DIGESTIBILITY OF AMINO ACIDS IN DIETS AND INGREDIENTS FED TO PIGS AND POULTRY

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INTRODUCTION

Feed ingredients consist of 6 classes of nutrients, which are water, carbohydrates, lipids, protein, minerals, and vitamins. Energy is not a nutrient, but energy can be obtained when animals oxidize absorbed end-products from the digestion of carbohydrates, lipids, and protein, whereas water, minerals, and vitamins do not contribute to the energy status of the animal.

The nutritional value of a feed ingredient is determined by the relative contribution of each class of nutrients. For practical handling purposes, most feed ingredients are dehydrated and contain less than 15% moisture, and there are only minor variations among feed ingredients in the concentration of water. Concentrations of vitamins are quantitatively negligible and are usually not included in the evaluation of a feed ingredient, although there are some notable differences. As an example, yeast contains significant quantities of most B-vitamins, but this is an exception to the rule that the concentration of vitamins in feed ingredients is usually not taken into account in the evaluation of that ingredient. However, concentrations of the remaining 4 classes of nutrients (i.e., carbohydrates, lipids, proteins, and minerals), vary greatly among different ingredients, and often, ingredients are classified according to the levels of the most dominant nutrients they contain. Cereal grains are often classified as high-starch ingredients, because they typically contain between 50 and 70% starch. Most oilseed meals are classified as high-protein ingredients because they typically contain between 30 and 50% crude protein. A number of co-products from the grain processing industries are often classified as high fiber ingredients because these ingredients often contain more than 25 or 30% dietary fiber. Thus, the nutritional value varies considerably among feed ingredients according to the concentration of the nutrients each ingredient contain.

Protein is determined in feed ingredients by measuring the concentration of nitrogen (**N**) and because proteins on average contain 16% N, the concentration of protein in a feed ingredient is determined by multiplying the concentration of N by 6.25. Most of the protein in feed ingredients consists of amino acids, but there are also N-containing compounds in most feed ingredients that do not contain amino acids and are known as "non-protein-nitrogen" or "NPN". The NPN fraction has no value for pigs, poultry, fish and other monogastric animals, but is easily utilized by ruminant animals. For monogastric animals, only the amino acid containing protein has value because animals need amino acids for protein synthesis. All proteins in feed ingredients are synthesized from a common set of 20 amino acids, and these 20 amino acids are also needed for protein synthesis by animals. The 20 amino acids that are present in feed are connected together in different sequences to form large feed proteins that often consist of several hundred amino acids. These amino acids are connected together by peptide bonds.

There are large differences among feed ingredients in the concentration of protein and amino acids. Cereal grains have a low concentration and oilseed meals have the greatest concentration of protein and amino acids among feed ingredients of plant origin. Pulse crops such as peas and beans and many of the co-products from the grain processing industries often have a medium concentration of protein that is less than in the oilseed meals, but greater than in the cereal grains. However, some animal proteins such as fish meal, blood meal, meat meals, and some of the milk products contain more protein and amino acids than most ingredients of plant origin.

The 20 amino acids needed by animals for protein synthesis are usually divided into "indispensable" and "dispensable" amino acids. The dispensable amino acids can be synthesized by the animals and they need, therefore, not be included in the diets. In contrast, the indispensable amino acids cannot be synthesized by animals and the diets, therefore, need to contain these amino acids. Most of the indispensable amino acids are similar in pigs and poultry and include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. For poultry, glycine is also considered an indispensable amino acid because, in addition to being required for protein synthesis, it is also needed to synthesize uric acid, which is the primary N excretion product in birds. In diet formulations, it is important to make sure that adequate amounts of all the indispensable amino acids are included to enable the animal to maximize protein synthesis. Most feed ingredients of plant origin that are commonly used in diet formulations are low in lysine, threonine, tryptophan, and methionine relative to the requirements by pigs and poultry. These amino acids are, therefore, often called the first limiting amino acids in the diets. However, soybean meal is a unique ingredient because it has a relatively high concentration of both lysine and tryptophan. In contrast, the concentration of methionine is relatively low in soybean meal, but corn protein has a relatively high concentration of methionine. Corn and soybean meal are, therefore, complementary proteins in diets.

DIGESTION OF PROTEIN AND ABSORPTION OF AMINO ACIDS

Protein digestion in pigs starts in the stomach (Fig. 1) where pepsinogen, which is secreted by the Chief cells in the Fundic region, is activated to pepsin by H ions. Pepsinogen is a pro-enzyme (also called a zymogen) which has no proteolytic capability. It therefore needs to be activated before it can start digesting peptide bonds, and this activation occurs in the lumen of the stomach when pepsinogen comes in contact with the H ions from HCl that is also secreted in the Fundic region of the stomach. This is similar in poultry (Fig. 2) where digestion starts in the proventriculus. 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. This also occurs in poultry but to a lesser extent than in pigs. It has been shown that protein and amino acid digestibility increases in young chicks up until about two weeks of age.

After gastric digestion, small and larger oligopeptides proceed to the small intestine where pancreatic enzymes (i.e., trypsin, chymotrypsin, elastase, and carboxypeptidase). All the pancreatic enzymes are secreted as inactive pro-enzymes, but when reaching the lumen of the small intestine, the brush border enzyme Enterokinase will activate the pro-enzyme trypsinogen into trypsin, and trypsin will then activate the other pancreatic pro-enzymes into their active forms. Amino peptidase is an enzyme secreted by the intestinal brush border, which also participates in protein digestion in the small intestine. The combined actions of the pancreatic enzymes and amino peptidase result in hydrolysis of 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 hydrolized 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 in pigs and primarily as uric acid in poultry. The carbon skeletons are used for ketogenesis or gluconeogenesis and thus provide energy for the animal.

DETERMINATION OF AMINO ACID DIGESTIBILITY

For a dietary amino acid to be retained in tissue protein in an animal, the amino acid needs to be ingested by the animal and absorbed from the intestinal tract. While the absorption of amino acids is a concept that can easily be defined, it is difficult to measure. Instead, the digestibility of amino acids is measured. Digestibility is defined as the difference between the amount of a certain amino acid ingested by the animal and the amount that is excreted in the feces or ileal fluids of the animal divided by the amount that is ingested (Sauer and Ozimek, 1986). By multiplying the fraction calculated this way by 100, the digestibility value is calculated. Thus, digestibility values are calculated by measuring the undigested quantity of dietary amino acids rather than the portion that was digested. It is assumed that the digestible amount of dietary amino acids equals the amount that was absorbed.

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. The nitrogen from the amino acids metabolized by the microorganisms may be absorbed and mostly excreted in the urine or may subsequently be excreted as microbial protein in the feces. If the nitrogen from the amino acids is absorbed, this results in amino acid disappearance and the amount of amino acids excreted in the feces is lower than what the pig or chicken did not digest. Consequently, amino acid digestibility is overestimated and digestibility values are too high. If the amino acids wind up in microbial protein, they may or may not be the same amino acid that entered the large intestine. Thus, determination of amino acid digestibility is believed to be more accurate if determined at the end of the ileum than in the feces (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). To determine all of these values, it is necessary that ileal digesta are collected, and as a consequence, techniques for collecting ileal digesta had to be developed.

