this technique in studying AA digestibility and utilization in animal nutrition. For instance, Hodgkinson et al. (2003) indicated that the homoarginine technique underestimates ileal endogenous lysine and nitrogen flows, compared with other methods. On the contrary, in the study by Friesen et al. (2006), the TID of some AA exceeded 100 %, which was attributed to the possibility that the homoarginine technique may overestimate the endogenous flow of some AA. Furthermore, it has been argued that the guanidination procedure may change the capacity of digestive enzymes to hydrolyze the guanidinated protein (Hodgkinson et al. 2003), which can potentially affect the availability of AA and peptides for absorption (Pomar et al. 2008). However, in the study by Nyachoti et al. (2002), it was demonstrated that the process of guanidination per se does not influence the digestibility of protein in growing pigs. Similarly, Boucher et al. (2009) reported a high degree of consistency in nitrogen and AA composition of ileal endogenous secretions. These authors surmised that it is unlikely that the homoarginine technique underestimates ileal endogenous lysine followed by overestimating homoarginine concentration because the formation of homoarginine is specific to the ε-amino group of lysine (Boucher et al. 2009). Based on the findings of these studies and digestive physiology, it can be argued that dietary composition can influence endogenous gut losses of protein and AA (Bergen and Wu 2009). Accurate analysis of AA in both diets and intestinal digesta should be performed to correctly determine dietary AA intake, endogenous AA flow in the small intestine, and digestibilities of AA in feedstuffs (Wu et al. 2013).

Comparison of homoarginine labeling with other methods further supports the use of the homoarginine method to measure the TID of AA and endogenous flow of AA (Boucher et al. 2009; Hodgkinson et al. 2003). Rutherfurd and Moughan (1997, 2005) concluded that the homoarginine technique provides more accurate estimates of available lysine in soy products and some purified dairy-based protein sources (including whole milk protein, whey protein concentrate, evaporated milk, sports formula, and lactose-hydrolyzed milk powder) than do other available methods. According to Souffrant et al. (1997), the endogenous AA flows determined with the homoarginine technique are quantitatively greater than those determined with the $^{15}N$-perfusion technique, and the homoarginine technique provides more consistent values in comparison to data obtained with the $^{15}N$-perfusion technique. Roos et al. (1994) reported similar findings and also suggested that incorporation of $^{15}N$ into endogenous proteins and re-entry into the intestinal lumen via secretions and cell sloughing are the major cause for the lower digestibility values obtained with the $^{15}N$ perfusion technique than with the homoarginine technique. Compared with the furosine method for determining reactive lysine, the homoarginine method is positively correlated with standardized lysine

digestibility and, therefore, can be used to predict lysine digestibility from soybean meal, distillers dried grains with solubles, and fish meal samples (Boucher et al. 2009). Furthermore, the effect of microbial phytase on the AID and TID of AA in pigs determined with the homoarginine technique gives similar values as the casein-based method (Pomar et al. 2008; Traylor et al. 2001).

Various studies indicated that the homoarginine technique can be used to estimate TID of AA in addition to lysine in diets on the basis of endogenous lysine flow and the composition of AA in endogenous secretions relative to lysine (de Lange et al. 1990; Siriwan et al. 1994). Rutherfurd and Moughan (2005) reported that the differences in the TID of 80 % acid-stable AA (including lysine) between the homoarginine technique and the conventional methods were less than 3 % and argued that such a small difference was not meaningful in practical terms. However, there were larger differences in the TID of the other 20 % acid-stable AA (including histidine) between the homoarginine technique and the conventional methods (Rutherfurd and Moughan 2005). Taken together, the homoarginine technique can be used to determine the TID of lysine and the majority of other acid-stable AA in diets and ingredients fed to animals.

