CHAPTER 2
REVIEW OF LITERATURE

2.1 MORPHOLOGY OF THE PORCINE DIGESTIVE TRACT

2.1.1 Overview of the gastrointestinal tract

The gastrointestinal tract of the pig is a tubular canal, beginning at the mouth and passing through the body to the anus. The greater part of this tube is lined by endoderm, but at the anterior and posterior ends, ectoderm has been invaginated to form a stomodeum and proctodeum respectively. The wall of the alimentary tract is primarily muscle and connective tissue, together with blood vessels and nerves (Grove and Newell, 1955). Primary components of the alimentary canal are the mouth, oesophagus, stomach, intestines and accessory glands (salivary glands, liver and pancreas). A schematic diagram of the digestive tract of the pig is shown in Figure 1.

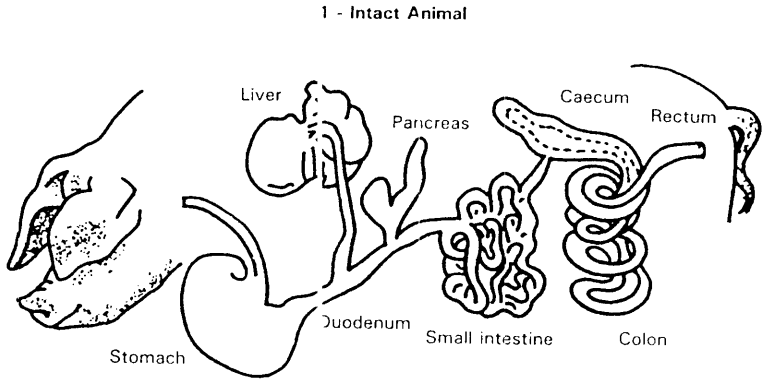

Figure 1. Digestive tract of the pig (Rhône-Poulenc, 1989)

2.1.2 Buccal cavity and oesophagus

Three salivary glands (parotid, submaxillary, sublingual) are present in the buccal (oral) cavity. Secretion by these glands, controlled by the autonomic nervous system act to, a) initiate starch digestion (through the action of amylase), and b) moisten and

lubricate the food bolus as it passes through the oesophagus to the stomach (Low and Zebrowska, 1989). Salivary volume and composition (amylase concentration) has been shown to vary in response to food and other stimulants in the mouth (Kudryavtsev, 1935; cited by Kidder and Manners, 1986), tending to increase rapidly in the presence of strong acids, and for prolonged duration in the presence of dry cereal diets (Kvasnitskii, 1951; cited by Kidder and Manners, 1986). The residence time for food in the buccal cavity is extremely short, followed by passage to the oesophagus (Low and Zebrowska, 1989).

Movement of food through the oesophagus is an involuntary response, facilitated by two layers of spiral muscular tissue (Low and Zebrowska, 1989), and secretion of mucus from tubuloacinar glands (Longland, 1991).

2.1.3 Stomach

The stomach is a dilated portion of the tubular digestive tract (Grove and Newell, 1955). It is both a temporary storage organ, and the first major region of digestive activity. Proteolysis is initiated, and much of the physical structure of foods is disrupted here, making chemical structures more available for enzymic hydrolysis in the small intestine (Low and Zebrowska, 1989; Longland, 1991). A pig's stomach has four functionally distinct regions (oesophageal, cardiac, gastric and pyloric) (Longland, 1991) as shown in Figure 2.

The oesophageal region is glandless, often cornified and is associated with a population of lactobacilli. Surrounding this area is the cardiac region, which occupies about one-third of the total luminal area. It is soft and smooth to touch, and throughout the submucosa are cardiac glands which secrete an alkaline, enzyme-free mucus which protects the mucosa from autodigestion. The central gastric (or fundal) region represents a further one-third of the stomach and consists of a cellual composite producing protective mucus, pepsins and hydrochloric acid; it is brown-red in colour

and consists of many folds, or rugae. The pyloric region also contains mucus-secreting cells, and is a yellowish, smooth structure (Low and Zebrowska, 1989).