TECHNIQUES FOR COLLECTING ILEAL DIGESTA

Several techniques are available for the collection of ileal fluid. These techniques either allow for the total collection of digesta or partial collection combined with the use of an inert marker to calculate digesta passage.

Re-entrant cannulas

The original cannula described by Cunningham et al. (1962) was a so-called ileo-ileo re-entrant cannula. With this type of cannula, the ileum is transected approximately 5 cm cranial to the cecum, and two cannulas are inserted into the two intestinal ends and exteriorized. The two cannulas are then connected outside the body wall by rubber- or plastic tubing. Ileal digesta can be collected by replacing the tubing on the cranial cannula with a collection bag. Several problems were associated with this type of cannula because of difficulties in maintaining a uniform flow of digesta through the cannula and with blockage of the cannula (Cunningham et al., 1963). To overcome these problems, Easter and Tanksley (1973) developed an ileocecal re-entrant cannula that bypassed the ileocecal valve, which proved to eliminate some of the difficulties observed with the ileo-ileo cannulas. These authors also demonstrated that grain-soybean meal diets could be fed on an ad libitum basis to the pigs, thus allowing for digesta collection under circumstances that parallel practical feeding conditions. However, for high-fiber diets or diets with large feed particle size, blockage of the cannula was a major problem causing the animals to go off feed (Sauer and Ozimek, 1986; Sauer and de Lange, 1992). In addition, concerns about intestinal motility associated with the use of re-entrant cannulas have been raised.

T-cannulas

The T-cannula was first described by Furuya et al. (1974). The cannula consisted of a flange placed inside the intestine, and a barrel with an internal diameter of 8 mm extending through the body wall allowing for collection of digesta. This procedure does not require a total transection of the intestine, and is thus believed to be less invasive than the re-entrant cannula. Modifications to the original cannula have been proposed by Decuypere et al. (1977), Gagallo and Zimmerman (1980), Hamilton et al. (1985), and Wubben et al. (2001). Cannulas with an inner diameter of the barrel of 10 to 16 mm have usually been used. For digestibility experiments, they are installed in the distal ileum 10 to 20 cm cranial to the ileo-cecal valve. Kesting et al. (1986) proposed a cannula barrel with an inner diameter of 20 to 25 mm to be used in experiments in which digestion of forage samples was studied. Smaller T-cannulas for experiments involving baby-pigs also have been described (Decuypere et al., 1977; Walker et al., 1986; Li et al., 1993; Kien et al, 1997). For sows, larger cannulas were developed and succesfully used (Stein et al., 1998). The use of a T-cannula does not allow for a quantitative collection of digesta. Therefore, indigestible markers are used in association with this technique, and a representative sampling is assumed.

The T-cannula is the most commonly used method for collecting digesta from the small intestine (Moughan, 2003). The main reason for its popularity is the relatively easy surgical procedures required for the installation of the cannula and a minimal trial-to-trial variation in the values obtained by using this technique (Knabe et al., 1989). Growth rates of cannulated pigs have been shown to be comparable to those of intact pigs, and no major differences in fecal digestibilities of dry matter, crude protein, or lysine were found between cannulated pigs and intact pigs (Jørgensen et al., 1985). In addition, if the T-cannula is correctly installed, pigs usually have no health problems and the cannulas can easily be maintained during the entire growing period from 25 to 130 kg allowing for multiple collections in the same animal. In sows, T-cannulas were maintained for 3 parities (Stein et al., 1998). Dislodgment of cannulas has been reported in a few studies. It was suggested to ameliorate this problem by exteriorizing the cannula between the last two ribs (Wubben et al., 2001). However, dislodgment is mainly a problem if the cannula is made out of flexible material such as polyethylene or nylon tubing. In the author's laboratory, cannulas produced from stainless steel are used and pigs very rarely loose these cannulas.

Although T-cannulation has been most extensively used in swine, the procedure has also been described for avians (van Leuwen et al., 2000); canines (Walker et al., 1994), felines (Mawby et al., 1999), and equines (Peloso et al., 1994).

Post valve T-cannulas

The so-called post-valve-T-cecum cannulation (PVTC) procedure was described by van Leeuwen et al. (1991). Using this technique, the cecum is partially removed and replaced by the PVTC cannula which is positioned opposite to the ileo-cecal valve. The cannula is closed by a plug, but during collections, the plug is removed, and the ileo-colic valve protrudes into the cannula, allowing for the collection of digesta. Studies have shown that 99% of an indigestible marker (chromium oxide) was recovered in the digesta, indicating that a complete quantitative collection of digesta is possible with this procedure (van Leeuwen et al., 1991). However, other research teams have reported less than 100% recovery of digesta from pigs equipped with a PVTC cannula (Hodgkinson et al., 2000; Yin et al., 2000). Therefore, it is recommended that a marker is included in digestibility studies using the PVTC cannula (Moughan, 2003).

A modification to the PVTC cannulation procedure was presented by Mroz et al. (1991) in the form of the so-called steered ileo-cecal valve (SICV) procedure. In addition to the cannula placed in the cecum across from the ileo-colic valve, these researchers also placed a silicon covered metal ring in the distal ileum approximately 10 cm posterior to the ileo-cecal valve. Via a nylon cord secured to the metal ring, the distal end of the ileum can be pulled into the cannula during collection periods, thus eliminating the possibility for any digesta to bypass the cannula. The original SICV procedure was later slightly modified and proved to be effective in collection of ileal digesta in several experiments (Radcliffe et al., 2006).

Ileorectal anastomy

Ileorectal anastomy (IRA) was first described by Fuller and Livingstone (1982). Using this approach, the terminal ileum is connected to the rectum so that the colon is by-passed, thus

allowing for rapid collections of ileal materials. In the so-called end-to-side anastomy, the distal ileum is attached to the side of the colon just prior to the rectum while in the so-called end-to-end anastomy, the colon is completely cut off, and the terminal ileum is attached directly to the rectum. Using this procedure, it may be necessary to install a T-cannula that acts as a chimney for any gases produced as a result of microbial activity in the isolated colon (Sauer and de Lange, 1992). To avoid contamination of the digesta from the isolated colon, the end to end procedure is preferred (Laplace et al. (1994; Moughan, 2003). The main advantages of using the IRA procedure are that this procedure is less labor intensive than the cannulation procedures and it is very easy to collect the ileal digesta because it is expelled from the rectum. Because total collection of digesta can be accomplished, the need for using markers is eliminated. Diets high in fiber can be fed to the animals without difficulty (Sauer and de Lange, 1992). However, because of the lack of a functional colon, the absorption of sodium and magnesium is impaired (Köhler et al. 1992b; Hennig et al., 1997), and weights of liver, spleen, kidneys, and adrenal glands may be higher in IRA pigs than in intact pigs (Köhler et al. 1992b; Salgado et al., 2002). Lower daily growth rates and gain to feed ratios in IRA pigs also have been reported as compared to intact animals (Köhler et al. 1992a). Apparent and true ileal digestibility coefficients for most amino acids were 2 to 8% lower in IRA pigs compared to pigs prepared with a T-cannula (Leterme et al., 1990; Köhler et al., 1991). An effect of time after surgery on endogenous losses of amino acids was also reported, indicating that the microbial population in the small intestine may adapt to the loss of the colon (Hess and Seve, 1999). Finally, some ethical and animal welfare concerns have been raised with the IRA procedure (Moughan, 2003), and so far, this technique has not been used in North America in experiments involving pigs.