## Conclusion and perspectives

Homoarginine is virtually absent from conventional foods of plant and animal origin, and is present at exceedingly low concentrations in animal tissues (May et al. 2015; Kayacelebi et al. 2014, 2015). Homoarginine is formed from the lysine residue in food protein after its treatment with methylisourea before feeding and the subsequent hydrolysis of the guanidinated protein by proteases in the lumen of the small intestine. In the field of animal nutrition, the homoarginine technique has been used to measure the TID of lysine and other AA in swine diets and feed ingredients. In this method, the test feed ingredient is subjected to a guanidination treatment in which dietary lysine is converted to homoarginine. The absorbed homoarginine does not reappear in the digestive tract during the period of measurement of endogenous AA flows, and, therefore, the apparent digestibility of homoarginine is considered to represent the TID of lysine (Nyachoti et al. 1997a, b). Although much is known about the application of the homoarginine technique in animal nutrition, some concerns should be addressed. For example, the uniform guanidination of lysine in a protein source is required and the optimum conditions of guanidination for a certain protein source should be investigated if the homoarginine is employed widely to determine true digestibilities of AA in new feed ingredients. Furthermore, the TID of AA varies with different feed ingredients and physiological status in animals. In our studies, we found that various stresses (e.g., birth, weaning, and mycotoxins contaminated feed) and dietary supplementation with certain AA can affect AA absorption and metabolism as well as animal health (Yin et al. 2013, 2014a, b, 2015). Based on the available evidence, the homoarginine technique can be used to determine the TID of lysine and the majority of other acid-stable AA in swine diets and ingredients. Further studies are needed to validate its utility for studying AA digestibility in diets fed to ruminants, poultry, fish, and other animal species.

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## References

Adedokun SA, Parsons CM, Lilburn MS et al (2007) Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. Poult Sci 86:2598–2607

Aoyagi S, Baker DH (1994) Dietary L-homoarginine has no lysine bioactivity in chicks. Poult Sci 73:1755–1757

Bergen WG, Wu G (2009) Intestinal nitrogen recycling and utilization in health and disease. J Nutr 139:821–825

Boucher SE, Pedersen C, Stein HH et al (2009) Evaluation of the furosine and homoarginine methods for determining reactive lysine in rumen-undegraded protein. J Dairy Sci 92:3951–3958

Caine WR, Sauer WC, Verstegen MW et al (1998) Guanidinated protein test meals with higher concentration of soybean trypsin inhibitors increase ileal recoveries of endogenous amino acids in pigs. J Nutr 128:598–605

Chang HM, Tsai CF, Li CF (1999) Quantification of racemization of amino acids in alkaline-treated duck eggs by micellar capillary electrophoresis. J Agric Food Chem 47:479–484

de Lange CF, Souffrant WB, Sauer WC (1990) Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the 15N-isotope dilution technique. J Anim Sci 68:409–418

de Vrese M, Middendorf K, Hagemeister H (1994) Prevention of amino acid racemization during guanidination–a prerequisite for measurement of protein digestibility by homoarginine labeling. Z Ernahrungswiss 33:310–312

Fan MZ, Sauer WC (2002) Determination of true ileal amino acid digestibility and the endogenous amino acid outputs associated with barley samples for growing-finishing pigs by the regression analysis technique. J Anim Sci 80:1593–1605

Fontaine J, Zimmer U, Moughan PJ et al (2007) Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. J Agric Food Chem 55:10737–10743

Friesen MJ, Kiarie, Nyachoti CM (2006) Ileal amino acid digestibility and reactive lysine content in peas (Pisum sativum) fed to growing pigs. Anim Feed Sci Technol 129:210–223

Hendriks WH, Sritharan K (2002) Apparent ileal and fecal digestibility of dietary protein is different in dogs. J Nutr 132:1692S–1694S

Hodgkinson SM, Souffrant WB, Moughan PJ (2003) Comparison of the enzyme-hydrolyzed casein, guanidination, and isotope dilution methods for determining ileal endogenous protein flow in the growing rat and pig. J Anim Sci 81:2525–2534

Huang GS, Sauer WC, Diebold G et al (2003) Estimates of ileal recovery of endogenous protein and amino acids in protein supplements for pigs by means of the homoarginine method. In: Progress in Research on Energy and Protein Metabolism. EAAP Scientific Series No. 109, pp 605–608

Kayacelebi AA, Beckmann B, Gutzki FM et al (2014) GC–MS and GC–MS/MS measurement of the cardiovascular risk factor homoarginine in biological samples. Amino Acids 46:2205–2217

