DIGESTIBLE AMINO ACIDS: DETERMINATION AND USE

Hans H Stein

l. INTRODUCTION

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 coefficient is calculated. Thus, digestibility coefficients 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.

Only amino acids that can be incorporated into tissue proteins are bioavailable. Bioavailability is defined as the dietary proportion of amino acids that are absorbed in a chemical form that is suitable for protein synthesis (Batterham, 1992; Lewis, 1992). It has been demonstrated that some amino acids under certain circumstances may be absorbed in a form that precludes utilization by tissues (van Barneveld et al., 1994). Therefore, not all digested amino acids are bioavailable and a distinction between the terms “digestibility” and “bioavailability” should be made (Sauer and Ozimek, 1986; Knabe, 1991, Moughan, 2003). Bioavailability of amino acids may be measured using slope ratio techniques. However, such procedures are tedious and costly. Therefore, for practical feed formulation, digestibility coefficients are measured and used as an indication of the quantities of dietary amino acids that are available to the animal.

The objective of the current contribution is to review how digestibility coefficients are measured and how they may be used in diets for non-ruminant animals.

2. APPARENT AMINO ACID DIGESTIBLITY COEFFICIENTS

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). 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 to diets formulated on the basis of total dietary amino acid contents (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 coefficients (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 (cf. Jondreville et al., 1995; NRC, 1998; Rademacher et al., 1999).

2.1 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.

2.1.1. 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.

2.1.2. 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).

2.1.3. 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.

Both the PVTC and the SICV techniques provide a reliable method for obtaining ileal fluids from pigs.

2.1.4. 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).

2.1.5. 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).

2.1.6. 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).

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, 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). Thus, this procedure seems to provide a reliable technique for determining amino acid digestibility coefficients. Especially in animals that represent a low economical value, the technique may be useful. Therefore, the slaughter technique is often used in studies involving laboratory animals.

2.2 Methodologies for calculating apparent ileal digestibility coefficients

The simplest and easiest method to use 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 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), the feed ingredient under investigation usually provides approximately 90% 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)), 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 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 coefficients tend to show a quadratic-plateau response to increasing dietary crude protein levels (Eggum, 1973; Donkoh and Moughan, 1994; Fan et al., 1994). Therefore, it is preferable that assay diets contain at least 14 to 16% crude protein. If the dietary protein concentration is lower, the digestibility coefficients may be underestimated. In experiments involving most cereal grains, it is not possible to formulate assay diets containing 14 to 16 % crude protein if the direct method is used.

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 stuff, and the assay diet consists of a mixture of the basal diet and the assay feed stuff (Fan and Sauer, 1995a and b). The mixture is formulated to ensure that the crude protein concentration in the assay diet is at least 14 to 16%. The digestibility coefficients of the assay feed stuff is calculated by difference, assuming there is no interaction between the digestibility coefficients in the basal and the assay feed ingredient. However, the reliability of the digestibility coefficients 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). Theoretically, AID in cereal grains obtained using the difference method should be higher than those obtained using the direct method. However, recent data from the author’s laboratory indicate that this is not always the case (Bohlke, 2002). Therefore, at this point it is not recommended to use the difference method for calculating AID in cereal grains. However, the procedure may be used for the calculation of AID in feed ingredients with low palatability or with very high fiber concentrations (Knabe et al. 1989).

3. 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.

3.1. 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 (Wünche et al., 1987; Boisen and Moughan, 1996a; Stein et al., 1999b).

3.2. 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. Recently, Pedersen et al. (2002) provided strong evidence to support this hypothesis.

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; Rayadurg and Stein, 2003), 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) found 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, 1997c). 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).

3.3 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), 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).

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 and Nyachoti, 2003).

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.

4. STANDARDIZED AND TRUE DIGESTIBILITY COEFFICIENTS

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). To ameliorate this problem, it has been suggested that AID 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). The 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).

5. FACTORS AFFECTING DIGESTIBILITY COEFFICIENTS

5.1 The effect of age and physiological status on amino acid digestibility

While young pigs are highly efficient in digesting milk proteins (Mavromichalis et al., 2001), amino acid digestibility coefficients 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 demonstrated that pigs heavier than 60 kg digest proteins to the same degree as do older pigs (Stein, 1998), but it is not clear when the digestive capacity of the pig reaches a plateau. Recent data suggest that AID are constant in pigs from 40 to 130 kg (Sulabo et al., 2003, unpublished).

It has been reported that the AID are similar in restricted-fed gestating sows and growing pigs given free access to feed (Stein et al., 1999a). However, the SID were higher in gestating sows than in growing pigs (Stein et al., 2001). The reason for this difference was that gestating sows had higher endogenous losses than did the growing pigs (Stein et al., 1999b) because of a lower feed intake. Therefore, it was concluded that the physiological status of the animal as such does not influence amino acid digestibility.

5.2 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 (Rayadurg and Stein, 2003). However, further increases in feed intake do not change the AID (Sauer et al., 1982; Haydon et al., 1984; Albin et al., 2001b; Rayadurg and Stein, 2003). 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; Rayadurg and Stein, 2003). Because the SID are calculated by correcting AID for the basal endogenous losses, the SID are linearly decreased as feed intake is increased (Rayadurg and Stein, 2003). 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. The implication of these differences is that growing pigs and lactating sows that are used to measure amino acid digestibility coefficients 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 coefficients 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.

5.3 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 has been shown to 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 has also been shown to significantly affect the endogenous losses of amino acids (Leterme et al., 1996c) as has the viscosity of fiber (Larsen et al., 1993). In an experiment involving three sources of insoluble fiber, Mariscal-Landin et al. (1995) found 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 has increased AID in a few studies (Imbeah and Sauer, 1991; Li and Sauer, 1994). However, these increases were only significant at relatively high levels of dietary fat (i.e. 10 to 15%). 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. Recently it was reported that the response to dietary fat to some degree may be influenced by the source of fat used in the diets (Albin et al., 2001a). No effect of the dietary level of fat on the endogenous losses of amino acids was found (de Lange et al., 1989). There are still several unanswered questions surrounding this topic and more research on the effect of dietary fat on amino acid digestibility is needed.

The 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). 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.