An adaptation to the IRA-technique is often used to gain access to ileal fluids in poultry. Because of the short colon in poultry, the importance of the microbial fermentation in this part of the GI-tract is thought to be negligible. On the other hand, substantial quantities of microbes are present in the ceca of poultry. Therefore, a method for removing the ceca has been developed (Parsons, 1985; Green and Kiener, 1989). This procedure is known as the precision-fed cecectomised cockerel procedure and involves surgical removal of the ceca and total collection of excreta from birds fed experimental diets. The nitrogen containing fractions of the urine are neglected (van Leuwen et al., 2000).

The mobile nylon bag technique

The earliest studies of digestion in livestock and humans involved the oral administration of small perforated metal tubes or linen bags filled with feeding materials (Reaumur, 1756, cited from: Sauer et al., 1983; Spallanzani, 1782, cited from: Sauer et al., 1983). More recently, Petry and Handlos (1978) tried to repeat these early experiments using small nylon bags. Results from this experiment yielded digestibility coefficients that were considerably higher than those obtained by conventional methods, presumably because of a prolonged retention time in the stomach. Sauer et al. (1983) introduced the so-called Mobile Nylon Bag Technique (MNBT). Using this technique, feed ingredients are placed in small nylon bags that are inserted into the small intestine through a duodenal cannula, thus overcoming the problem of extended retention time in the stomach. By doing so, it became necessary to predigest the samples in HCl and pepsin. The contents of the bags are digested during the passage of the intestinal tract. The bag is eventually recovered in the feces

and any undigested material can be analyzed. If the technique is combined with the IRA, small intestine nutrient disappearance may be calculated (Viljoen et al., 1997; Yin et al., 2002).

Factors that have been shown to influence the accuracy, with which digestibility coefficients can be determined using the MNBT, include the pre-digestion time, the pepsin concentration, the pore size of the nylon bags, and the particle size of the feed stuff in the bag (Cherian et al., 1988, 1989; Yin et al., 2002). The bags recovered in the feces should not be washed prior to analysis (Qiao and Thacker, 2001). Results obtained with this technique have varied. The estimated digestibility of organic matter and DM has been reported to be lower using the MNBT compared to conventional methods (Taverner and Campbell, 1985). Digestibility coefficients for cereal grains obtained by MNBT are lower than those obtained in conventional studies, but a good agreement has been shown for protein concentrates (Sauer et al., 1989; de Lange et al., 1991).

The slaughter technique

The simplest way of collecting ileal contents is by removing the ileum from the animals and collecting the ileal contents. Using this technique, the animals are usually fed the experimental diets for 5 to 7 d, and a marker is included in the diet. The ileum is then removed from the animals under anesthesia to minimize the shedding of epithelial cells into the gut lumen which may occur with electrical stunning (Batterham, 1994). The animals are sacrificed after the ileum has been removed. The contents of the distal 20 to 150 cm of the ileum is flushed out using distilled water or physiological saline (Butts et al., 1992), and the digestibility of amino acids is calculated using the chromium content as a marker. Because digesta can be sampled only once in each animal, the timing of sampling relative to feeding is crucial. Donkoh et al. (1994) concluded that the lowest variance in the estimated digestibility coefficients were obtained if sampling occurred 9 h after the start of feeding. The length of the terminal ileum that is sampled seems to be of little importance. In pigs, any distance up to 140 cm from the ileo-cecal valve can be used without influencing the digestibility coefficients (Kies et al., 1986; Donkoh et al., 1994). A similar technique has been described for broiler chickens (Garcia et al., 2007).

The main advantages of using the slaughter technique are that it is a relatively quick way of sampling the animals and no surgical procedures are involved (Batterham, 1994). The major disadvantages of the procedure are that only one sample can be obtained per animal and the potential difficulty in obtaining a representative sample (Moughan, 1993). In two experiments, values for AID obtained using the slaughter technique were compared to those obtained in T-cannulated pigs. In both cases, there was an excellent agreement between the results obtained using either technique (Moughan and Smith, 1987; Donkoh et al., 1994). However, in broiler chickens, values obtained with the slaughter technique are sometimes different from values obtained with cecectomized roosters, but it is possible this is a result of the different ages between broilers and roosters (Garcia et al., 2007).

APPARENT ILEAL AMINO ACID DIGESTIBILITY

In the early work by Kuiken and Lyman (1948), apparent total tract digestibility coefficients were measured by analyzing the amino acid contents of the fecal material and subtracting this from the dietary content of amino acids. However, it has been demonstrated that amino acids may be digested by microbes in the large intestine and subsequently turned into microbial protein that is excreted in the feces (Darragh et al., 1991). The microbes may also deaminate some amino acids in the large intestine with a subsequent production and absorption of ammonia that is excreted as urea in the urine (Just et al., 1981; Wünsche et al., 1982). Therefore, the amino acid composition of undigested proteins is altered in the large intestine and total tract digestibility coefficients often over- or under-estimate amino acid absorption.

To avoid the manipulation by microbes in the hind gut, Cunningham et al. (1962) developed and described a cannula that was surgically inserted into the distal ileum of growing pigs. Using this technique, it was possible to collect ileal fluids at the distal ileum and, thus, study the digestion of protein prior to the end of the small intestine. This led to the calculation of ileal digestibility coefficients. Because the digesta collected at the distal ileum contain protein and amino acids of endogenous origin along with undigested feed protein, these values are most correctly referred to as apparent ileal digestibility coefficients (**AID**; Nyachoti et al., 1997; Mosenthin et al., 2000; Stein et al., 2007). It has been reported that AID have a higher correlation with deposited protein in growing pigs than have total tract digestibility coefficients (Just et al., 1985). It also has been demonstrated that diets formulated on the basis of AID more accurately predict the digestibility of amino acids compared with diets formulated on the basis of total dietary amino acid concentrations (Tanksley and Knabe, 1984). It is, therefore, generally accepted that AID more precisely reflect the feeding value of dietary proteins than do values based on total tract digestibility (Sauer and de Lange, 1992; Batterham, 1994). For that reason, AID have been measured in most commonly used feedstuffs, and summaries of these values have been presented (Sauvant et al., 2004; NRC, 2012).