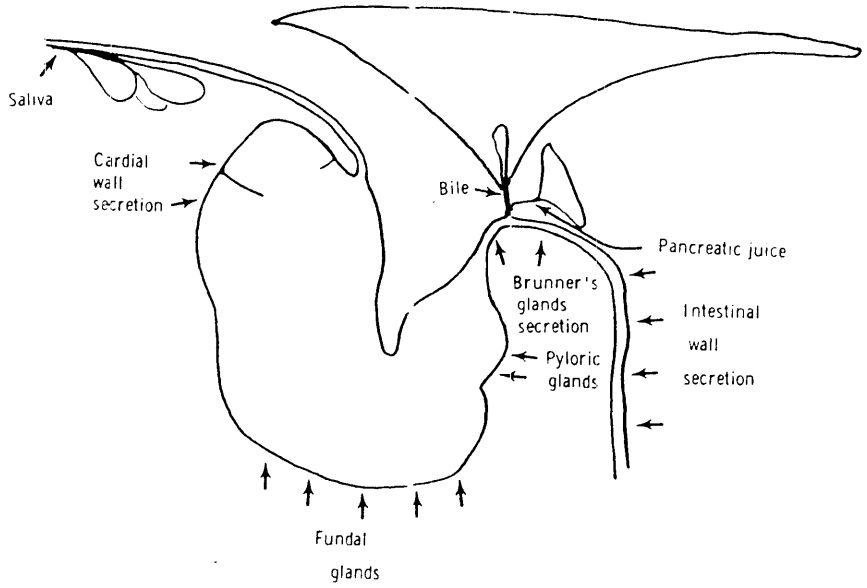

Figure 2. Gastro-intestinal glands of the pig (Kidder and Manners, 1986)

Secretions in the stomach are principally acidic, originating from the fundic and pyloric regions, however a low volume (125ml/24 hours, Kidder and Manners, 1986) of alkaline secretion occurs in the cardial region. Acidic secretions are stimulated by ingestion of food, in which case cardial alkaline secretion is temporarily suppressed (Kidder and Manners, 1986).

The principle site of acid secretion in the stomach is the fundic region. Fundic secretions contain proteolytic enzyme precursors such as pepsinogen, which can only be activated and function at a very acid pH (Kidder and Manners, 1986).

2.1.4 Small intestine

A mature pig has approximately 18 lineal meters of small intestine, of which about 85% is the jejunum, the remainder is approximately equally divided between the

duodenum and ileum (Low and Zebrowska, 1989; Longland, 1991). Bile fluid enters the duodenum 2 to 5 cm distal to the pylorus, and the pancreatic duct opens a further 10cm beyond this (Low and Zebrowska, 1989).

The small intestine is the major site of enzymatic digestion, and absorption of end products of digestion in the pig. Absorption of nutrients is facilitated by villi which cover the length of the small intestine and which greatly increase its surface area. A microbial population is present in the small intestine, becoming progressively greater in activity toward the large intestine (Longland, 1991).

2.1.5 Large intestine

The large intestine is 4 to 4.5 m long, it has a much greater diameter than that of the small intestine and consists of a short blind-ended caecum which continues into the colon at the point of ileal attachment (Low and Zebrowska,, 1989; Longland, 1991). Microbial digestion is most active in the large intestine, the end products of which are absorbed across the mucosa.

2.2 NUTRIENT DIGESTION BY THE PIG

With the exception of mineral salts and some carbohydrates, most solid food offered to pigs is insoluble or, even if capable of mixing with water, forms a colloidal solution which can not be absorbed and is therefore unavailable for metabolism. Food must be rendered soluble and diffusible before it can be absorbed by the pig (Figure 3). It must be transformed from the colloid to the crystalloid condition through digestion. Digestion is a process of enzymatic action and hydrolysis, during which the molecular size of feed substances is progressively reduced until a true solution is achieved (Grove and Newell, 1955).

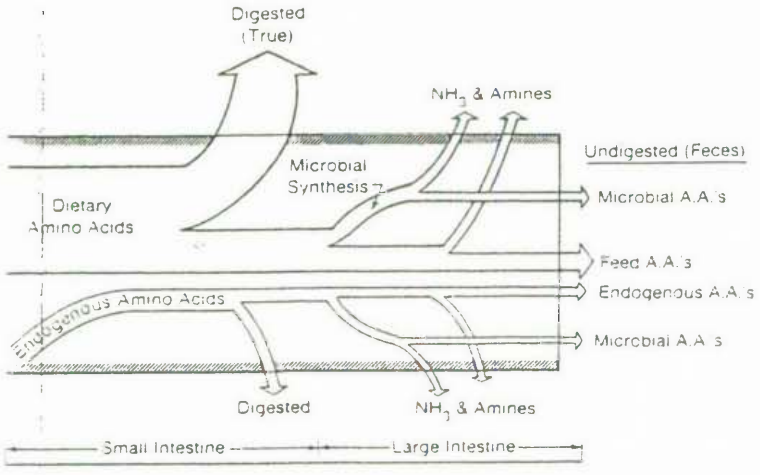

Figure 3. Protein and amino acid digestion by the pig (Sauer et al., 1989)

2.2.1 Carbohydrate Digestion

2.2.1.1 Carbohydrate classification

Plant carbohydrates are the predominant nutrient component of feedstuffs for pigs, generally contributing over 70% of the dry matter. Familiar carbohydrates include starch, sugars and cellulose (Grove and Newell, 1955). Starch is the major source of dietary energy for adult pigs in most parts of the world (Kidder and Manners, 1986).

Carbohydrates are a complex group of compounds, whose chemical structure and physiological affect on the pig varies considerably. Carbohydrates may be categorised according to one of five chemically distinct groups (Graham, 1991).