6. USE OF DIGESTIBILITY COEFFICIENTS

It is still relatively common to formulate diets based on the total concentrations of amino acids in feed ingredients. Where corn and soybean meal are the only amino acid contributing ingredients in the diets, this may not cause any major complications. However, where other amino acid sources are included in the formula, inaccuracies in diet formulations are introduced. This is particularly true where crystalline amino acids are used.

Formulating diets based on AID is practiced in several countries and is also gaining popularity in North America. 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, the 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). This underestimation is believed to impair the additivity of digestibility coefficients in a mixed diet (Jansman et al., 2002). However, there is only limited experimental evidence to support this hypothesis.

To overcome the problems with a lack of additivity for AID, SID may be the most appropriate digestibility coefficients to use in practical diet formulation (Jondreville et al., 1995; Nyachoti et al., 1997; Jansman et al., 2002). Because the basal endogenous losses are disregarded in the calculations of SID, these values are believed to be additive, although this assumption has not been thoroughly investigated. Nonetheless, the use of SID in feed formulations has been promoted and is gaining in popularity. The main disadvantage of this approach is that the SID do not represent the portion of the dietary amino acids that are absorbed. In addition, because of the dependence of SID on the level of feed intake, errors may be introduced if animals are fed at a level of feed intake that is different from the level used to calculate the SID (Stein et al., 1999b). Under practical feeding conditions, this may sometimes be the case, and experiments that address this particular concern need to be undertaken.

In computer models used to simulate amino acid metabolism, the endogenous cost of digestion is often predicted by the model (Batterham, 1994). Using AID under these circumstances will lead to a double correction for endogenous losses of amino acids. Therefore SID or true digestibility coefficients are the most appropriate to use under such circumstances.

Values for true digestibility coefficients should not be used in practical feed formulation because these values do not account for the specific endogenous losses induced by the individual feed ingredients. However, because there is a substantial energy cost associated with the synthesis of endogenous protein, true digestibility coefficients may be important where experiments aimed at measuring net energy values of feed ingredients are conducted. Nyachoti et al. (1997) calculated that up to 13% of the maintenance energy requirement of pigs is used for the synthesis of endogenous protein. True digestibility coefficients also are used where the absorption of specific dietary amino acid is studied. However, the accuracy with which true digestibility coefficients are estimated needs to be improved.

7. CONCLUSIONS

Valuable progress has been made during the last two decades in our understanding of amino acid digestibility. Estimating amino acid absorption is most correctly accomplished by measuring ileal digestibility coefficients. Several techniques are available to gain access to ileal fluids with the T-cannula and the PVTC cannula being the most popular choices in pigs, and cecectomy being the method 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 coefficients 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 ration of apparent ileal digestibility coefficients obtained in individual feed ingredients have been raised. Therefore, standardized ileal digestibility coefficients are now recommended (Mosenthin et al., 2000; Jansman et al., 2002). To calculate such 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, the peptide alimentation procedure, or by mathematical regression. Standardized ileal digestibility coefficients obtained in individual feed ingredients are believed to be additive in a mixed ration, but this hypothesis still needs experimental verification. Because the level of feed intake greatly influences the basal endogenous losses of animals, standardized ileal digestibility coefficients are also influenced by the level of feed intake. Nonetheless, standardized ileal digestibility coefficients are presently the method of choice to be used in diet formulations and amino acid requirement studies for non-ruminant animals.

True digestibility coefficients are calculated by correcting the ileal output of amino acids for the total endogenous losses. These losses may be estimated using the homoarginine procedure or the isotope dilution procedure. Both of these techniques have some shortcomings in their ability to precisely estimate endogenous losses and improvements in these procedures are needed. True digestibility coefficients are used in studies where the exact absorption of dietary amino acids is being measured and sometimes also in computer modeling. However, true digestibility coefficients should not be used in practical feed formulation or amino acid requirement studies. Many of the values in the literature that are reported as true digestibility coefficients are in fact standardized digestibility coefficients.

8. REFERENCES

Albin, DM., M. R. Smiricky, J. E. Wubben, and V. M. Gabert. 2001a. The effect of dietary level of soybean oil and palm oil on apparent ileal digestibility and postprandial flow patters of chromium oxide and amino acids in pigs. Can. J. Anim. Sci. 81:495-503.

Albin, DM., J. E. Wubben, M. R. Smiricky, and V. M. Gabert. 2001b. The effect of feed intake on ileal rate of passage and apparent amino acid digestibility determined with or without correction factor in pigs. J. Anim. Sci. 79:1250-1258.

Angkanaporn, K., V. Ravindran, Y. Mollah, and W. L. Bryden. 1997. Homoarginine influences voluntary feed intake, tissue basic amino acid concentrations and arginase activity in chickens. J. Nutr. 128:1128-1136.

Ball, R. O. 2002. Definition of amino acid requirements in pigs: Partitioning between gut and muscle. Canadian Society of Animal Science – SCSA, July 21, 2002, Quebec City, Canada, p 17-25.

Bartelt, J., H. Bergner, W. Drochner, C. Kijora, and K. P. Gotz. 1994. Untersuchungen zum Einfluss einer Unterschiedlichen Protein- und Faseraufnahme auf den Ilealen Fluss an Endogenem Protein bei ^{15}N-markierten Schweinen. Arch. Anim. Nutr. 46:305-319.

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

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

Batterham, E. S. 1994. Ileal digestibilities of amino acids in feedstuffs for pigs. P. 113-131 In D'Mello, J. P.F (Ed.): Amino Acids in Farm Animal Nutrition. CAB International, Wallingford, UK.

Bohlke, R. A. 2002. Calcium, phosphorus, and aminoacid digestibilities in low phytate corn by growing pigs. Masters Thesis. South Dakota State University. 66 pp.

Boisen, S. and P. J. Moughan. 1996a. Dietary influences on endogenous ileal protein and amino acid loss in the pig - A review. Acta Agric. Scand., Sect A, Animal Sci. 46:154-164.

Boisen, S. and P. J. Moughan. 1996b. Different expressions of dietary protein and amino acid digestibility in pig feeds and their application in protein evaluation: A theoretical approach. Acta. Agric. Scand. Sect. A, Anim. Sci. 46:165-172.

Butts, C. A., P. J. Moughan, and W. C. Smith. 1991. Endogenous amino acid flow at the terminal ileum of the rat determined under conditions of peptide alimentation. J. Sci. Food Agric. 55:175-187.