Methodologies for calculating apparent ileal digestibility values

The simplest and easiest method for calculating values for AID is the so-called direct method. Using this approach, the assay feedstuff provides all the nitrogen and amino acids in the assay diet, and the AID values are measured directly in the diet. In experiments in which the AID of cereal grains are determined (e.g. Lin et al., 1987; Green et al., 1987; Stein et al., 1999a; Pedersen et al., 2007), the feed ingredient under investigation usually provides 90 to 97% of the assay diet with the remaining part of the diet being provided by non-protein additives such as vitamins and minerals, oil, and possibly sucrose. Where AID are to be determined in protein concentrates (e.g., Green and Kiener, 1989; Fan et al., 1996; Stein et al., 1999a; Cervantes Pahm and Stein, 2008; Gonzalez-Vega and Stein, 2012; Sulabo and Stein, 2013), the test feed ingredient usually provides only 20 to 50% of the total diet, and starch and sucrose are used as non-protein energy source in these diets. The direct method has been widely used in digestibility experiments for a wide variety of feed ingredients, and the majority of the AID values provided in the literature are obtained using this method. However, the calculated AID of a feed ingredient are dependent on the crude protein level in the assay diet, and the digestibility values tend to show a quadratic-plateau response to increasing dietary crude protein levels (Eggum, 1973; Donkoh and Moughan, 1994; Fan et al., 1994;

Eklund et al., 2010). Therefore, it is preferable that assay diets contain at least 14 to 16% crude protein. If the dietary protein concentration is lower, the digestibility values may be underestimated. It is usually not possible to formulate diets containing cereal grains that contain 14 to 16% CP and AID values for cereal grains, are, therefore, usually underestimated. However, when these cereal grains are included in diets containing protein ingredients, such as soybean meal, diets with greater concentrations of crude protein are formulated, but because of the underestimation of the values for AID in the cereal, values for AID are not additive in mixed diets (Stein et al., 2005).

An alternative procedure that eliminates these concerns is the so-called difference method. Using this approach a basal diet and an assay diet are formulated. The basal diet contains the basal protein-containing feed ingredient, and the assay diet consists of a mixture of the basal diet and the assay feed ingredient (Fan and Sauer, 1995a; b). The mixture is formulated to ensure that the crude protein concentration in the assay diet is at least 14 to 16%. The digestibility value of the assay feed stuff is calculated by difference, assuming there is no interaction between the digestibility values in the basal and the assay feed ingredient. However, the reliability of the digestibility values obtained with the difference method depends on the level of contribution of each amino acid from the assay feed ingredient; the higher the contribution of each amino acid, the more reliable are the results (Fan and Sauer, 1995a). The difference procedure is often used to calculate AID in feed ingredients with low palatability or with very high fiber concentrations (Knabe et al. 1989; Sulabo et al., 2013).

ILEAL ENDOGENOUS LOSSES OF AMINO ACIDS

The protein and amino acids collected at the distal ileum of an animal contains not only undigested dietary protein and amino acids, but also protein of endogenous origin. The calculated AID, therefore, underestimate the true digestibility of the dietary protein. To obtain true digestibility coefficients of dietary proteins, the quantities of protein and amino acids collected at the distal ileum needs to be separated into undigested dietary proteins and proteins and amino acids of endogenous origin. This is usually accomplished by estimating the endogenous portion of the ileal output. By subtracting this portion from the total output, the quantities of undigested feed protein may be estimated.

Origin and composition of endogenous protein and amino acids.

Endogenous nitrogen mainly consists of nitrogen from digestive enzymes, mucoproteins, desquamated cells, serum albumin, peptides, free amino acids, amines and urea (Moughan and Schuttert, 1991). The main sources of endogenous protein are saliva, gastric secretions, pancreatic juice, bile acids, and intestinal secretions (Low and Zebrowska, 1989; Tamminga et al., 1995). The intestinal secretions account for more than 60% of total endogenous secretions (Low and Zebrowska, 1989). These consist mainly of desquamated epithelium cells and mucin secreted by the goblet cells as well as other glycoconjugates secreted by the enterocytes (Lien et al., 1997b). Saliva and gastric-, pancreatic-, and bile secretions each contribute 8 to 10% of total endogenous output. It has been estimated that 70 to 80% of the endogenous proteins that are secreted into the GI-tract of an animal are hydrolyzed and re-absorbed before reaching the distal ileum (Souffrant et

al., 1993; Krawielitzki et al., 1994; Fan and Sauer, 2002). The remaining endogenous nitrogen is mainly from deconjugated bile salts and mucin glucoprotein, because these components are largely resistant to proteolysis and, therefore, escape re-absorption (Taverner et al., 1981; Moughan and Schuttert, 1991; Lien et al. 1997b). Glycine accounts for more than 90% of the total amino acid content of bile acid, and mucin glycoprotein is rich in proline, glutamic acid, aspartic acid, serine, and threonine. There is also evidence that proline, glycine, threonine, serine, aspartic acid, and glutamic acid are more slowly absorbed from the intestinal lumen than are most other amino acids (Taverner et al., 1981). These amino acids are mainly absorbed as constituents of small peptides and subsequently hydrolyzed intracellularly in the enterocyte. However, this process is slow and, therefore, the net absorption rates of these amino acids are lower than those of other amino acids (Holmes et al., 1974). It also has been suggested that the activity of pyrroline-5-carboxylate reductase (the enzyme that catalyzes proline synthesis) is higher than that of the proline-degrading enzyme, proline oxidase (Mariscal-Landin et al., 1995). Therefore, proline will accumulate in the enterocytes and diffuse into the lumen. Gardner (1975) provided evidence for a substantial flux of proline and glycine from the enterocytes into the intestinal lumen. Because of these mechanisms, endogenous protein usually has a high content of proline, glycine, threonine, serine, aspartate, and glutamate. Several estimates of the amino acid composition of endogenous protein have been published (Table 2; Wünche et al., 1987; Boisen and Moughan, 1996a; Stein et al., 1999b).

Techniques used to measure endogenous losses

Endogenous protein was originally defined as the amount of protein excreted in the feces of animals fed a protein-free diet (Mitchell, 1924). Due to the modification of protein that takes place in the hind gut, digesta are now being collected at the distal end of the ileum, and the amount of protein and amino acids excreted at this point after the ingestion of a protein-free diet is considered being of endogenous origin. This method is the most commonly used procedure for estimating endogenous protein. However, a fair amount of criticism has been directed towards this procedure. Because of the "unphysiological" nature of the protein-free state, it has been suggested that the amount of protein secreted into the ileum after feeding such a diet does not accurately represent the amount of protein secreted when a protein-containing diet is fed (Low, 1980; Moughan et al., 1992a, Moughan, 2003). In several experiments, it has been concluded that the protein-free diet underestimates the endogenous losses of amino acids (de Lange et al., 1990; Moughan and Rutherfurd, 1990; Butts et al., 1991; Donkoh et al., 1995; Hodgkinson et al., 2000). However, careful examination of published data does not support the hypothesis of a systematic underestimation of the endogenous protein and amino acid losses following the feeding of a protein-free diet. On the other hand, the fact that standardized digestibility coefficients for glycine and proline exceeding 100% have been calculated (e.g., Sauer et al., 1977; Stein et al., 2001) clearly indicates that the endogenous losses of these two amino acids are overestimated after the ingestion of a protein free-diet. However, Taverner et al. (1981) suggested that glycine and proline are exceptional, and that the endogenous losses of the other amino acids can be accurately estimated after feeding a protein-free diet. Data from Pedersen et al. (2002) provided strong evidence to support this hypothesis and the protein-free diet is therefore often recommended for determination of basal endogenous losses of protein (Stein et al., 2007).