1. mono- or disaccharides; which are mainly glucose but also include fructose, sucrose, lactose and maltose.
2. oligosaccharides; particularly the raffinose series (raffinose, stachyose and verbascose)
3. storage polysaccharides; primarily starch and fructans.
4. cell wall storage polysaccharides; mannans, galactans and xyloglucans
5. cell wall structural polysaccharides; cellulose, hemicellulose, pectins

Cell wall storage, and cell wall structu al polysaccharides are often referred to as non-starch polysaccharides (NSP). They are closely associated with lignin and together they form the dietary fibre complex (G aham, 1991).

2.2.1.2 Overview of carbohydrate d gestion in the pig

Storage carbohydrates, such as starch (or glycogen) and certain disaccharides may be hydrolysed by host enzymes to co nstituent monomers prior to absorption, whereas oligosaccharides and non-starch poly saccharides (NSP) of plant cell walls (such as cellulose, hemicellulose and pectins) must be fermented by gut microflora to volatile fatty acids (VFA) and gases prior to absorption (Longland, 1991). Free sugars can be absorbed directly and completely in he case of all common free sugars other than lactose (Kidder and Manners, 1986).

Starch is not as completely digested (94-98% apparent ileal digestibility) by the terminal end of the small intestine as sugars (99-100% apparent ileal digestibility), but is completely digested and absorbed by the end of the large intestine (Kidder and Manners, 1986). The principle site of cereal starch conversion to sugars is the duodenum, with the resultant sugars largely absorbed by the terminal ileum. Whilst starch is largely digested and absorbed in the terminal ileum, its absorption occurs at a much slower rate than that of glucose, leading to a slower rise in blood sugar levels.

2.2.1.3 Action of salivary alpha-amylase

The sequence of carbohydrate digestion, commences with degradation of starch to dextrins, maltose and maltotriose in the buccal (oral) cavity. This process occurs under the influence of salivary alpha-amylase within the pH range 3.8 to 9.4, but most efficiently at pH 6.9 (Bernfeld *et al*., 1948; cited by Kidder and Manners, 1986). Amylolytic activity continues in the oesophagus and oesophageal region of the stomach until the digesta mixes with gastric fluids of low pH (hydrochloric acid

secretions) and the pH of the digesta decreases to less than 3.6 (Longland, 1991; Kidder and Manners, 1986).

2.2.1.4 Bacterial fermentation in the stomach

Bacterial fermentation in the stomach, small and large intestine (Kidder and Manners, 1986; Longland, 1991) of the pig contribute significantly to carbohydrate digestion. Lactobacillus and Bifidobacterium species of bacteria are present in the gastric region of the stomach with varying prolificacy. It has been shown (Ledinek, 1970; cited by Kidder and Manners, 1986) that the bacterial population in the stomach will increase when dietary ingredients containing high levels of readily fermentable carbohydrates such as processed starch are offered to pigs. Bacterial fermentation of sugar, starch and hemicellulose in the oesophageal region of the stomach, yields lactic acid (Kidder and Manners, 1986; Longland, 1991), however this activity is rapidly suppressed when gastric secretions of low pH reduce the pH of the digesta.

2.2.1.5 Activity of duodenal secretions

Duodenal secretion of pancreatic juice, bile and secretions from Brunner's gland, are stimulated by the presence of digesta of low pH in the small intestine (Longland, 1991). These secretions (primarily pancreatic juice) are alkaline in nature, serving to almost neutralise the acidity of the small intestine. Studies by Friend *et al.* (1963; cited by Kidder and Manners, 1986) have confirmed that the total organic acid concentration in the small intestine is lower than in any other part of the alimentary tract. High pH in the small intestine facilitates the activity of pancreatic alpha-amylase (pancreatic juice is high in alpha-amylase) leading to renewed hydrolysis of starch in this region. The small intestine is the primary site for digestion of storage carbohydrates (Longland, 1991). Dextrins and sugars produced by amylase activity, together with those present in the diet, are acted upon by carbohydrases (lactase, trehalase, and maltase) (present in the mucosa of the small intestine) to yield

monosaccharides which are actively absorbed by the mucosa of the small intestine and pass into capillaries leading to the portal vein (Kidder and Manners, 1986).

2.2.1.6 Intestinal fermentation

Bacteria present in the small intestine of the pig possess a higher capacity for fermentation of dietary starch than more fibrous carbohydrates, which implies that microbes present in the foregut will degrade dietary starch and free sugars *in vivo* more readily than fibre (Graham, 1991) . The extent of, and the metabolic "cost" of this degradation in terms of loss of energy to the pig is undefined, however Drochner (1991) reports that each unit of energy digested in the hindgut, occurs with an efficiency of 0.8 relative to digestion in the upper digestive tract.

Carbohydrates (soluble sugars, starch and some cellulose) passing to the large intestine are fermented by micro-organisms yielding volatile fatty acids (primarily acetic acid) which are then absorbed through the mucosa of the large intestine and into the portal vein (Kidder and Manners, 1986). Diet composition has been found (Argenzio and Southworth, 1974; cited by Kidder and Manners, 1986) to influence volatile fatty acid production in the hindgut of the pig, implying that diet composition can also affect microbial population and activity in this region.