Kayacelebi AA, Willers J, Pham VV (2015) Plasma homoarginine, arginine, asymmetric dimethylarginine and total homocysteine interrelationships in rheumatoid arthritis, coronary artery disease and peripheral artery occlusion disease. Amino Acids 2015 Jan 25. [Epub ahead of print] PMID: 25618752

Kluess J, Schoenhusen U, Souffrant WB et al (2010) Impact of diet composition on ileal digestibility and small intestinal morphology in early-weaned pigs fitted with a T-cannula. Animal 4:586–594

Kong C, Adeola O (2010) Apparent ileal digestibility of amino acids in feedstuffs for White Pekin ducks. Poult Sci 89:545–550

Libao-Mercado AJ, Yin Y, van Eys J et al (2006) True ileal amino acid digestibility and endogenous ileal amino acid losses in growing pigs fed wheat shorts- or casein-based diets. J Anim Sci 84:1351–1361

Marty BJ, Chavez ER, de Lange CFM (1994) Recovery of amino-acids at the distal ileum for determining apparent and true ileal amino-acid digestibilities in growing pigs fed various heat-processed full-fat soybean products. J Anim Sci 72:2029–2037

May M, Kayacelebi AA, Batkai S (2015) Plasma and tissue homoarginine concentrations in healthy and obese humans. Amino Acids. 2015 Feb 6. [Epub ahead of print] PMID: 25655383

Moughan PJ, Souffrant WB, Hodgkinson SM (1998) Physiological approaches to determining gut endogenous amino acid flows in the mammal. Arch Tierernahr 51:237–252

National Research Council (NRC) (2012) Nutrient requirements of swine, 11th edn. National Academy Press, Washington, DC

Nyachoti CM, de Lange CF, Schulze H (1997a) Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. J Anim Sci 75:3206–3213

Nyachoti CM, deLange CFM, Schulze H (1997b) The homoarginine method for determining true ileal lysine digestibilities in casein, barley and canola meal fed to growing pigs. EAAP Publication, pp 408–412

Nyachoti CM, de Wiele EMM, de Lange CFM et al (2002) Evaluation of the homoarginine technique for measuring true ileal amino acid digestibilities in pigs fed a barley-canola meal-based diet. J Anim Sci 80:440–448

Pahm AA, Pedersen C, Hoehler D et al (2008a) Factors affecting the variability in ileal amino acid digestibility in corn distillers dried grains with solubles fed to growing pigs. J Anim Sci 86:2180–2189

Pahm AA, Pedersen C, Stein HH (2008b) Application of the reactive lysine procedure to estimate lysine digestibility in distillers dried grains with solubles fed to growing pigs. J Agric Food Chem 56:9441–9446

Perryman KR, Dozier WA 3rd (2012) Apparent metabolizable energy and apparent ileal amino acid digestibility of low and ultra-low oligosaccharide soybean meals fed to broiler chickens. Poult Sci 91:2556–2563

Pomar C, Gagne F, Matte JJ et al (2008) The effect of microbial phytase on true and apparent ileal amino acid digestibilities in growing-finishing pigs. J Anim Sci 86:1598–1608

Ravindran V, Imbeah M, Angkanaporn K et al (1996) Guanidination of lysine in cottonseed protein. J Agric Food Chem 44:1812–1815

Ravindran V, Morel PCH, Rutherfurd SM et al (2009) Endogenous flow of amino acids in the avian ileum as influenced by increasing dietary peptide concentrations. Br J Nutr 101:822–828

Rochell SJ, Applegate TJ, Kim EJ et al (2012) Effects of diet type and ingredient composition on rate of passage and apparent ileal amino acid digestibility in broiler chicks. Poult Sci 91:1647–1653

Roos N, Pfeuffer M, Hagemeister H (1994) Labeling with N-15 as compared with homoarginine suggests a lower prececal digestibility of casein in pigs. J Nut 124:2404–2409

Rutherfurd SM, Moughan PJ (1997) Application of a new method for determining digestible reactive lysine to variably heated protein sources. J Agric Food Chem 45:1582–1586