To alleviate the possible negative impact of feeding a protein-free diet, Moughan et al. (1990) suggested that endogenous losses be estimated by the so-called peptide alimentation technique. Using this approach, the animals are fed a protein-containing diet with casein being the only source of amino acids and nitrogen. An enzymatically hydrolyzed casein (EHC) consisting of a mixture of free amino acids and low MW (< 5,000 DA) oligopeptides is used. This allows for the separation of endogenous protein and any undigested dietary proteins in the digesta by centrifugation followed by ultrafiltration. All amino acids of dietary origin are supposed to be contained in the low molecular weight supernatant (MW < 10,000 DA), while amino acids of endogenous origin are captured in the high molecular weight precipitate plus retentate (MW > 10,000 DA). In three experiments, estimates for endogenous losses in young pigs fed either a protein-free diet or an EHC-diet were compared, and higher losses were obtained in pigs fed the EHC-diet as opposed to the protein-free diet (Moughan et al., 1992a; Butts et al., 1993a; Leterme et al., 1996b). However, the possibility that the casein peptides are not completely separated out of the digesta using this procedure has been raised (Seve and Henry, 1995).

Because amino acids from intact casein are thought to be 100% absorbed prior to the distal ileum it has been suggested that a diet based on intact casein rather than an EHC-diet may be used to measure endogenous losses in pigs (Leterme et al., 1996b). Although this procedure has been used in several experiments (Traylor et al., 2001; Fastinger and Mahan, 2002) it still needs to be experimentally evaluated. There is evidence that not all amino acids are completely absorbed prior to the distal ileum (Chung and Baker, 1992; Souffrant et al., 1997; Pedersen et al., 2002). If this is true, then this approach will lead to an overestimation of endogenous losses for some amino acids.

Determining the endogenous losses of amino acids by linear regression was proposed by Carlson and Bayley (1970). Using this approach, a series of diets containing graded levels of protein are formulated and fed to pigs. The linear relationship between the ileal output of amino acids and the dietary input is then established, and the endogenous level of amino acids is estimated by extrapolation back to zero protein intake. The endogenous losses of amino acids obtained using the regression technique are close to those obtained using a protein-free diet (Taverner et al., 1981; Mariscal-Landin et al., 1995; Pedersen et al., 2002). Because at least three diets are needed, it is more time consuming and costly to use the regression techniques than to use the protein-free diet.

The use of homoarginine for the estimation of true amino acid digestibilities was suggested by Hagemeister and Ebersdobler (1985). The procedure involves the transformation of dietary lysine into homoarginine. Body protein does not contain homoarginine and dietary homoarginine absorbed from the gastrointestinal tract is assumed to be completely metabolized in the liver by arginase yielding urea and lysine. Therefore, homoarginine is assumed not to be incorporated into endogenous protein (Hagemeister and Ebersdobler, 1987; Rutherfurd and Moughan, 1990; Roos et al., 1994), and all homoarginine recovered at the distal ileum is considered of dietary origin. Therefore, the true homoarginine digestibility can be calculated. By assuming that the portion of dietary lysine that was not converted to homoarginine was absorbed at a similar rate, the endogenous losses of lysine can be calculated. It is also assumed that homoarginine *per se* does not affect any digestive processes or animal behavior, and that homoarginine is not preferentially metabolized by the gut microflora (Imbeah et al., 1996). These assumptions have all been

evaluated (Smitz et al., 1991; Siriwan et al., 1994). The major advantage of using the homoarginine technique is that the true digestibility of lysine can be measured directly. However, there are several major drawbacks associated with this technique. In chickens and rats, feed intake is markedly depressed in animals fed diets containing homoarginine (Tews and Harper, 1986; Moughan and Rutherfurd, 1991; Angkanaporn et al., 1997). Because the level of feed intake significantly affects endogenous amino acid output in pigs (Butts et al., 1993b; Stein et al., 1999b; Moter and Stein, 2004), any decrease in feed intake caused by homoarginine would seriously devaluate the validity of data obtained using this technique. In addition, only the endogenous losses of lysine can be directly estimated by this procedure. To obtain data for the remaining amino acids, the assumption that all amino acids are absorbed at the same rate as homoarginine has to be made (Imbeah et al., 1996; Caine et al., 1997). The validity of this assumption is questionable and needs to be experimentally verified.

Labeling of dietary protein using ^{15}N was introduced by Krawielitski et al. (1977) as a mean of separating amino acids of dietary and endogenous origin. By collecting ileal digesta from animals fed ^{15}N-labeled proteins, the digestibility of amino acids can be calculated. The technique has been used in humans (Mahe et al., 1994; Gausseres et al., 1997) and in animals (Roos et al., 1994; Leterme et al., 1996a; Souffrant et al., 1997). However, values obtained with this technique may underestimate the true absorption of amino acids because of rapid incorporation of dietary amino acids into endogenous protein. Leterme et al. (1996a) showed that labeled dietary nitrogen in pigs appeared in the blood within 10 min. after feeding the labeled meal. After 50 min, the label appeared in pancreatic enzymes, after 90 min in bile, and after 4 h in ileal mucins. This recycling of the labeled dietary protein seems to seriously compromise the use of this technique.

As an alternative to labeling the dietary nitrogen, the animal's nitrogen pools can be labeled. Oral administration of $^{15}NH_4$ salts for a prolonged period of time can uniformly label the animal's tissue, plasma, and urine after stopping the oral doses (Krawielitski et al., 1990; Bartelt et al., 1994). Using this approach, the endogenous amino acids are labeled, while the dietary amino acids are unlabelled, thus allowing for a separation in ileal digesta. The same principle was introduced by Souffrant et al. (1981, 1986), but instead of orally administrating the label, the animals were intravenously infused with ^{15}N-leucine for 8 days. By assuming that the ^{15}N-enrichment in the TCA-soluble fraction of blood is similar to that in endogenous protein, the contribution of endogenous protein to the ileal output of protein can be calculated. This technique only measures the recovery of total endogenous nitrogen and a few other amino acids in ileal digesta, and not the recovery of all the individual amino acids (de Lange et al., 1990). Only if the amino acid composition of endogenous protein is assumed to be constant, and that values obtained after feeding a protein-free diet are also representative of the composition of endogenous protein after feeding a protein-containing diet, can the endogenous contribution of individual amino acids be calculated (de Lange et al., 1990). Two basic requirements of this technique have to be met: attainment of steady-state and the choice of the right precursor pool (Moughan et al., 1992b). Usually, 7 to 10 days of infusion are sufficient to obtain a steady-state condition in blood (de Lange et al., 1990; Schulze et al., 1995a). The TCA-soluble fraction of systemic blood has been used as the precursor pool in most experiments involving this technique. However, Hess et al. (1997) showed that after 23 d of infusion, the ^{15}N-enrichment in systemic blood was 50 to 100% higher than in liver and pancreatic

