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13

ASPECTS OF AMINO ACID DIGESTIBILITY IN FEED INGREDIENTS FED TO PIGS

F. N. ALMEIDA AND H. H. STEIN
Department of Animal Sciences, University of Illinois, Urbana, Illinois, USA

Introduction

Knowledge about the nutritional quality of feed ingredients is imperative for success in nutrition of pigs. For the protein fraction, it is generally agreed that the most accurate estimate of the quality of a feed ingredient is described by the digestibility of protein and amino acids in the ingredient. The objective of this chapter is to review digestion and absorption of protein and amino acids in pigs, to provide information about factors that influence protein and amino acid digestibilities in feed ingredients fed to pigs, and to discuss factors that may negatively impact protein and amino acid digestibility by pigs.

Digestion and absorption of protein and amino acids

Protein digestion in pigs starts in the stomach where pepsinogen, which is secreted by the Chief cells in the Fundic region, is activated to pepsin by H ions. In this stage, between 15 and 50% of all peptide bonds in proteins are hydrolyzed by pepsin, thus forming small oligopeptides. Activation of pepsinogen is best achieved at pH 2, and this may be a challenge for young pigs because HCl secretion is limited compared with that in older pigs. Therefore, activation of pepsinogen in young pigs may be limited and digestion of proteins may not be as effective as in older pigs, but there are limited data to demonstrate the extent to which protein digestion is impaired in young pigs. After gastric digestion, small and larger oligopeptides proceed to the small intestines where pancreatic enzymes (i.e., trypsin, chymotrypsin, elastase, and carboxypeptidase) and aminopeptidase,

which is secreted from the small intestinal brush border, hydrolyze most of the peptide bonds in the oligopeptides. The resulting free amino acids, di-peptides, and tri-peptides are subsequently absorbed into the enterocytes using four different active transport systems. After absorption, di-peptides and tri-peptides are broken down to free amino acids in the enterocytes by the action of di-peptidases and tri-peptidases, respectively. The majority of the free amino acids then leave the enterocytes via the basolateral membrane and are subsequently taken up by the hepatic portal vein and transported to the liver. These amino acids are used for synthesis of proteins that may be used for maintenance or for production by the animal. Excess amino acids are not stored in the body. Instead, excess amino acids are deaminated and metabolized, and the N is excreted in the urine in the form of urea. The carbon skeletons are used for ketogenesis or gluconeogenesis and thus provide energy for the animal.

Amino acid digestibility

Absorption of amino acids takes place only in the small intestine. Amino acids that pass the last portion of the small intestine (the ileum) into the large intestine can no longer be absorbed by the animal and may be metabolized by microorganisms in the large intestine and subsequently excreted as microbial protein in the feces. Thus, determination of amino acid digestibility is believed to be more accurate if determined at the end of the ileum than in the faeces (Stein et al., 2007). Amino acid digestibility is generally expressed as apparent ileal digestibility (AID), true ileal digestibility (TID), or standardized ileal digestibility (SID; Stein et al., 2007; Urbaityte et al., 2009).

APPARENT ILEAL DIGESTIBILITY

Values for AID of amino acids are used to describe the net disappearance of protein and amino acids from the digestive tract proximal to the distal ileum (Stein et al., 2007). To determine AID values, pigs are surgically fitted with a T-cannula in the distal ileum from which ileal digesta are collected using standard procedures (Almeida et al., 2011). The concentration of protein and amino acids in the ileal digesta is then subtracted from the concentration of protein and amino acids in the diet, and the difference is divided by the concentration of protein and amino acids in the diet (Stein et al., 2007). Values for the AID of protein and amino acids, however, are not to be additive in mixed diets because they do not account for the

endogenous losses of protein and amino acids, which is the main disadvantage of the use of values for AID (Stein et al., 2005). This issue, however, may be overcome by correction for endogenous protein and amino acids losses.