Rutherfurd SM, Moughan PJ (2005) Digestible reactive lysine in selected milk-based products. J Dairy Sci 88:40–48

Rutherfurd SM, Darragh AJ, Hendriks WH et al (2006) True ileal amino acid digestibility of goat and cow milk infant formulas. J Dairy Sci 89:2408–2413

Schmitz M, Hagemeister H, Erbersdobler HF (1991) Homoarginine labeling is suitable for determination of protein absorption in miniature pigs. J Nutr 121:1575–1580

Siriwan P, Bryden WL, Annison EF (1994) Use of guanidinated dietary-protein to measure losses of endogenous amino-acids in poultry. Br J Nutr 71:515–529

Souffrant WB, Fevrier C, Laplace JP et al (1997) Comparison of methods to estimate ileal endogenous nitrogen and amino acids in piglets. EAAP Publication, pp 591–595

Steen AD, Jorgensen BB, Lomstein BA (2013) Abiotic racemization kinetics of amino acids in marine sediments. PLoS One 8:e71648

Stein HH, Pedersen C, Wirt AR et al (2005) Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J Anim Sci 83:2387–2395

Stein HH, Seve B, Fuller MF et al (2007) Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J Anim Sci 85:172–180

Traylor SL, Cromwell GL, Lindemann MD et al (2001) Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. J Anim Sci 79:2634–2642

Woyengo TA, Cowieson AJ, Adeola O et al (2009) Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. Br J Nutr 102:428–433

Wu G (2013a) Functional amino acids in nutrition and health. Amino Acids 45:407–411

Wu G (2013b) Amino Acids: Biochemistry and Nutrition. CRC Press, Boca Raton

Wu G (2014) Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. J Anim Sci Biotechnol 5:34

Wu G, Wu ZL, Dai ZL et al (2013) Dietary requirements of "nutritionally nonessential amino acids" by animals and humans. Amino Acids 44:1107–1113

Wu G, Bazer FW, Dai ZL et al (2014) Amino acid nutrition in animals: protein synthesis and beyond. Annu Rev Anim Biosci 2:387–417

Xue PC, Ragland D, Adeola O (2014) Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J Anim Sci 92:3937–3944

Yin YL, Huang RL, Zhong HY et al (1991) Influence of different cannulation techniques on the pre-cecal digestibility of protein, amino acids and cell wall constituents from diets, containing different protein meal, in pigs. Anim Feed Sci Technol 35:271–281

Yin YL, Zhong HY, Huang RL et al (1993) Nutritive value of feedstuffs and diets for pigs. I. Chemical composition, apparent ileal and fecal digestibility. Anim Feed Sci Technol 44:1–27

Yin YL, Chen CM, Zhong HY et al (1994) Digestibility of energy, cell wall constituents, crude protein and amino acids of the Chinese oil seed meals for pigs. Anim Feed Sci Technol 45:283–298

Yin YL, McEvoy J, Souffrant WB et al (2000) Apparent digestibility (ileal and overall) of nutrients and endogenous nitrogen losses in growing pigs fed wheat or wheat by-products without or with xylanase supplementation. Livest Prod Sci 62:119–132

Yin YL, Huang RL, Zhong HY et al (2002) Evaluation of mobile bag technique for determining apparent ileal digestibilities of crude protein and amino acids in growing pigs. J Anim Sci 80:409–420

Yin J, Ren W, Liu G et al (2013) Birth oxidative stress and the development of an antioxidant system in newborn piglets. Free Radic Res 47:1027–1035

Yin J, Ren W, Duan J et al (2014a) Dietary arginine supplementation enhances intestinal expression of SLC7A7 and SLC7A1 and ameliorates growth depression in mycotoxin-challenged pigs. Amino Acids 46:883–892

Yin J, Wu MM, Xiao H et al (2014b) Development of an antioxidant system after early weaning in piglets. J Anim Sci 92:612–619

Yin J, Duan J, Cui Z et al (2015) Hydrogen peroxide-induced oxidative stress activates NF-κB and Nrf2/Keap1 signals and triggers autophagy in piglets. RSC Adv 20:15479–15486