Butts, C. A., P. J. Moughan, and W. C. Smith. 1992. Composition of endogenous ileal digesta nitrogen from the rat - the use of distilled water for digesta collection. J. Sci. Food Agric. 59:415-417.

Butts, C. A., P. J. Moughan, W. C. Smith, and D. H. Carr. 1993a. Endogenous lysine and other amino acid flows at the terminal ileum of the growing pig (20 kg body weight): The effect of protein-free, synthetic amino acid, peptide and protein alimentation. J. Sci. Food Agric. 61:31.

Butts, C. A., P. J. Moughan, W. C. Smith, G. W. Reynolds, and D. J. Garrick. 1993b. The effect of food dry mater intake on the endogenous ileal amino acid extraction determined under peptide alimentation in the 50 kg liveweight pig. J. Sci. Food Agric. 62:235-243.

Caine, W. R., W. C. Sauer, S. Taminga, M. W. A. Verstegen, and H. Schulze. 1997. Apparent ileal digestibilities of amino acid in newly weaned pigs fed diets with protease-treated soybean meal. J. Anim. Sci. 75:2962-2969.

Carlson, K. H. and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100:1353-1362.

Cherian, G., W. C. Sauer, and P. A. Thacker. 1988. Effects of predigestion factors on the apparent digestibility of protein for swine determined by the mobile nylone bag technique. J. Anim. Sci. 66:1963-1968.

Cherian, G., W. C. Sauer, and P. A. Thacker. 1989. Factors affecting the apparent digestibility of protein for swine when determined by the mobile nylon bag technique. Anim. Feed Sci. Technol. 27:137-146.

Chung, T. K. and D. H. Baker. 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: Comparison of values obtained with ileal-canulated pigs and cecectomized cockerels. J. Anim.Sci. 70:3781-3790.

Cunningham, H. M., D. W. Friend, and J. W. G. Nicholson. 1962. Note on a re-entrant intestinal fistula for digestion studies with pigs. Can. J. Anim. Sci. 42:112-113.

Cunningham, H. M., D. W. Friend, and J. W. G. Nicholson. 1963. Observations on digestion in the pig using a re-entrant intestinal fistula. Can. J. Anim. Sci. 43:215-225.

Darragh, A. J., P. D. Cranwell and P. J. Moughan. 1991. Methionine absorption from the hind-gut of piglets. P. 186 in Batterham, E. S. (Ed.): Manipulating pig production III. p 186. Australasian Pig Science Association.

Decuypere, J. A., I. J. Vervaeke, H. K. Henderickx and N. A. Dierick. 1977. Gastro-intestinal cannulation in pigs: A simple technique allowing multible replacements. J. Anim. Sci. 46: 463-468.

de Lange, C. F. M., W. C. Sauer, R. Mosenthin, and W. B. Souffrant. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. 67:746-754.

de Lange, C. F. M., W. B. Souffrant, and W. C. Sauer. 1990. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. J. Anim. Sci. 68:409-418.

de Lange, C. F. M. , W. C. Sauer, L. A. den Hartog, and J. Huisman. 1991. Methodological studies with the nylon bag technique to determine protein and energy digestibilities in feedstuffs for pigs. Livest. Prod. Sci. 29:213-225.

de Lange, C. F. M. , W. C. Sauer, W. B. Souffrant, and K. A. Lien. 1992. ^{15}N-Leucine and ^{15}N-isoleucine isotope dilution techniques versus the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein and amino acids in digesta collected from the distal ileum in pigs. J. Anim. Sci. 70:1848-1856.

Donkoh, A., and P. J. Moughan. 1994. The effect of dietary crude protein content on apparent and true ileal nitrogen and amino acid digestibilities. Br. J. Nutr. 72:59-68.

Donkoh, A., P. J. Moughan, and W. C. Smith. 1994. Comparison of the slaughter method and simple T-piece cannulation of the terminal ilum for determining ileal amino acid digestibility in meat and bone meal for the growing pig. Anim. Feed Sci. Technol. 49:43-56.

Donkoh, A., P. J. Moughan, and P. C. H. Morel. 1995. Comparison of methods to determine the endogenous amino acid flow at the terminal ileum of the growing rat. J. Sci. Food Agric. 67:359-366.

Donkoh, A., P. J. Moughan, and W. C. Smith. 1994. Comparison of the slaughter method and simple T-piece cannulation of the terminal ilum for determining ileal amino acid digestibility in meat and bone meal for the growing pig. Anim. Feed Sci. Technol. 49:43-56.

Easter, R. A. and T. D. Tanksley Jr. 1973. A technique for re-entrant ileocecal cannulation of swine. J. Anim Sci. 36:1099-1103.

Eggum, B. O. 1973. A study of certain factors influencing protein utilization in feedstuffs in rats and pigs. Ph.D Thesis. 406. Beretning fra Forsøgslaboratoriet, Statens Husdyrbrugsforsøg, Copenhagen, Denmark.

Eggum, B. O. 1995. The influence of dietary crude fiber on protein digestion and utilization in monogastrics. Arch. Anim. Nutr. 48:89-95.

Fan, M. Z. and W. C. Sauer. 1995a. Determination of apparent ileal amino acid digestibility in barley and canola meal for pigs with the direct, difference, and regression methods. J. Anim. Sci. 73:2364-2374.

Fan, M. Z. and W. C. Sauer. 1995b. Determination of apparent ileal amino acid digestibility in peas for pigs with the direct, difference, and regression methods. Livest. Prod. Sci. 44:61-72.

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

Fan, M. Z., W. C. Sauer, and C. F. M. de Lange. 1995a. Amino acid digestibility in soybean meal, extruded soybean and full-fat canola for early weaned pigs. Anim. Feed Sci. Technol. 52:189-203.

Fan, M. Z., W. C. Sauer, and W. M. Gabert. 1996. Variability of apparent ileal amino acid digestibility in canola meal for growing-finishing pigs. C. J. Anim. Sci. 76:563-569.

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: Effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859.

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

Fastinger, N. D., and D. C. Mahan. 2002. Effect of soybean meal particle size on amino acid and energy digestibility in grower-finisher swine. J. Anim Sci. 81:697-704.