ENDOGENOUS PROTEIN AND AMINO ACID LOSSES

Endogenous protein and amino acid losses are composed of protein and amino acids resulting from mucoproteins, sloughed cells, digestive enzymes, microbial protein, amides, and ingested hair (Souffrant, 1991; Nyachoti et al., 1997). Endogenous protein and amino acid losses may be divided into 2 categories: 1) basal endogenous losses, which relates to the physical flow of feed dry matter through the gastrointestinal tract and represent the minimum amount of protein and amino acids inevitably lost by the pig; and 2) specific endogenous losses, which is stimulated by specific characteristics of feed ingredients such as fibre and antinutritional factors, and represent the losses above the basal endogenous losses (Schulze et al., 1995; Stein et al., 2007).

Methods used to determine basal endogenous losses of protein and amino acids include feeding a protein-free diet, the regression procedure, and the peptide alimentation procedure (Fuller, 1991; Jansman et al., 2002; Moughan, 2003). Use of the N-free diet is the simplest and easiest of these procedures and is, therefore, the most commonly used procedure. However, it is recognized that the N-free procedure may overestimate endogenous losses of proline and glycine (Stein et al., 2007).

Total endogenous losses of protein and amino acids, which include basal plus specific losses, may be estimated using the homoarginine method or the isotope tracer technique (Krawielitzki et al., 1977; Hagemeister and Ebersdobler, 1985). Both of these procedures are expensive and tedious to use and only estimate the loss of one or a few amino acids and they are, therefore, not commonly used in practical feed ingredient evaluation (Stein et al., 2007).

TRUE ILEAL DIGESTIBILITY

Values for the TID of amino acids are determined by correcting AID values for total endogenous losses of protein and amino acids. True ileal digestibility values, therefore, represent the amount of dietary protein and amino acids that disappear from the gastrointestinal tract proximal to the distal ileum (Stein et al., 2007). However, because of the difficulties in determining the total endogenous losses,

values for TID of amino acids are not commonly determined for feed ingredients and these values are not used in practical feed formulation.

STANDARDIZED ILEAL DIGESTIBILITY

Values for the SID of amino acids represent the quantities of dietary protein and amino acids that disappear from the gastrointestinal tract proximal to the distal ileum but, in this case, AID values are corrected for the basal endogenous losses of protein and amino acids (Stein et al., 2007). For this reason, it is expected that values for the SID of protein and amino acids are greater than values for AID and less than values for the TID. Utilization of SID values may overcome some of the limitations of using AID and TID values as observed by Stein et al. (2005). Specifically, SID values are additive in mixed diets, which is of great practical importance for the feed industry because the SID of AA in mixed diets can be predicted from the SID of AA in the individual ingredients.

Factors affecting amino acid digestibility

Many factors may influence the digestibility of amino acids in feed ingredients. These include factors intrinsic to feed ingredients such as nutritional composition and antinutritional factors. Other factors relate to the physiological condition of the pig (i.e., weanling, growing, gestation, lactation), or to management practices, which include, but are not limited to, feed intake of animals. Processing of feed ingredients also may have a direct effect on amino acid digestibility.

PHYSIOLOGICAL ASPECTS

Body weight may influence the efficacy of pigs to digest protein and amino acids (Nitrayová et al., 2006). Little research, however, has been conducted to compare the digestibility of protein and amino acids among pigs at different physiological states.

Early studies revealed that the AID of protein and amino acids in growing pigs is less than the AID of protein and amino acids in lactating sows (Stein et al., 1999a). When compared with gestating sows, however, the AID of protein and amino acids in growing pigs is similar for most amino acids although the AID of histidine, lysine, threonine, and tryptophan are greater for gestation sows (Stein et al., 1999a). The assumptions for the differences observed were that the endogenous

protein and amino acid losses may have been different among each group of pigs. Thus, pigs with less endogenous losses would have greater AID of amino acids (Stein et al., 1999a). This hypothesis was later confirmed by Leterme and Théwis (2004), and Presto and Lindberg (2010) who observed that endogenous losses of protein and amino acids are lower in growing pigs than in finishing pigs. The AID of protein and amino acids was greater for pigs at 61.7 kg than for pigs at 20.6 kg (Nitrayová et al., 2006). Similarly, the AID of protein and amino acids in piglets fed canola meal was lower than in growing pigs (Mariscal-Landín et al., 2008).