tissues as well as in intestinal mucosa and serosa. This observation indicates that the enrichment in systemic blood may not be representative for the enrichment in cells synthesizing endogenous protein. An accurate estimate of the enrichment in intestinal mucin is crucial because mucin protein contributes a major part of total endogenous losses. However, endogenous proteins synthesized from dietary protein within the enterocytes are not labeled, which may lead to inaccuracies. The fact that Hess et al. (1997) reported that the ^{15}N-enrichment in intestinal mucosa and serosa were considerably lower than in systemic blood suggests that this may be of great importance. The time of blood sampling seems to be important for an accurate estimate of the ^{15}N-enrichment in the precursor pool. By sampling hourly over a 12 h period instead of only once after feeding, a closer agreement between the ^{15}N-enrichment in blood and mucin was obtained (Lien et al., 1997a). It has also been suggested that the enrichment of portal blood is more representative for the enrichment in secretory tissue than the enrichment in systemic blood (Hess et al., 1997). An overestimation of the ^{15}N-enrichment in the precursor pool could potentially lead to an underestimation of total endogenous losses as indicated by the very low estimates of endogenous losses reported in recent experiments (Mosenthin et al., 1993; Gabert et al., 1997).

Because the ^{15}N-label from leucine can be transaminated and incorporated into valine and isoleucine and all the dispensable amino acids, the endogenous output can be estimated based on the ^{15}N-enrichment in various pools. However, more than a two-fold difference in the estimates of endogenous protein and amino acid has been reported depending on whether the estimates were based on the enrichment in plasma nitrogen, plasma leucine, or plasma isoleucine (de Lange et al., 1992; Lien et al., 1997a; c). Because the highest enrichment is usually obtained in the leucine pool, the lowest estimates for endogenous output are reached if the calculations are based on leucine, and the highest estimates are reached if the calculations are based on total nitrogen or isoleucine. The use of ^{15}N-leucine as a marker for the whole endogenous nitrogen pool also has been questioned, because of the unique metabolism of the branched chained amino acids (Leterme et al., 1997). These authors suggested that a multiple ^{15}N-labeled amino acid infusion should be used rather than ^{15}N-leucine to get accurate estimates for endogenous protein losses.

In conclusion, several obstacles and uncertainties are associated with the use of the isotope dilution technique, and some of the results obtained with this technique have to be questioned (Leterme et al., 1997). Therefore, changes to the current methodology are necessary before accurate estimates of endogenous output of protein and amino acids can be obtained using this technique (Lien et al. 1997c; Leterme et al., 1998; Moughan, 2003).

Partitioning of endogenous losses

The fact that an animal fed a protein-free diet continues to lose protein at the distal ileum indicates that a certain amount of protein will always be lost regardless of the diet fed. This portion of the ileal output is of endogenous origin and can be referred to as the basal or non-specific endogenous loss (Seve et al., 1994; Jansmann et al., 2002; Stein and Nyachoti, 2003; Stein et al., 2007), or the minimum endogenous loss (Nyachoti et al., 1997). The basal endogenous protein and amino acid loss is believed to be largely affected by the feed intake of the animal (Butts et al., 1993b; Moter and Stein, 2004). However, the basal loss may also be influenced by the BW or the age of the animal, in particular at low levels of DMI (Nyachoti et al., 1997).

In addition to the basal loss of endogenous protein, most feed ingredients induce a specific endogenous loss (Seve et al., 1994; Boisen and Moughan, 1996b; Jansmann et al., 2002). This portion of endogenous losses is induced by components in the feed ingredient itself, and varies among feed ingredients (Seve et al., 1994). The dietary fiber content and the level of anti-nutritional factors are responsible for the largest part of the specific endogenous losses (Stein et al., 2007).

The protein-free diet, the peptide alimentation technique, and the regression technique all estimate the basal endogenous losses. The homoarginine and the isotope dilution techniques estimate the total endogenous losses, i.e. basal and specific losses combined (Stein et al., 2007).

STANDARDIZED AND TRUE ILEAL DIGESTIBILITY

Because of the problems associated with estimating AID in feed ingredients that are low in protein concentration, questions about the additivity of AID obtained in individual feed ingredients in a mixed diet have been raised (Boisen and Moughan, 1996b; Jansman et al., 2002; Stein et al., 2005). To ameliorate this problem values for AID may be corrected for the basal endogenous protein and amino acid loss (Jondreville et al., 1995; Jansman et al., 2002). By doing so, the effect of the dietary CP content is removed, and values that more correctly represent the digestibility of the feed ingredient can be derived. Such values are described by the term "standardized ileal digestibility coefficients" (SID). Because these values are not corrected for the specific endogenous losses, they should not be referred to as true digestibility coefficients (Nyachoti et al., 1997; Stein et al., 2007). Values for SID are calculated by subtracting the basal endogenous losses from the ileal output of amino acids, and this value is then expressed in relation to the dietary input of amino acids.

Under certain circumstances, it may be necessary to estimate the true amino acid digestibility of a feed ingredient. To do so, the total endogenous output (basal and specific) has to be subtracted from the ileal output of protein, and the calculated difference then represents the undigested dietary protein. Alternatively, if the dietary proteins are labeled, then they can be separated from the endogenous protein in ileal digesta. By relating the undigested feed protein to the dietary input, the true digestibility coefficients of the amino acids can be calculated. Because the specific endogenous output cannot be determined by feeding a protein-free diet or by regression analysis, true digestibility coefficients can only be calculated by way of the homoarginine technique or the isotope dilution technique. The term "true digestibility" has sometimes (i.e., Chung and Baker, 1992; NRC. 1998) incorrectly been used to describe SID. True digestibility has also sometimes been referred to as real digestibility (de Lange et al., 1990).

FACTORS AFFECTING DIGESTIBILITY COEFFICIENTS

The effect of age and physiological status on amino acid digestibility

Whereas young pigs are highly efficient in digesting milk proteins (Mavromichalis et al., 2001), amino acid digestibility values for soybean protein are low in young pigs, but increases with the age of the animal (Wilson and Leibholz, 1981; Caine et al., 1997). The reason for the low digestibility of proteins of vegetable origin in young pigs is that the activity of some of the protein digesting enzymes is low in early life (Moughan, 1993).