Fuller, M. F. and R. M. Livingstone. 1982. Annual Report of studies in animal nutrition and allied sciences. Rowett Research Institute, Scotland. 39:45.

Furuya, S., S. Takahashi, and S. Omori. 1974. The establishment of T piece cannula fistulas into the small intestine of the pig. Jpn. J. Zootech. Sci. 45:42-44.

Gabert, V. M., N. Canibe, H. Jørgensen, B. O. Eggum, and W. C. Sauer. 1997. Use of ^{15}N-isotope dilution technique to determine endogenous amino acids in ileal digesta in growing pigs. Acta Agric. Scand., Sect. A, Anim. Sci. 47:168-177.

Gardner, M. L. G. 1975. Absorption of amino acids and peptides from a complex mixture in the isolated small intestine of the rat. J. Physiol. 253:233-256.

Gargallo, J. and D. R. Zimmermann. 1980. A simple intestinal cannula for swine. Am. J. Vet. Res. 41:618-619.

Gausseres, N., Mahe, S., R. Benamouzig, C. Luengo, F. Ferrier, J. Rautureau, and D. Tome. 1997. [^{15}N]-labeled pea flour protein nitrogen exhibits good ileal digestibility and postpandrial retention in humans. J. Nutr. 127:1160-1165.

Green, S. and T. Kiener. 1989. Digestibilities of nitrogen and amino acids in soyabean-meal, sunflower, meat and rapeseed meals measured with pigs and poultry. Anim. Prod. 48:157-179.

Green, S., S. L. Bertrand, M. J. C. Duron, and R. A. Maillard. 1987. Digestibility of amino acids in maize, wheat and barley meal measured in pigs with ileo-rectal anatomosis and isolation of the large intestine. J. Sci. Food Agric. 41:29-43.

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

Hagemeister, H. and H. Ebersdobler. 1987. Messung der präzäkalen Verdaulichkeit von Protein mit der Homoarginine-Methode. Arch. Anim. Nutr. 37:451-466.

Hamilton, C. R., C. R. Dove, G. M. Zinn, and T. L. Veum. 1985. Simultaneous cecostomy and ileal cannulation with a modified flexible T cannula in gilts. Am.J. Vet. Res. 46:942-944.

Haydon, K. D., D. A. Knabe, and T. D. Tanksley, Jr. 1984. Effect of level of feed intake on nitrogen, amino acid and energy digestibilities measured at the end of the small intestine and over the total digestive tract of growing pigs. J. Anim. Sci. 59:717-724.

Hennig, U., W. B. Souffrant, J. P. Laplace, C. Fevrier, and M. Anke. 1997. Retention of minerals by pigs with four models of ileorectostomy compared with intact animals. P. 344-347 in Laplace, J. P., C. Fevrier, and A. Barbeau(Ed.): Digestive physiology in pigs. EAAP Publ. No 88. St. Malo, France.

Hess, V., and B. Séve. 1999. Effects of body weight and feed intake on basal ileal endogenous losses in growing pigs. J. Anim. Sci. 77:3281-3288.

Hess, V., J. N. Thibault, and B. Seve. 1997. Apparent, true and real digestibility of intact or hydrolyzed isolated pea protein. P. 587-590 in Laplace, J. P., C. Fevrier, and A. Barbeau (Ed.): Digestive physiology in pigs. EAAP Publ. No 88. St. Malo, France.

Hodgkinson, S. M., and P. J. Moughan. 2000. Amino Aicds: Digestibility, availability and metabolism. P. 125-132 in Moughan, P. J., M. W. A. Verstegen, and M. I. Risser-Reyneveld (Ed.): Feed evaluation – Principles and practice. Wageningen Press, Wageningen, The Netherlands.

Holmes, J. H., H. S. Bayley, and P. A. Leadbeater. 1974. Digestion of protein in small and large intestine of the pig. Br. J. Nutr. 32:479-489.

Imbeah, M. and W. C. Sauer. 1991. The effect of dietary level of fat on amino acid digestibilities in soybean meal and canola meal and on rate of passage in growing pigs. Livest. Prod. Sci. 29:227-239.

Imbeah, M., K. Angkanaporn, V. Ravindran, and W. L. Bryden. 1996. Investigations on the guadination of lysine in proteins. J. Sci. Food Agric. 72:213-218.

Jansman, A. J. M., A. A. Frohlich, and R. Marquardt. 1994. Production of proline-rich proteins by parotid glands of rats is enhanced by feeding diets containing tannins from faba beans (Vicia faba L.). J. Nutr. 124:249-258.

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

Jondreville, C., J. Van den Broecke, F. Gatel, and S. Van Cauwenberghe. 1995. Ileal digestibility of amino acids in feedstuffs for pigs. Eurolysine/ITFC publication, France. 52 pp.

Just, A., H. Jørgensen, and J. A. Fernandez. 1981. The digestive capacity of the ceacum-colon and the value of nitrogen absorbed from the hind gut for protein synthesis in pigs. Br. J. Nutr. 46:209-219.

Just, A., H. Jørgensen, and J.A. Fernandez. 1985. Correlations of protein deposited in growing female pigs to ileal and faecal digestible protein and amino acids. Livest. Prod. Sci. 12:145-159.

Jørgensen, H., J. A. Fernandez, and A. Just. 1985. Comparative digestibility experiments with normal and cannulated pigs. P. 348-351 in Just, A., H. Jørgensen and J. A. Fernandez (Ed.): Digestive physiology in the pig. 580. Beretning fra Statens Husdyrbrugsforsøg. p 348-351. Copenhagen, Denmark.

Kesting, U., H. Klukas, H. Engelmann, and G. Bolduan. 1986. Methodische Untersuchungen zur ilealen Flussmessung mittels grosslumiger Darmkanülen beim Schwein. Arch. Anim. Nutr. 36:793-802.

Kien, C. L., A. H. Ailabouni, R. D. Murray, P. A. Powers, R. E. McLead, and J. Kepner. 1997. Technical note: Pig model for studying nutrient assimilation by the intestine and colon. J. Anim. Sci. 75:2161-2164.

Kies, A. K., P. J. Moughan, W. C. Smith. 1986. The apparent and true ileal digestibility of nitrogen and amino acids in lactic casein for the growing pig. Anim. Feed Sci. Technol. 16:169-178.