2.2.2 Protein Digestion

2.2.2.1 Enzymic And Hydrolytic Activity In The Stomach

Hydrolysis of dietary protein is initiated in the stomach through the action of various pepsins and hydrochloric acid (HCl). Pepsin is secreted into the gastric area as the inactive precursor, pepsinogen, which is then hydrolysed to pepsin by removal of a peptide from the N-terminal end of the molecule in the presence of HCl (Longland,

1991). This occurs rapidly and effectively at pH 2.0, and more slowly as pH approaches 4.0.

Pepsin activity is governed by gastric pH, with dual optimal function at pH 2.0 and 3.5. Pepsin activity declines above pH 3.6 with no activity beyond pH 6.0. Pepsins secreted by the fundic mucosa (pepsins A and D) function more effectively at the higher pH, whereas pepsins secreted by the pyloric mucosa (pepsins B and C), where the pH is lower, require a more acid environment for optimal activity (Longland, 1991). The pH of digesta within the fundic and pyloric regions of the stomach falls rapidly to pH 5.0 and then progressively to pH 2.0. Peptic hydrolysis of protein is therefore initially slow and is related to both gastric pH and the type of protein ingested. At least half of the protein which leaves the stomach is usually in peptide form. (i.e. in peptides with 10 or less amino-acids) and much of this is soluble in trichloroacetic acid (Longland, 1991).

The proteolytic capability of young pigs is limited to gastric mucosal secretion of chymosin (renin) (which is secreted as prochymosin an inactive precursor), the main function of which is to clot milk at pH < 5.5 (Longland, 1991).

2.2.2.2 Proteolysis in the small intestine

High pH in the small intestine extinguishes pepsin activity, however pancreatic and intestinal brush border secretions of proteolytic enzymes continues proteolysis (Longland, 1991). Three groups of proteases are secreted into the small intestine; endopeptidases, exopeptidases, and aminopeptidases. Endopeptidases and exopeptidases are secreted by the pancreas, the former hydrolysing any susceptible link in the peptide chain, the latter cleaving only the terminal bonds from the carboxyl end of the peptide chains (Longland, 1991). Primary pancreatic endopeptidases are trypsin, chymotrypsin and elastase. The exopeptidases are the carboxypeptidases A and B. Aminopeptidases, produced by the mucosa of the small intestine are located

on the brush border and in the cytoplasm (Longland, 1991). Pancreatic proteases are secreted into the duodenum as inactive precursors, where a duodenal mucosal secretion of enterokinase activates trypsinogen to trypsin. Once this activation begins, trypsin formation is autocatalytic. When activated, trypsin also catalyses activation of other pancreatic proteases (Longland 1991). Activated proteases and peptidases reduce the size of the peptides to a chain comprising two or three amino acids, which are either hydrolysed on the mucosal surface or are absorbed as such together with free amino acids into the enterocytes of the small intestine (Low, 1990).

2.2.2.3 Absorption of amino acids

There is no evidence of active uptake of amino acids in either the stomach or large intestine (Low, 1990). The small intestine is the site of all amino acid absorption, however there is also substantial endogenous loss of amino acids, peptides and proteins into the digestive tract in this region (see Figure 3). Endogenous proteins include shed epithelial cells, enzymes, plasma proteins and mucin. Bacterial biomass has also been shown to contribute greatly to the mixture of proteins found throughout the digestive tract, especially at the terminal end of the small intestine (Drochner, 1991) and in the large intestine.

Proteins, peptides and amino acids which enter the large intestine are subjected to a wide variety of microbial metabolic processes, many of which lead to an apparent disappearance of amino acids, however, no absorption of peptides or amino acids occurs in this region (Zebrowska, 1973; cited by Taverner and Curic, 1983)

2.2.3 Effect Of Age, Dietary Fibre And Microbial Fermentation On Digestive Function

2.2.3.1 Factors affecting digestive function in the young pig

Porcine digestive and absorptive capacities are largely determined by the physical capacity of the gut, the nature and amount of secretions it can provide (e.g. acid, enzymes, bicarbonate and bile), development of mechanisms to control these secretions and the digestive and absorptive capacity of the mucosal surface of the small intestine (Cranwell and Moughan 1989). Characteristics such as stomach and intestinal weight per unit of body weight, increase rapidly after weaning, whilst microbial fermentation may not develop peak efficiency for many months (Fernandez *et al.*, 1986).

Nielsen (1962; cited by Fernandez *et al.*, 1986) reported that the small intestine of pigs weighing 20kg was fully developed whilst the digestive capacity of the hindgut was still developing in pigs weighing 150kg. Hence age of pig may be the primary determinant of digestive efficiency of many nutrients (particularly oligosaccharides and NSP) in which microbial fermentation is required.