It has been reported that the AID are similar in restricted-fed gestating sows and growing pigs given free access to feed, but the SID of AA were greater in gestating sows than in growing pigs (Stein et al., 1999a; 2001). The reason for this difference was that gestating sows had greater endogenous losses than did the growing pigs (Stein et al., 1999b) because of a lower feed intake. In contrast, lactating sows which were allowed ad libitum access to feed have SID values that are not different from the values for growing pigs (Stein et al., 2001). Therefore, it was concluded that the physiological status of the animal as such does not influence amino acid digestibility.

Effect of level of feed intake on amino acid digestibility

The AID of protein and amino acids will increase as feed intake increases from a level that is close to the maintenance requirement for energy and to an amount that is approximately twice that level (Moter and Stein, 2004). However, further increases in feed intake do not change the AID (Sauer et al., 1982; Haydon et al., 1984; Albin et al., 2001b; Moter and Stein, 2004; Chastanet et al., 2007). The reason for the reduced AID by animals fed at a low level of feed intake is that the basal endogenous losses of amino acids at the end of the small intestine are elevated in animals on a low level of feed intake (Butts et al., 1993b; Stein et al., 1999b; Moter and Stein, 2004). Because the SID are calculated by correcting AID for the basal endogenous losses, values for SID are linearly decreased as feed intake is increased (Moter and Stein, 2004). Thus, due to the impact of the endogenous amino acid losses, the influence of the level of feed intake on SID are opposite of the influence of feed intake on the AID. Later research documented that pigs fed at a level of at least 3 times the estimated energy requirement will have AID values of AA that are not different from that of pigs allowed ad libitum access to feed (Chastanet et al., 2007). It was also observed that values for AID are not influenced of whether the feed is provided in one meal per day or divided into 2 meals (Chastanet et al. 2007). The implication of these observations is that growing pigs and lactating sows that are used to measure amino acid digestibility values should be allowed ad libitum access to their diets because this is usually the way they are fed under commercial conditions. When amino acid digestibility values are measured for gestating sows, the animals should be restricted in their feed intake to reflect the feed intake of gestating sows kept under commercial conditions.

Effect of chemical composition of the feed ingredient or diet

Several dietary factors have been shown to influence the digestibility of amino acids. The concentration of protein and crude protein in the assay diets influences the AID as was discussed

above. The level of dietary fiber MAY decrease AID (Mosenthin et al., 1994; Lenis et al., 1996), but in other experiments, no effect of the addition of fiber was observed (Sauer et al., 1991; Li et al., 1994). The reason for this disagreement may be that different sources of fiber were used. Mosenthin et al. (1994) included pectin in the diets, while sources of cellulose were used in the other experiments. Soluble fibers such as pectin usually reduce nutrient digestibility in the small intestine, but insoluble fibers have only a minor effect on nutrient absorption prior to the distal ileum (Eggum, 1995). The reason for the decreased AID in diets containing soluble fibers may be that the endogenous losses of amino acids and amino sugars increase as the levels of fiber increase (Schultze et al., 1994; Seve et al., 1994; Schultze et al., 1995c). The source of fiber may also affect the endogenous losses of amino acids (Leterme et al., 1996c) as may the viscosity of fiber (Larsen et al., 1993). In an experiment involving three sources of insoluble fiber, Mariscal-Landin et al. (1995) observed that endogenous losses of amino acids were affected by the dietary fiber level until approximately 4% fiber was included in the diet. At higher fiber levels, no further increase in endogenous losses was observed. Similar observations have been made in other experiments (de Lange et al., 1989; Leterme et al., 1992).

Dietary fat also increases values for AID and SID of AA (Imbeah and Sauer, 1991; Li and Sauer, 1994; Albin et al., 2001a; Cervantes-Pahm and Stein, 2008; Kil et al., 2011). The reason for this increase is thought to be an increased retention time in the small intestine for diets high in fat, thus giving the proteolytic enzymes more time to hydrolyze dietary proteins. There is, however, no influence of dietary fat on the endogenous losses of amino acids (de Lange et al., 1989).

Effects of anti-nutritional factors on ileal amino acid digestibility have been investigated in several experiments. Inclusions of trypsin inhibitors in diets based on pea protein decreased the AID of nitrogen (le Guen et al., 1995; Goebel and Stein, 2011). This reduction in digestibility is caused by an increased loss of endogenous and exogenous protein and amino acids (Barth et al., 1993). Likewise, soybean lectins (Schulze et al., 1995b) and polyphenols (Shahkhalili et al., 1990) have been shown to increase endogenous losses of protein and thereby decrease AID. The effects of dietary levels of tannins were investigated in experiments with rats. Jansman et al. (1994) observed an increased secretion of proline-rich proteins from the parotid glands as tannins from faba beans were included in the diets. These proteins interact with dietary condensed tannins to reduce their anti-nutritional effects. However, they are not being completely hydrolyzed and re-absorbed before reaching the end of the ileum, thus increasing the endogenous losses of protein and decreasing the apparent ileal digestibility. Yu et al. (1996) also reported decreased AID of protein and amino acids as cottonseed condensed tannins were included in the diets. It may, therefore, be concluded that dietary anti-nutritional factors increases the specific endogenous losses of amino acids. Therefore, both the AID and the SID are reduced in diets or feed ingredients containing such factors.

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USE OF DIGESTIBILITY VALUES

Formulating diets based on AID is practiced in several countries. These values take into account that differences between feed ingredients exist. Feed ingredients with low digestibility coefficients are not overestimated using this approach. The additional endogenous losses introduced by high concentrations of fiber or anti-nutritional factors are debited against the feed ingredient and credit is given only to the portion of the feed ingredient that is digested and absorbed. Therefore, values for AID represent the net absorption of amino acids from a given feed ingredient. The main criticism of using AID in diet formulations is the underestimation of amino acid digestibility in feed ingredients with low protein concentrations (Mosenthin et al., 2000; Jansman et al., 2002; Stein et al., 2007). This underestimation impairs the additivity of digestibility values in mixed diets (Jansman et al., 2002; Stein et al., 2005).

To overcome the problems with a lack of additivity for AID, values for SID may be used. Because the basal endogenous losses are disregarded in the calculations of SID, these values are additive in mixed diets (Stein et al., 2005). Values for SID are, therefore, the values that should be used in practical feed formulation (Stein et al., 2007) and these values are, therefore, usually used in diets fed to pigs and poultry.

CONCLUSIONS

Valuable progress has been made during the last few decades in our understanding of amino acid digestibility. Estimating amino acid absorption is most correctly accomplished by measuring ileal digestibility values. Several techniques are available to gain access to ileal fluids with the T-cannula being the most popular choices in pigs, and the slaughter procedure or cecectomy being the methods of choice in poultry. By subtracting the amounts of amino acids in ileal digesta from the quantities of amino acids ingested by the animal, apparent ileal digestibility values are calculated. For a single feed ingredient, these values most precisely estimate the quantities of amino acids absorbed from the small intestine. However, concerns about poor additivity in a mixed diet of values for AID obtained in individual feed ingredients have been documented, and these values should, therefore, not be used in practical diet formulation. Instead, diets are most correctly formulated based on values for standardized ileal digestibility because these values are additive in mixed diets. To calculate SID values, the basal endogenous losses of protein and amino acids need to be subtracted from the total ileal output. This necessitates the estimation of basal endogenous losses. These losses may be estimated using a protein-free diet. Because the level of feed intake greatly influences the basal endogenous losses of amino acids, SID values are also influenced by the level of feed intake. It is, therefore, important that animals are fed close to their voluntary feed intake if values for basal endogenous losses or values for amino acid digestibility are determined. The only exception to this would be if values are measured in gestating sows, where it would be more appropriate to restrict the daily feed intake to approximately 1.5 times the estimated requirement for energy.