Knabe, D. A., D. C. LaRue, E. J. Gregg, G. M. Martinez, and T. D. Tanksley, Jr. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim Sci. 67:441-458.

Krawielitzki, K., T. Völker, S. Smulikowska, H. D. Bock, and J. Wünsche. 1977.Weitere Untersuchungen zum Multikompartment-Modell des Proteinstoffwechsels. Arch. Tierernähr. 27:609-627.

Krawielitzki, K., T. Zebrowska, R. Schadereit, and J. Kowalczyk, U. Hennig, J. Wünsche, and U. Herrmann. 1990. Determination of nitrogen absorption and nitrogen secretion in different sections of the pig's intestine by digesta exchange between ^{15}N labelled and unlabelled animals. Arch. Anim. Nutr. 40:25-37.

Krawielitzki, K., F. Kreienbring, T. Zebrowska, R. Schadereit, and J. Kowalczyk. 1994. Estimation of N absorption, secretion, and reabsorption in different intestinal sections of growing pigs using the ^{15}N isotope dilution method. P 79-82 in Souffrant, W. B. and H. Hagemeister (Ed.): Digestive physiology in pigs, vol. I.EAAP publ. No. 80. Bad Doberan, Germany.

Kuiken, K. A. and C. M. Lyman. 1948. Availability of amino acids in some foods. J. Nutr. 36:359-368.

Köhler, T., L. A. den Hartog, J. Huisman, R. Mosenthin, and M. W. A. Verstegen. 1991. Digestibility measurements in pigs by using post-valve T-caecum cannulation or end to side ileo-rectal anastomosis. J. Anim. Physiol. Anim. Nutr. 66:278-286.

Köhler, T., R. Mosenthin, M. W. A. Verstegen, J. Huisman, L. A. den Hartog, and F. Ahrens. 1992a. Effect of ileo-rectal anastomosis and post valve T-caecum cannulation on growing pigs. 1. Growth performance, N-balance and intestinal adaptation. Br. J. Nutr. 68:293-303.

Köhler, T., R. Mosenthin, M. W. A. Verstegen, T. Wensing, L. A. den Hartog, and J. Huisman. 1992b. Effect of ileo-rectal anastomosis and post valve T-caecum cannulation on growing pigs. 2. Blood variables and mineral balances. Br. J. Nutr. 68:305-315.

Laplace, J. B., W. B. Souffrant, U. Hennig, E. Chabeauti and C. Fevier. 1994. Measurement of preceacal dietary protein and plant cell wall digestion in pigs; comparison of four surgical procedures for ileo-rectal anastomosis. Livest. Prod. Sci. 40:313-328.

Larsen, F. M., P. J. Moughan, and M. N. Wilson. 1993. Dietary fiber viscosity and endogenous protein excretion at the terminal ileum of growing rats. J. Nutr.123:1898-1904.

le Guen, M. P., J. Huismann, J. Gueguen, G. Beelen and M. W. A. Verstegen. 1995. Effects of a concentrate of pea antinutritional factors on pea protein digestibility in piglets. Livest. Prod Sci. 44:157-167.

Lenis, N. P., P. Bikker, J. van der Meulen, J. Th. M. van Diepen, J. G. M. Bakker, and A. W. Jongbloed. 1996. Effect of dietary neutral detergent fiber on ileal digestibility and portal flux of nitrogen and amino acids and on nitrogen utilization in growing pigs. J. Anim. Sci. 74:2687-2699.

Leterme, P., L. Pirard, and A. Thewis. 1992. A note on the effect of wood cellulose level in protein-free diets on the recovery and amino acid composition of endogenous protein collected from the ileum in pigs. Anim. Prod. 54:163-165.

Leterme, P., T. Monmart, A. Thewis, and P. Morandi. 1996b. Effect of oral and parenteral N nutrition vs N-free nutrition on the endogenous amino acid flow at the ileum of the pig. J. Sci. Food Agric. 71:265-271.

Leterme. P., B. Seve, and A. Thewis. 1998. The current 15N leucine infusion technique is not suitable for quantitative measurements of ileal endogesnous amino acid flows in pigs. J. Nutr. 128:1961-1968.

Leterme, P., A. Thewis, Y. Beckers, and E. Baudart. 1990. Apparent and true ileal digestibility of amino acids and nitrogen balance measured in pigs with ileo-rectal anastomosis or T-cannulas, given a diet containing peas. J. Sci. Food Agric. 52:485-497.

Leterme, P., A. Thewis, E. Francois, P. van Leeuwen, B. Wathelet, and J. Huisman. 1996a. The use of ^{15}N-labeled dietary proteins for determining true ileal amino acid digestibilities is limited by their rapid recycling in the endogenous secretions of pigs. J. Nutr. 126:2188-2198.

Leterme, P., A. Thewis, P. van Leuwen, T. Monmart, and J. Huismann. 1996c. Chemical composition of pea fibre isolates and their effect on the endogenous amino acid flow at the ileum of the pig. J. Sci. Food Agric. 72:127-134.

Leterme, P., A. Thewis, and B. Seve. 1997. The ^{13}C- vs the ^{15}N-leucine infusion technique for the determination of the ileal endogenous N losses in pigs. P. 596-599 in Laplace, J. P., C. Fevrier, and A. Barbeau (Ed.): Digestive physiology in pigs. EAAP Publ. No 88. St. Malo, France.

Lewis, A. J. 1992. Determination of the amino acid requirements of animals. P. 67-85 in Nissen, S.(Ed.): Modern methods in protein nutrition and metabolism. Academic Press Inc., San Diego, Ca.

Li, S. and W. C. Sauer. 1994. The effect of dietary fat content on amino acid digestibility in young pigs. J. Anim. Sci. 72:1737-1743.

Li, S., W. C. Sauer, and M. Z. Fan. 1993. The effect of dietary crude protein level on amino acid digestibility in early-weaned pigs. J. Anim. Physiol. Anim. Nutr. 70:26-37.

Li, S., W. C. Sauer, and R. T. Hardin. 1994. Effect of dietary fiber level on amino acid digestibility in young pigs. Can. J. Anim. Sci. 74:327-333.