Cranwell and Moughan (1989) report experiments in which the relative weight of the small intestine of pigs weaned at 21 days of age, increased by 84-98% within the subsequent 21 days. Other studies have demonstrated, that whilst the relative length of the small intestine decreases with age in the post-weaning period, the diameter and holding capacity of this structure undergoes a 3.5 and 43 fold increase between birth and 8-10 weeks of age respectively (Cranwell and Moughan, 1989).

2.2.3.2 Gastric and intestinal organic acids in the young pig

Friend *et al.* (1963)(cited by Kidder and Manners, 1986) have studied the level of organic acids in the stomach of weaned pigs from 1 week to maturity, and reported that the total organic acids in the stomach amounted to 150 meq/l in mature pigs and

30-92 meq/l in early weaned pigs. Lactic acid (i.e. product of microbial fermentation) contribution to total molar concentration was over 90% of the gastric organic acid in most pigs of all ages, but on cereal diets, this fell to 50%. The remaining VFA were approximately half propionic, with progressively lower concentrations of butyric and valeric acids. In older pigs, formic acid contributed 3.6 to 4.5% of the organic acids.

Intestinal studies by the same authors (Kidder and Manners, 1986) found organic acid levels of 110 meq/l in the caecum and colon of young (1 week old) pigs, rising to 300 meq/l in mature pigs. The primary component of these VFA's were acetic acid, followed by propionic, butyric, lactic and valeric acids in reducing amounts. Argenzio and Southworth (1974; cited by Kidder and Manners, 1986) found that proportional composition of the organic acids were not affected by position along the large intestine, but were influenced by diet.

2.2.3.3 Effect of age on microbial activity

Fermentation in the hindgut of the pig is a function of, the quantity of readily fermentable or digestible dietary carbohydrate, the microbial population and the transit time of digesta through the large intestine. When diets containing high levels of readily fermentable carbohydrates are offered to pigs, there will be relatively more fermentation in oesophageal region of the stomach and small intestine and less in the large intestine, when compared with diets containing high levels of dietary NSP or oligosaccharides.

Fernandez *et al.* (1986) compared the digestive ability of growing pigs (40-60kg) to that of large adult sows (180kg) using 26 foodstuffs and diets. Without exception the digestive capacity of the sow was found to be superior to that of the growing pig, on average digesting 0.15 more crude protein, 0.10 more crude fat, 0.30 more crude fibre and 0.09 more gross energy than the younger pigs.

Regression analyses (Fernandez *et al.*, 1986) have demonstrated that increasing amounts of dietary crude fibre will increase the relative difference in digestibility coefficients between sows and growing pigs, and that the difference in gross energy digestibility is negatively correlated with the concentration of soluble carbohydrate, suggesting that the largest part of the difference in digestive capacity between sows and growing pigs originates from the greater hind-gut fermentation capability of the sow.

2.2.3.4 Effect of dietary fibre on digestive function

The effect of crude fibre from different cereals on gut fill, digestibility and sites of digestion of nutrients by growing pigs (20 to 90kg) has been reported by the Standing Committee on Agriculture (1987). In these experiments it was found that a 1% increase in dietary fibre depressed the digestibility of gross energy by 3.5% and depressed efficiency of utilisation of metabolisable energy by 0.7%. The proportion of digested energy which disappeared in the caecum-colon increased with increasing content of dietary crude fibre, partially explaining the negative influence of crude fibre on the efficiency of utilisation of metabolisable energy by the pig.

Taverner and Curic (1983) conducted experiments with *L. angustifolius, L. albus, P. sativum* and soyabeans in wheat-based diets to determine the effect of each ingredient on the ileal and faecal digestibility of energy and N. They found significant ($P<0.01$) interactions between ileal and faecal digestibility coefficients for gross energy and nitrogen among these diets. They also found considerable differences in the sites of energy digestibility (ileal energy digestibility; wheat 80%, narrow-leaf lupin 44%, white lupin 56%, soyabean meal 65%, peas 79%). It was calculated that the ratio of net energy:metabolisable energy decreased by 0.5% for each one percent proportion of energy which disappeared from the hind-gut.

2.3 AMINO ACID DIGESTIBILITY AND AVAILABILITY

2.3.1 Use of digestibility coefficients

The nutritive value of any ingredient supplying protein to the pig may be determined by its a) total, b) digestibile, or c) available amino acid composition. Each method of assessment has a greater or lesser importance in assessing the relative value of differing feedstuffs, particularly with regard to amino acids which are likely to be limiting performance.

Digestible nutrient content (amino acids and energy) is commonly used as a relative comparison of the nutritive value of one feedstuff with another. The accuracy with which this measure predicts nutritive value is determined by the site and extent to which the nutrient disappears from the digestive tract of the pig (Taverner and Curic, 1983). It is well documented (Zebrowska, 1973; Taverner and Curic, 1983) that amino acids and energy which disappear from the stomach and small intestine of the pig are effectively utilised by the pig for growth and maintenance, whereas gross energy and amino acids which disappear from the large intestine are less available and unavailable to the pig respectively.