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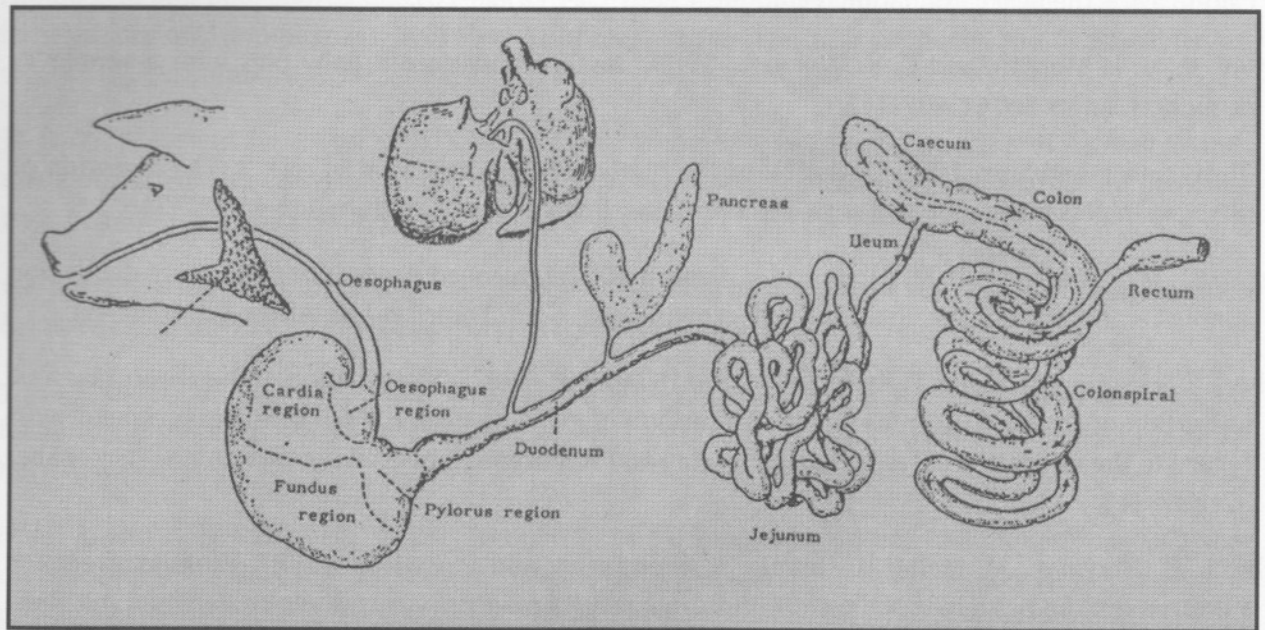
Figure 1. Digestive tract of the pig

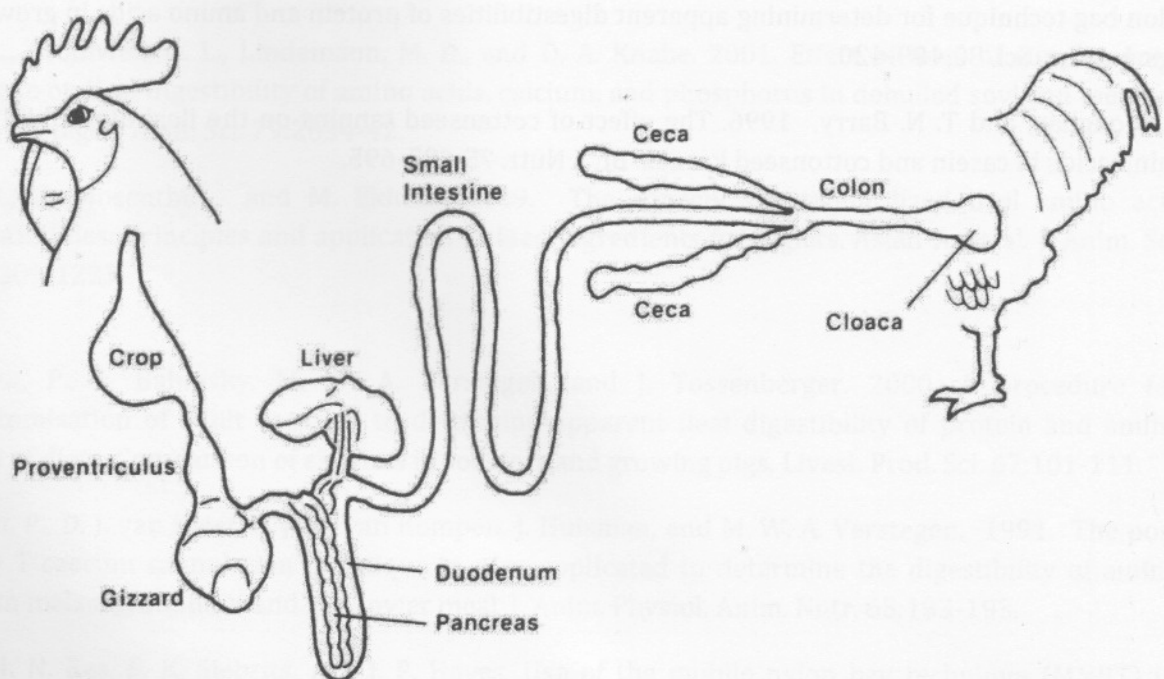**Figure 2.** Digestive tract of poultry

Table 1. Enzymes used in protein digestion

Location	Enzymes secreted
Stomach	Pepsins
Pancreas	Trypsin, Chymotrypsin, Elastase, Carboxypeptidase A & B
Small intestine, brush border	Enterokinase, Aminopeptidase
Small intestine, enterocytes	Di-peptidase, Tri-peptidase

Table 2. Composition of endogenous protein in growing pigs (from Stein et al., 1999b)

Item	%
Crude protein (N x 6.25)	100
Indispensable amino acids	
Arginine	2.93
Histidine	1.29
Isoleucine	3.19
Leucine	4.88
Lysine	3.48
Methionine	1.01
Phenylalanine	2.82
Threonine	4.35
Tryptophan	1.27
Valine	3.99
Total, indispensable amino acids	29.21
Dispensable amino acids	
Alanine	4.81
Aspartic acid	7.20
Cysteine	1.94
Glutamic acid	9.26
Glycine	6.85
Proline	14.38
Serine	3.70
Tyrosine	2.46
Total, dispensable amino acids	50.61