Lien, K. A., W. C. Sauer, and M. E. R. Dugan. 1997a. Evaluation of the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digesta of pigs: Effect of the pattern of blood sampling, precursor pools, and isotope dilution technique. J. Anim. Sci. 75:159-169.

Lien, K. A., W. A. Sauer, and M. Fenton. 1997b. Mucin output in ileal digesta of pigs fed a protein-free diet. Z. Ernährungswiss. 36:182-190.

Lien, K. A., W. C. Sauer, R. Mosenthin, W. B. Souffrant, and M. E. R. Dugan. 1997c. Evaluation of the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digestion of pigs: Effect of dilution in the precursor pool for endogenous nitrogen secretion. J. Anim. Sci. 75:148-158.

Lin, F. D., D. A. Knabe, and T. D. Tanksley, Jr. 1987. Apparent digestibility of amino acids, gross energy and starch in corn, sorghum, wheat, barley, oat groats and wheat midlings for growing pigs. J. Anim. Sci. 64:1655-1663.

Low, A. G. 1980. Nutrient absorption in pigs. J. Sci. Food Agric. 31:1087-1130.

Low, A. G. and T. Zebrowska. 1989. Digestion in Pigs. P. 53-121 in Bock, H. D., B. O. Eggum, A. G. Low, O. Simon, and T. Zebrowska (Ed.) Protein metabolism in farm animals. Oxford University Press, Oxford, GB.

Mahe, S., N. Roos, R. Benamouzig, H. Sick, A. Baglieri, J. F. Huneau, and D. Tome. 1994. True exogenous and endogenous nitrogen fractions in the human jejunum after ingestion of small amounts of ^{15}N-labeled casein. J. Nutr. 124:548-555.

Mariscal-Landin, G., Seve, B., Colleaux, Y. and Y. Lebreton. 1995. Endogenous amino nitogen collected from pigs with end-to-end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. J. Nutr. 125:136-146.

Mavromichalis, I., T. M. Parr, V. M. Gabert, and D. H. Baker. 2001. True ileal digestibility of amino acids in sow's milk for 17-day-old pigs. J. of Anim. Sci. 79:707-713.

Mawby, D. I., A. G. Matthew, E. A. Mears, T. D. Moyers, and D. J. Krahwinkel. 1999. Complications of ileal cannulation in cats. Lab Anim Sci. 49:406-410.

Mitchell, H. H. 1924. A method of determining the biological value of protein. J. Biol. Chem. 58:873-882.

Mosenthin, R., W. C. Sauer and F. Ahrens. 1994. Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. J. Nutr. 124:1222-1229.

Mosenthin, R. W. C. Sauer, R. Blank, J. Huisman, M. Z. Fan. 2000. The concept of digestible amino acids in diet formulation for pigs. Livest. Prod. Sci. 64:265-280.

Mosenthin, R., W. C. Sauer, K. A Lien, and C. F. M. de Lange. 1993. Apparent, true and real ileal protein and amino acid digestibilities in growing pigs fed two varieties of fababeans (Vicia faba L.) different in tannin content. J. Anim. Physiol. Anim. Nutr. 70:253-265.

Moughan, P. J. 1993. Towards an improved utilization of dietary amino acids by the growing pig. P. 117-136 in Cole, D. J. A., W. Haresign, P. C. Garnsworthy(Ed.): Recent developments in pig nutrition 2. Nottingham University Press, Nottingham, UK.

Moughan, P. J. 2003. Amino acid digestibility and availability in food and feedstuffs. P. 199-221 in In Ball, R. O. (E.d): Digestive Physiology in pigs, Proc. 9th intl. Symposium. Vol. 1.

Moughan, P. J. and W.C. Smith. 1987. A note on the effect of cannulation of the terminal ileum of the growing pig on the apparent ileal digestibility of amino acids in ground barley. Anim. Prod. 44:319-321.

Moughan, P. J. and S. H. Rutherfurd. 1990. Endogenous flow of total lysine and other amino acids at the distal ileum of the protein- or peptide-fed rat: The chemical labeling of gelatin protein by transformation of lysine to homoarginine. J. Sci. Food Agric. 52:179-192.

Moughan, P. J. and S. H. Rutherfurd. 1991. Endogenous lysine flow at the distal ileum of the protein-fed rat: Investigation of the effect of protein source using radioactively labelled acetylated lysine or lysine transformed to homoarginine. J. Sci. Food Agric. 55:163-174.

Moughan, P. J. and G. Schuttert. 1991. Composition of nitrogen-containing fractions in digesta from the distal ileum of pigs fed a protein-free diet. J. Nutr. 121:1570-1574.

Moughan, P. J., A. J. Darragh, W. C. Smith, and C. A. Butts. 1990. Percloric and trichloroacetic acids as precipitants of protein in endogenous ileal digesta from the rat. J. Sci. Food Agric. 52:13-21.

Moughan, P. J., G. Schuttert, and M. Leenaars. 1992a. Endogenous amino acid flow in the stomach and small intestine of the young growing pig. J. Sci. Food Agric. 60:437-442.

Moughan, P. J., P. J. Buttery, C. P. Essex, and J. B. Soar. 1992b. Evaluation of the isotope dilution technique for determining ileal endogenous nitrogen excretion in the rat. J. Sci. Food Agric. 58:165-172.

Mroz, Z., A. W. Jongbloed, P.A. Kemme, H. Everts, A. M. van Vuuren, and R. Hoste. 1991. Preliminary evaluation of a new cannulation technique (steered ileo-caecal valve) for quantitative collection of digesta from the small intestine of pigs. P. 334-339 in Verstegen, M. W. A., J. Huisman, and L. A. den Hartog (Ed.): Digestive physiology in pigs. EAAP publication No. 54. p 334-339. Pudoc Wageningen. Netherlands.

NRC. (1998). Nutrient Requirements of Swine (10th Ed.) National Academy Press, Washington, DC.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77:149-163.

Parsons, C. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grain with solubles. J. Agric. Sci., Camb. 104:469-472.

Pedersen, C., S. Boisen, and J. A. Fernandez. 2002. Studies on the effect of dietary crude protein supply on the composition of ileal endogesnous crude protein loss in growing pigs. Acta Agric. Scand. Sect. A. Anim. Sci. 52:141-149.