2.3.2 Definition of amino acid "availability" and "digestibility"

The terms amino acid `availability' and amino acid `digestibility' are often used synonymously in the literature, however each is a different measure of the nutritive value of a feedstuff. Amino acid digestibility may be defined as the difference between the quantity of a specific amino acid in a diet or feedstuff, and the quantity of that amino acid in the ileal digesta (or faeces), divided by the amount in the diet (Sauer *et al.*, 1989; Sauer and Ozimek, 1986). Amino acid availability may be defined as the proportion of the total amino acid in a diet or feedstuff which is digested and absorbed in a form suitable for protein synthesis by the pig (Batterham, 1992a).

Unlike amino acid digestibility, which is determined by laboratory analysis of both feedstuff and digesta, amino acid availability can only be determined by techniques which measure the utilisation of an amino acid when it is limiting in a diet (Batterham, 1992a). Availability can only be determined for one amino acid at a time, and is generally assessed by growth experiments, in which the response to increasing increments of the test amino acid in a feedstuff is compared to the response observed to the standard free amino acid (Batterham, 1992a). The statistical design of the treatments is referred to as a slope-ratio assay and in essence expresses the slope of the response of the pig to the diets containing the test amino acid as a proportion of the slope of the response to the free amino acid (Batterham, 1992a).

2.3.3 Total amino acids vs amino acid "digestibility" and "availability"

Formulating diets of differing ingredient composition, on the basis of equivalent total amino acid composition, does not necessarily lead to diets of comparable nutritive value for the pig. An understanding of the amino acid digestibility of individual ingredients, and subsequent formulation of diets on this basis, is widely used as a more accurate method of producing diets of differing ingredient composition, yet similar estimated nutritive value. There are limitations to the accuracy with which amino acid digestibility can predict the nutritive value of a feedstuff, the key limitation being the relationship which exists between digestibility and availability. The closeness of this relationship varies from feedstuff to feedstuff, however, generally speaking, the relationship is closer in cereal feedstuffs and protein concentrates which have not been heat treated (Batterham, 1992b). Table 2 shows the relative differences between ileal digestibility and availability of lysine in soyabean and cottonseed meals.

Amino acid digestibility is less expensive to determine, more easily interpreted and faster than determination of amino acid availability, and for this reason digestible nutrient content has become the predominant comparative measure of nutritive value for many feedstuffs.

Table 2. Comparison of ileal digestibility and availability of amino acids in cotton seed meal and soyabean meal to growing pigs

	Ileal digestibility	Availability
Cottonseed meal No. 1	0.58	0.27
Cottonseed meal No. 2	0.68	0.30
Cottonseed meal No. 3	0.72	0.29
Soyabean meal	0.89	0.90

From Batterham (1992b)

2.4 DETERMINATION OF AMINO ACID DIGESTIBILITY

2.4.1 Ileal vs faecal determination of amino acid digestibility

As defined earlier, amino acid digestibility may be determined either by analysis of digesta extracted from the pig at the terminal ileum, or by analysis of the faeces. Faecal amino acid digestibility is a simple technique requiring no invasive practices for collection of the sample (faeces). On the other hand ileal assessment of amino acid digestibility, by definition, dictates that some surgical techniques are required to permit collection of the digesta.

The faecal analysis method for determination of amino acid digestibility was developed by Kuiken and Lyman (1948; cited by Sauer and Ozimek, 1986) and was widely adopted in early studies of amino acid digestibility by pigs. Despite advantages related to the simplicity of this technique, it has been shown to be significantly flawed in a number of important areas.

In the first instance, it has been shown on many occasions, that amino acid absorption from the large intestine does not occur in a form which is available to the pig. Early work by Zebrowska (1973; cited by Sauer *et al.*, 1989) demonstrated the superiority of

ileal to faecal measures of amino acid digestibility by showing that whilst intact and enzymatically hydrolysed casein were digested and absorbed when infused into the distal part of the ileum of pigs fed a protein-free diet, that the amino acids within the casein were rapidly and completely excreted in the animal's urine. In contrast, however, when casein was given orally to the animals, the level of free amino acids in the blood increased whilst the level of plasma urea was low.

More recently, Schmitz *et al.* (1991) conducted an experiment in which pigs were either caecally infused with homoarginine, or supplied orally with either 100%, 10% or 5% of the caecally infused level of homoarginine. Blood assays were conducted at various times after infusion/consumption and it was found that the blood level of homoarginine in the animals which were orally infused increased clearly and rapidly at 100% and 10%, but was unchanged in animals which were caecally infused. It was concluded that amino acid absorption from the large intestine was less than 10% of that infused into the caecum.