FEED INTAKE

Voluntary feed intake of pigs is associated with many factors, such as gender, group size, and lysine level in the diet (Hyun et al., 1997) and the level of feed intake affects the endogenous losses of protein and amino acids in growing pigs and gestating sows (Stein et al., 1999b; Moter and Stein, 2004). Consequently, values for the SID of protein and amino acids, which is calculated by correcting AID values for basal endogenous losses, are also affected by feed intake (Stein et al., 2001; Moter and Stein, 2004). Specifically, values for the AID of protein and some amino acids increases as the feed intake level increases, whereas the SID of protein and most amino acids decreases with increasing levels of feed intake (Moter and Stein, 2004). These observations were later confirmed for weanling pigs, where SID of protein and amino acids decrease if the level of feed intake increases above 2 times the maintenance requirement for energy (Goerke et al., 2012). However, values for SID of protein and amino acids are identical if pigs are fed 3 times the level of maintenance energy or if they are allowed ad libitum access to feed (Chastanet et al., 2007). It is, therefore, recommended that pigs are fed at a level of at least 3 times the energy requirement for maintenance when experiments to determine protein and amino acid digestibility are conducted.

FIBRE

The concentration of fibre in diets fed to pigs is negatively correlated with the digestibility of some amino acids (Lehnen et al., 2011). Specifically, crude fibre decreases the AID of phenylalanine, lysine, leucine, proline, and serine. Neutral detergent fibre also reduces the AID of arginine, histidine, isoleucine, lysine, and methionine, and acid detergent fibre reduces the AID of aspartate, glutamate, alanine, phenylalanine, and proline. Lignin was also negatively correlated with the AID of glutamate, alanine, and phenylalanine (Lehnen et al., 2011).

Some of the reduction in the AID of amino acids by dietary fibre may be attributed to an increase in N excretion from endogenous origin (Huang et al., 2001). Dietary fibre may increase sloughing of cell in the small intestines, in addition to increased mucus production (Huang et al., 2001). Dietary fibre also affect gelling and viscosity properties that may decrease the mixing of intestinal contents, and therefore, impair interactions between proteins and enzymes, thereby reducing digestibility (Huang et al., 2001). In addition, an unstirred water layer may form as a result of increased concentration of dietary fibre, thus reducing absorption and consequently reducing the AID of amino acids (Huang et al., 2001).

ANTINUTRITIONAL FACTORS

Antinutritional factors are defined as natural occurring plant metabolites or compounds formed as a result of processing of feed ingredients that may negatively affect utilization of nutrients by the animal, and consequently reduce productive performance (Gilani et al., 2005). Among the natural occurring metabolites that directly affect protein and amino acid digestibility are gossypol, phytic acid, and trypsin inhibitors.

Gossypol, a naturally occurring polyphenolic compound in cotton seeds, is present in the plant in the forms of bound gossypol or free gossypol (Martin, 1990). Bound gossypol is non-toxic to pigs, but free gossypol is associated with growth depression and reduced lysine digestibility in pigs (Martin, 1990). Free gossypol is believed to bind lysine, thus reducing its availability. Increasing levels of cottonseed meal from 25 to 50, and to 75% in diets fed to pigs caused a reduction on the digestibility of amino acids (Li et al., 2000). The detrimental effects of free gossypol on pig performance, however, may be reduced if ferrous sulfate is added to diets in a 1:1 ratio (Martin, 1990).

The majority of phosphorus in plant feed ingredients such as maize and maize co-products is bound to phytic acid (Eeckhout and de Paepe, 1994; Selle and Ravindran, 2008; Almeida and Stein, 2012). Because of the negative charges of phosphate groups in phytic acid, complexes between phytic acid and proteins may be formed in feed ingredients (Lehnen et al., 2011). Phytic acid may also form complex molecules with free amino acids in the gastro intestinal tract (Kies et al., 2001). For these reasons, the digestibility of amino acids may be reduced as a result of high concentrations of phytic acid in some feed ingredients. Phytases are enzymes commonly added to pig diets to hydrolyze phytic acid and release phosphorus to be used by the pig (Almeida and Stein, 2012). Thus, it has been hypothesized that phytases may also reduce the binding of phytic acid to proteins and, therefore, increase the digestibility of amino acids (Mroz et al., 1994). Results