Peloso, J. G., J. Schumacher, S. R. McClure, M. R. Crabill, D. V. Hanselka, D. D. Householder, and G. D. Potter. 1994. Technique for long-term ileal cannulation in ponies. Can. J. Vet. Res. 58:181-184.

Petry, H. and B. M. Handlos. 1978. Untersuchungen zur bestimmung der Verdaulichkeit von Nährstoffen und Futter Energie mit hilfe der nylon beutel Technick beim Schwein. Arch. Tierernähr. 28:531-543.

Rademacher, M., W. C. Sauer, and A. J. M. Jansman. 1999. Standardized ileal digestibility of amino acids in pigs. Degusa-Hülls AG publication, Frankfurt, Germany.

Rayadurg, V. and H. H. Stein. 2003. The effect of feed intake on amino acid digestibility in growing pigs. J. of Anim. Sci. 81(Suppl. 2):45.

Roos, N., M. Pfeuffer, and H. Hagemeister. 1994. Labelling with ^{15}N as compared with homoarginine suggests a lower prececal digestibility of casein in pigs. J. Nutr. 124:2404-2409.

Rutherfurd, S. N. and P. J. Moughan. 1990. Guadination of lysine in selected dietary proteins. J. Agric. Food Chem. 38:209-211.

Qiao, S., and P. A. Thacker. 2001. A comparison of washed and unwashed bags to determine the apparent fecal digestibility of dry matter, energy and crude protein in feeds for swine using the mobile nylon bag technique. Can. J. Anim. Sci. 81:371-378.

Salgado, P., J. M. Martins, I. Carvalho, M. Abreu, J. P. B. Freir, R. Toullec, J. P. Lalles, and O. Bento. 2002. Component digestibility of lupin (*Lupinus Augustifolius*) and Pea (*Pisum Sativum*) seeds and effects on the small intestine and body organs in anastomised and intact growing pigs. Anim. Feed Sci. Technol. 98:187-201.

Sauer, W.C. and L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical applications. A review. Livest. Prod. Sci. 15:367-388.

Sauer, W.C. and K. de Lange. 1992. Novel methods for determining protein and amino acid digestibilities in feedstuffs. P. 87-120 in Nissen, S.(Ed.): Modern methods in protein nutrition and metabolism. Academic Press Inc., San Diego, Ca.

Sauer, W. C., S. C. Stothers, and R. J. Parker. 1977. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57:775-884.

Sauer, W. C., A. Just, and H. Jørgensen. 1982. The influence of daily feed intake on the apparent digestibility of crude protein, amino acids, calcium and phosphorus at the terminal ileum and overall in pigs. Z. Tierphysiol., Tierernährg. Futtermittelkde. 48:177-182.

Sauer, W. C., H. Jørgensen, and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci. 63:233-237.

Sauer, W. C, L. A. den Hartog, J. Huisman, P. van Leeuwen, and C. F. M. de Lange. 1989. The evaluation of the mobile nylon bag technique for determining the apparent protein digestibility in a wide variety of feedstuffs for pigs. J. Anim. Sci. 67:432-440.

Sauer, W. C., R. Mosenthin, F. Ahrens, and L. A. den Hartog. 1991. The effect of source of fiber on ileal and fecal amino acid digestibility and bacterial nitrogen excretion in growing pigs. J. Anim. Sci. 69:4070-4077.

Schulze, H., P. van Leuwen, M. W. A. Verstegen, J. Huisman, W. B. Souffrant, and F. Ahrens. 1994. Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. J. Anim. Sci.72:2362-2368.

Schulze, H., C. A. Butts, P. J. Moughan, and M. W. A. Verstegen. 1995a. The ^{15}N-isotope dilution method for determining ileal endogenous nitrogen excretion in the young (10 kg liveweight) pig. J. Sci. Food Agric. 69:41-50.

Schulze, H., H. S. Saini, J. Huisman, M. Hessing, W. van den Berg, and M. W. A. Verstegen.1995b. Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. Sci. Food Agric. 69:501-510.

Schulze, H., van Leuwen, P., Verstegen, M. W. A., and J. W. O. van den Berg. 1995c. Dietary level and sources of neutral detergent fiber and ileal endogenous flow in pigs. J. Anim. Sci. 73:441-448.

Seve, B., and Y. Henry. 1995. Protein utilization in non ruminants. P. 59-82 in Nunes, A. F., A. V. Portugal, J. P. Costa, and J. R. Ribeiro(Ed.): Protein metabolism and nutrition. EAAP publ. No 81. Vale de Santerem, Portugal.

Seve, B., G. Mariscal-Landin, Y. Colleaux, and Y. Lebreton. 1994. Ileal endogenous amino acid and amino sugar flows in pigs fed graded levels of protein or fibre. P. 35-38 in Souffrant, W. B. and H. Hagemeister

(Ed.): Digestive physiology in pigs, vol. I. EAAP publ. No 80. Bad Doberan, Germany.

Shahkhalili, Y., P. A. Finot, R. Hurrel, and E. Fern. 1990. Effects of foods rich in polyphenols on nitrogen excretion in rats. J. Nutr. 120:346-352.

Siriwan, P., W. L. Bryden, and E. F. Annison. 1994. Use of guadinated dietary protein to measure losses of endogenous amino acids in poultry. Br. J. Nutr. 71:515-529.

Smitz, M., H. Hagemeister, and H. F. Erbersdobler. 1991. Homoarginine labeling is suitable for determination of protein absorption in minature pigs. J. Nutr. 121:1575-1580.

Souffrant, W. B., R. Köhler, R. Matkowitz, G. Gebhard, and H. Schmandke. 1981. Ernährungsphysiologishe Untersuchungen an Schweinen zur Beurteilung von modifizierten Proteinen. 2. Mitteilung: Bestimmung des endogenen N im Dünndarminhalt mit Hilfe der ^{15}N-Tracer technik. Arch. Tierernähr. 31:675-683.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Köhler, G. Gebhard, and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig: Preliminary results of a collaborative study. Arch. Anim. Nutr. 36: 269-274.

Souffrant, W. B., A. Rerat, J. P. Laplace, B. Darcy-Vrillon, R. Köhler, T. Corring, and G. Gebhard. 1993. Exogenous and endogenous contributions to nitrogen fluxes in the digestive tract of pigs fed a casein diet. III. Recycling of endogenous nitrogen. Reprod. Nutr. Dev. 33:373-382.