Sauer and Ozimek (1986) have shown that when dietary energy is limiting, undigested protein in the hindgut is degraded by microbes to ammonia and amines, which, although absorbed through the wall of the large intestine, are excreted primarily in urine. When dietary energy is plentiful, undigested nitrogen is utilised by microbes for synthesis of bacterial protein which is then expelled in the faeces.

All experiments have demonstrated that amino acids which disappear from the large intestine make no contribution to the amino acid/peptide nutrition of the animal. The only exception to this may be when an animal is offered diets which are limiting in nitrogen per se for the synthesis of dispensable amino acids, and thereby having a sparing effect on the utilisation of indispensable amino acids (Sauer *et al.*, 1989).

The faecal analysis technique is further flawed by the intense fermentation which occurs in the large intestine of the pig. This fermentation is so intense that up to 76% of the total faecal nitrogen may be of bacterial origin (Sauer and Ozimek, 1986). Furthermore, aggressive degradation of amino acids by bacteria in the large intestine leads to greater digestibility of most amino acids in most feedstuffs by the faecal technique than the ileal technique.

Microbial synthesis and utilisation of amino acids, particularly in the hindgut, is a significant (and unpredictable) contributor to amino acid composition of faeces, and therefore has a considerable effect on the estimated apparent amino acid digestibility of a feedstuff. In some cases, as reported by Tanksley *et al* (1982) and Low (1987) (cited by Sauer *et al.*, 1989), a net synthesis of lysine and methionine can occur through microbial fermentation. Whereas cystine, threonine and tryptophan disappear to a large extent (Batterham, 1992b).

Faecal digestibility estimates are no longer accepted as accurate predictors of nutritive value of a feedstuff, as the technique tends to produce misleading estimates of digestibility (Batterham, 1992b).

2.4.2 Techniques for collection of ileal digesta

2.4.2.1 Overview of techniques

Techniques for collection of ileal digesta are numerous, however each requires physiological disruption of the gastrointestinal tract of the pig. These techniques may be broadly classified as cannulation, ileo-rectal anastomosis and the slaughter technique. Use of the cannulation technique permits multiple or complete (depending upon type of cannula) sampling of ileal digesta over many days, and potentially throughout multiple experiments. Ileo-rectal anastomosis is a technique which also permits complete collection of digesta for extended periods. Serial slaughter, however,

is a procedure requiring euthanasia of the pig, and therefore provides a single "spot" sample of variable quantity.

2.4.2.2 Cannulation

Cannulation is a surgical technique in which the ileum is either partially or completely transected, allowing implantation of either a "simple T-piece" or "re-entrant" cannulae respectively. The simple T-piece cannula, placed 5-10 cm anterior to the ileocaecal valve is a commonly used technique. This technique avoids transection and disruption of the myoelectric complex of the of the small intestine, and for this reason is thought to have less adverse physiological affect on the animal and therefore less potential to alter digestive efficiency (Sauer and Ozimek 1986). A further advantage of this technique is retained function of the iloecaecal sphincter, permitting greater residence time for digesta in the small intestine (Moughan and Donkoh, 1991).

Because only partial sampling of digesta is possible, the success of the T-piece cannula technique depends upon, a) obtaining representative samples of digesta, and b) selecting an indigestible marker which is appropriate for the feedstuff which is to be assessed. Furthermore, in order to obtain representative samples, attention should be paid to such factors as, a) the frequency and duration of sampling in relation to the time and frequency of feeding, b) the internal diameter of the cannula, c) dry matter content and viscosity of the digesta, d) fibre content of the diet, e) the mesh size of the screen through which the diet has been ground, and f) the amount of digesta which is collected (Sauer and Ozimek, 1986).

Re-entrant cannulae may be classified according to where they transect the intestine of the pig. These combinations (shown in Figure 4) include; ileo-ileo (placed 30-40 cm anterior to the ileocaecal sphincter), ileo-caecal (one end placed 5-10cm anterior to the ileocaecal sphincter, the other entering the hindgut at the caecum) and the ileo-colic post-valve techniques (the anterior end of the cannula is inserted posterior to the

ileocaecal sphincter, hence preserving its function in regulating digesta flow to the hindgut, and the posterior end of the cannula enters the hindgut at the colon rather than the caecum) (Sauer *et al.*, 1989).

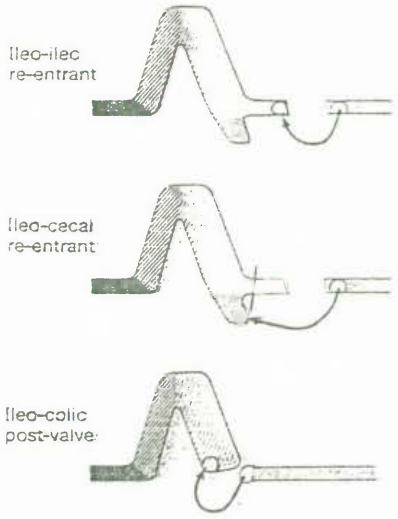

Figure 4. Representations of different cannulation techniques for collection of ileal digesta (Sauer et al., 1989)

Blockage of re-entrant (ileo-ileo and ileo-caecal) cannulae is a primary problem associated with these techniques. As is the case with simple T-piece cannulae, dietary fibre content, degree to which the diet has been ground, viscosity, and quantity of digesta, each influence the degree to which the cannula will become blocked. Blockage appears to be less common in pigs fitted with ileo-caecal than ileo-ileo cannulae (Sauer and Ozimek, 1986).