from a meta-analysis indicate that addition of phytase to diets increased the digestibility of arginine by 2%, but did not increase the digestibility of other amino acids (Lehnen et al., 2011). However, results of some experiments have indicated that phytase improves amino acid digestibility (Sands et al., 2007; Nortey et al., 2007; and Kiarie et al., 2010), whereas results of other experiments indicate that addition of phytase to diets fed to pigs does not improve amino acid digestibility (Cervantes et al., 2011; Yanez et al., 2011). Based on these observations, it is not clear if microbial phytase can improve the digestibility of amino acids in feed ingredients.

Trypsin inhibitors are present in many commonly fed feed ingredients such as soybean meal, peas, triticale, and rye (van Heugten, 2001), and they impair digestion and metabolism of protein, therefore, causing reduction in pig performance (van Heugten, 2001). The negative effects of trypsin inhibitors on protein digestibility in pigs are a result of the negative impact of these inhibitors on the activity of trypsin, chymotrypsin, and other pancreatic enzymes (Yen et al., 1977). Because of their proteic nature, however, trypsin inhibitors may be inactivated by heat processing, and therefore, soybean meal is usually heat processed before it is used in diets for pigs (Gilani et al., 2005; Goebel and Stein, 2011). Heat treatment of full fat soybeans also improves the AID and SID of amino acids (Goebel and Stein, 2011).

Glucosinolates are sulfur-containing plant metabolites present in Brassica that have been associated with reduced performance of pigs, when fed at high dietary levels (Tripathi and Mishra, 2007). It is recommended that dietary levels of glucosinolates are kept below 2μmol/g of diet (Tripathi and Mishra, 2007). Iodine deficiency, increased thyroid hormone levels, and thyroid hypertrophy have been observed in pigs when dietary concentration of glucosinolates exceeded the recommended maximum concentration (Bourdon and Aumaitre, 1990; Corino et al., 1991). Thyroid hormones play important role in metabolism and hyperthyroidism in humans and have been associated with increased oxidation of protein, loss of muscle mass, and increased protein catabolism (Martin et al., 1991; Møller et al., 1996; Riis et al., 2002). It has been show in humans that hypothyroidism causes both an increase in protein catabolism and anabolism, although the rate of protein catabolism is greater than that of anabolism (Riis et al., 2005). To our knowledge, the secondary detrimental effects of glucosinolates leading to hyperthyroidism and consequent effects on protein metabolism in pigs have not been demonstrated. Nevertheless, it is likely that reduced performance of pigs fed diets containing high levels of glucosinolates is a result of both reduced feed intake due to reduced palatability and also due to an increase in protein catabolism as observed in humans.

Heat damage of amino acids

The nutritional value of feed ingredients may be reduced during storage and processing (Friedman, 1996). This is probably a consequence of a combination of heat and humidity that leads to the Maillard reaction, which starts with the condensation between an amino group of an amino acid or protein and a carbonyl group of a reducing sugar. Lysine is an essential amino acid that has an -amino group that easily condenses with the carbonyl group of a reducing sugar (Nursten, 2005). In the initial stage of the reactions, Amadori products are formed. These products go through subsequently reactions called Strecker degradation, which later lead to the formation of pre-melanoidins and melanoidins at the final stage of the reactions (Nursten, 2005). Melanoidins are heterocyclic brown polymers that are responsible for color formation during heat processing of feed ingredients.