Souffrant, W. B. , C. Fevrier, J. P. Laplace, and U. Hennig. 1997. Comparison of methods to estimate ileal endogenous nitrogen and amino acids in piglets. P. 321-324 in Laplace, J. P., C. Fevrier, and A. Barbeau(Ed.): Digestive physiology in pigs. EAAP Publ. No 88. St. Malo, France.

Stein, H. H. 1998. Comparative amino acid digestibilities in growing pigs and sows. Ph.D. Thesis, Univ. of Illinois, Urbana.

Stein, H. H., and M. Nyachoti. 2003. Animal effects on ileal amino acid digestiblity. P. 223-241. In Ball, R. O. (E.d): Digestive Physiology in pigs, Proc. 9th intl. Symposium. Vol. 1.

Stein, H. H., S. Aref, and R. A. Easter. 1999a. Comparative protein and amino acid digestibilities in growing pigs and sows. J. Anim. Sci. 77:1169-1179.

Stein, H. H., S. W. Kim, T. T. Nielsen, and R. A. Easter. 2001. Standardized amino acid digestibilities in growing pigs and sows. J. Anim. Sci. 79:2113-2122.

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical note: A technique for inserting a simple T-cannula in the distal ileum of gestating sows. J. Anim. Sci. 76:1433-1436.

Stein, H. H., N. L. Trottier, C. Bellaver, and R. A. Easter. 1999b. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J. Anim. Sci. 77:1180-1187.

Tamminga, S., H. Schulze, J. van Bruchem, and J. Huisman. 1995. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch. Anim. Nutr. 48:9-22.

Tanksley, Jr., T. D. and D. A. Knabe. 1984. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. P. 75-95 in Haresign, W. and D. J. A. Cole (Ed.): Recent advances in animal nutrition. p 75-95.

Taverner, M. R. and R. G. Campbell. 1985. Evaluation of the mobile nylon bag technique for measuring digestibility of pig feeds. P 385-288 in Just, A., H. Jørgensen and J. A. Fernandez (Ed.): Digestive physiology in the pig. 580. Beretning fra Statens Husdyrbrugsforsøg. Copenhagen, Denmark.

Taverner, M. R., I. D. Hume, and D. J. Farrell. 1981. Availability to pigs of amino acids in cereal grains. 1. Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets. Br. J. Nutr. 46:149-158.

Tews, J. K. and A. E. Harper. 1986. Induction in rats of lysine imbalance by dietary homoarginine. J. Nutr. 116:1910-1921.

Traylor, S. L., Cromwell, G. L., Lindemann, M. D., and D. A. Knabe. 2001. Effect of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. J Anim Sci. 79:2634-42.

van Barneveld, R. J., E. S. Batterham, and B. W. Norton. 1994. The effect of heat on amino acids for growing pigs. 2. Utilization of ileal-digestible lysine from heat-treated field peas. Br. J. Nutr. 72:243-256.

van Leeuwen, P., L. Babinsky, M. W. A. Verstegen, and J. Tossenberger. 2000. A procedure for ileostomisation of adult roosters to determine apparent ileal digestibility of protein and amino acids of diets: Comparison of six diets in roosters and growing pigs. Livest. Prod. Sci. 67:101-111.

van Leeuwen, P., D. J. van Kleef, G. J. M. van Kempen, J. Huisman, and M. W. A. Verstegen. 1991. The post valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. J. Anim. Physiol. Anim. Nutr. 65:183-193.

Viljoen , J. M. N. Ras, F. K. Siebrits, and J. P. Hayes. Use of the mobile nylon bag technique (MNBT) in combination with the ileo-rectal anastomosis technique (IRA) to determine amino acid digestiblity in pigs. Livest. Prod. Sci. 51:109-117.

Walker, J. A., D. L. Harmon, K. L. Gross, and G. F. Collings. 1994. Evaluation of nutrient utilization in the canine using the ileal cannulation technique. J. Nutr. 124:2672S-2676S.

Walker, W. R., G. L. Morgan, and C. V. Maxwell. 1986. Ileal cannulation in baby pigs with a simple T-cannula. J. Anim. Sci. 62:407-411.

Wilson, R. H., and J. Leibholz. 1981. Digestion in the pig between 7 and 35 d of age. 3. The digestion of nitrogen in pigs given milk and soya-bean proteins. British Journal of Nutrition 45:337-346.

Wubben, J. E. , M. R. Smiricky, D. M. Albin, and V. Gabert. 2001. Improved procedure and canula design for simple T-canulation at the distal ileum in growing pigs. Cont. Topics in Lab. ANim. Sci. 40:27-31.

Wünsche, J., U. Henning, M. Meinl, F. Kreienbring, and H. D. Bock. 1982. Investigation of the absorption and utilization of amino acids infused into the caecum of growing pigs. I. N-balance measurement with regard to the utilization of lysine and isoleucine and requirement of growing pigs. Arch. Tierernähr. 32: 337-348.

Wünsche, J., U. Herrman, M. Meinl, U. Hennig, F. Kreinbring, and P. Zwierz. 1987. Einfluss exogener Faktoren auf die präzäkale Nährstoff- und Aminosäurenresorption, ermittelt an Sweinen mit Ileo-Rektalen-Anastomosen. Arch. Anim. Nutr. 37:745-764.

Yin, Y. L., J. D. G. McEvoy, H. Schulze, H. Henning, W. B. Souffrant, and K. J. McCracken. 2000. Apparent digestibility (ileal and overall) of nutrients as evaluated with PVTC-cannulated or ileo-rectal anatomized pigs fed diets containing two indigestible markers. Livest. Prod. Sci. 62:133-141.

Yin, Y. L., R. L. Huang, H. Y. Zhong, T. J. Li, W. B. Souffrant, and C. F. M. de Lange. 2002. Evaluation of mobile nylon bag technique for determining apparent digestibilities of protein and amino acids in growing pigs. J. Anim. Sci. 80:409-420.

Yu, F., P. J. Moughan, and T. N. Barry. 1996. The effect of cottenseed tannins on the ileal digestibility of amino acids in casein and cottonseed kernel. Br. J. Nutr. 75:683-695.