To overcome problems associated with blockage, methodology has been adopted in which finely ground diets have been offered to pigs on a restricted basis. It is therefore important to determine whether digestibility values obtained under restrictive feeding

and using finely ground diets, are applicable to practical situations (Sauer and Ozimek, 1986).

Ileo-colic post-valve cannulae become less frequently blocked than some others mentioned above. This technique preserves the function of the ileocaecal sphincter, prolonging the retention time of digesta in the small intestine by 60-90 minutes (Sauer *et al.*, 1989), allowing sampling of digesta according to its normal arrival in the large intestine. Using this technique, it is possible to measure nutrient digestibility in coarsely ground diets (Sauer and Ozimek, 1986).

2.4.2.3 Ileo-rectal anastomosis

The ileo-rectal anastomosis technique (often referred to as the ileo-rectal shunt) was suggested by Fuller and Livingstone (1982; cited by Sauer and Ozimek, 1986) as a rapid, routine technique for collection of ileal digesta. This procedure involves transection of the ileum (15 cm anterior to the ileocaecal sphincter), and reattachment of this section of the ileum to either, a) the side of the descending colon, just before the rectum, b) the rectum with complete exclusion *in situ* of the large intestine, or c) as in b with the addition of a simple T-piece cannula which is implanted in the colon to allow evacuation of gases produced by microbial activity. Figure 5 provides a schematic representation of these techniques.

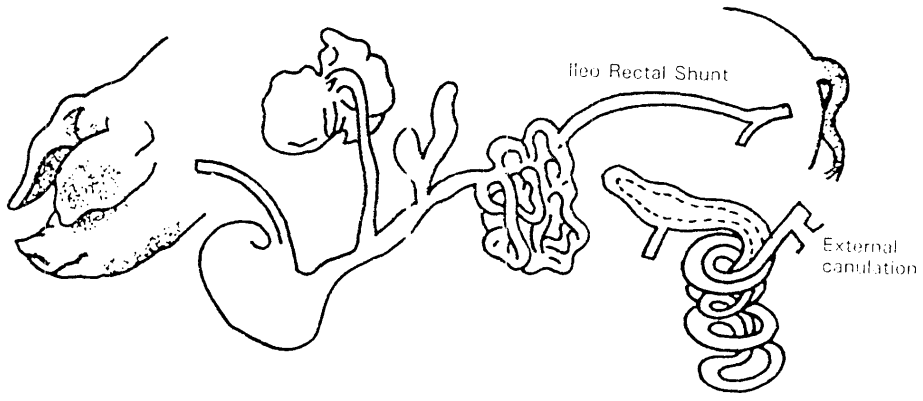

Figure 5. Ileo-rectal anastomosis (Rhône-Poulenc, 1989)

2.4.2.4 Slaughter

The slaughter technique is an alternative to all of the surgical procedures previously discussed. Using this method, the terminal ileum (usually a length 1.5m anterior to the ileocaecal valve) is dissected from either anaesthetised or euthanased pigs, and the digesta collected. This technique has the distinct advantage that there is only minimal disruption of the normal digestive function of the pig prior to collection, and that samples of digesta may be taken from many parts of the digestive tract. Criticism of this technique surrounds, a) difficulty in obtaining representative samples of digesta, b) the applicability of the indigestible marker to the ingredient, nutrient or diet being assessed, and c) the incidence and degree to which post-morteum secretions may alter the nutrient profile of digesta prior to collection.

2.4.2.5 Comparison of techniques

Studies by Zebrowska et al. (1977; citec by Sauer and Ozimek, 1986) comparing the simple T-piece, with re-entrant cannulation, found no difference between the techniques in most instances. however, out of 72 comparisons (four diets, 18 amino acid) which were made, 12 measurements were significantly different between the two techniques. Taverner *et al.* (1983) reported no difference between re-entrant and simple cannulae in pigs offered wheat, wheat/lupins and wheat/meat and bone meal diets.

Other studies (Kohler *et al.*, 1990) have compared digestibility of dry matter, nitrogen, crude fibre, ADF and NDF when determined using simple T-piece, post-valve T-caecum and ileo-caecal re-entrant cannulation. in cereal, pectin-rich, crude-fibre-rich and semisynthetic diets. In this instance dry matter digestibility in the cereal, pectin-rich and crude-fibre rich diets were higher using the re-entrant cannula. In the crude fibre-rich diet, digestibility of crude fibre, ADF and NDF, as well as ADF digestibility in the semisynthetic diet was also higher with the re-entrant cannula.