Distillers dried grains with solubles (DDGS) that were oven-dried at 50, 75, or 100°C had reduced concentration of reactive lysine (Pahm et al., 2008). When autoclaving DDGS for 45 min at 120°C, the digestibility of amino acids was reduced, especially that of lysine (Martinez-Amezcua et al., 2007), and it was suggested that the reduction in the digestibility of amino acids other than lysine was a result of the formation of Maillard reaction products that interfered with the absorption of other amino acids. Heat treatment of whey protein in the presence of lactose at temperatures that ranged from 75 to 121°C also caused a decrease in the availability of lysine from 75 to 45% (Desrosiers et al., 1989). González-Vega et al. (2011) reported that the SID of lysine by pigs was reduced from 93% (non-heated soybean meal) to 89.3 and 84.2% when soybean meal was autoclaved for 15 and 30 min, respectively, at a temperature of 125°C. In another experiment, Cozannet et al. (2010) observed that the SID of lysine in wheat DDGS was highly variable and that the samples with the least values for SID were darker and contained less lysine as a percent of crude protein, thus suggesting that color and lysine:crude protein ratio may be used as indicators of heat damage in wheat DDGS. As observed by Stein and Shurson (2009) and confirmed by Cozannet et al. (2010), when feed ingredients are heat damaged the concentration of lysine is reduced whereas the concentration of crude protein remains relatively constant. Therefore, the concentration of SID lysine in wheat DDGS fed to pigs may accurately be predicted ($R^2 = 0.86$) from the lysine:crude protein ratio (Cozannet et al., 2010). Kim et al. (2012) determined the SID of crude protein and amino acids in 21 sources of corn DDGS and observed that there is a positive correlation between the SID of lysine and the lysine:crude protein ratio, which further confirms the above theory. Cysteine and arginine also participate in the Maillard reactions (Ledl and Schleicher, 1990). Heat processing may cause oxidation of unsaturated lipids leading to formation of hydroperoxides (Meade et al., 2005).

Hydroperoxidases may oxidize cysteine, thus, limiting its utilization by the animal. In feed ingredients that have been heat damaged to a higher degree, pre-melanoidins may also react with cysteine and arginine (Finot, 1990). Cysteine may also go through Strecker degradation reactions producing hydrogen sulfide, ammonia, and acetaldehyde (Mottram and Mottram, 2002). The products of these reactions serve as intermediates to the formation of aroma compounds, such as thiazoles and disulphides, which are associated with the Maillard reactions (Mottram and Mottram, 2002). The participation of arginine in the Maillard reactions resulting from heat processing is associated with the formation of cross-links with lysine through imidazopyridinium bridges (Ledl and Schleicher, 1990).

There is, therefore, ample evidence that heat damage to feed ingredients may reduce the nutritional value of feed ingredients, specifically the concentration and digestibility of most amino acids and crude protein. Because many feed ingredients are heated during manufacturing or preparation, it is necessary to evaluate the nutritional quality of these feed ingredients in a quick and reliable manner to accurately use them in feeding programs.

Conclusions

Amino acids are indispensable nutrients that need to be available for pigs to synthesize protein. Most feed ingredients used in practical feed formulation contain protein that is digested in the stomach and small intestine with a subsequent absorption of amino acids. Absorption takes place only in the small intestine and amino acids that are not absorbed by the end of the small intestine will enter the large intestine and be used for the synthesis of microbial protein, which is excreted in the feces. Digestibility of amino acids, therefore, needs to be determined by the end of the small intestine. However, values for AID of amino acids are not always additive in mixed diets fed to pigs because of the influence of endogenous losses of amino acids on AID values. In contrast, values for SID of amino acids, which are calculated by correcting AID values for the basal endogenous losses, are additive in mixed diets and are, therefore, used in practical feed formulation.

Several anti-nutritional factors including gossypol, phytate, trypsin inhibitors, and glucosinolates may negatively impact protein and amino acid digestibility. However, heat treatment will reduce the concentration of trypsin inhibitors in feed ingredients and use of low-glucosinolate varieties will reduce the impact of glucosinolates on amino acid metabolism.

Processing of feed ingredients involving heat will often result in Maillard reactions, which involves the condensation between the amino group of Lys or other AA, and the carbonyl group of reducing sugars. Consequently, Lys becomes

unavailable to pigs, thus reducing the digestibility of this AA. The Maillard reactions are a series of complex reactions that remain to be fully understood, although much is known about the initial and intermediate stages. For several feed ingredients it has been shown that calculation of the lysine to crude protein ratio provides a reasonable estimate for heat damage in the ingredient.

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