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FACTORS INFLUENCING THE APPARENT FAECAL DIGESTIBILITY OF ENERGY AND ORGANIC MATTER IN WHEAT AND WHEAT BY-PRODUCTS (BRAN AND BROLL) FOR THE GROWING PIG

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ABSTRACT

The effect of several factors on the determination of the apparent faecal digestibility of dietary energy (ADE) and dietary organic matter (ADOM) for the growing pig was studied. The work was conducted in three parts.

In the first part of the overall study, the effects of collection method (total faeces collection versus chromic oxide as a marker) and duration of the faeces collection period were examined. Thirty kg liveweight pigs were subjected to a conventional balance study (7 days adaptation, 12 days faeces collection) and were for either a wheat- or wheat by-product- (broll/bran) based diet. ADE and ADOM were higher ($P<0.001$) for the wheat diet in comparison to the wheat by-product based diet and in general higher ($P<0.001$) ADE and ADOM values were found with total collection versus the marker. There was a significant ($P<0.001$) effect of duration of the collection period. Chromium recovery (%) increased, for both diets, over the first 3 to 4 days of the collection period, but thereafter was relatively constant.

The aim of the second part of the study was to determine the influence of the two factors, feeding level (6 or 11% of metabolic liveweight) and liveweight (25 or 90 kg), on ADE and ADOM in the two cereal based diets. Growing pigs were subjected to a conventional balance study and digestibility coefficients were calculated by reference to the indigestible marker, chromic oxide. There were no significant ($P>0.05$) effect of feeding level but a significant ($P<0.05$) though relatively small effect of animal liveweight, with digestibility being somewhat higher for the heavier pigs.

The third part of the overall study evaluated the effect of genotype on ADE and ADOM for the two cereal based diets. Four-month-old Large White x Landrace pigs,

(55 kg liveweight) and three-month-old Kune-Kune pigs (20 kg liveweight) were subjected to a conventional balance study with ADE and ADOM being determined based on a total collection of faeces. When for the wheat by-product based diet the Kune-Kune pigs showed a higher ($P<0.001$) digestibility of nutrients, no statistically significant difference between genotypes was found for the more digestible wheat based diet.

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GENERAL INTRODUCTION

The cost of feed accounts for two-thirds or more of total pig production costs, with the cereal component representing the greatest proportion of the feed cost. Cereal grains are the major energy source for pig diets in New Zealand and the digestible energy they contribute is one criterion used to characterize their nutritive value. Most of the variation observed in determined digestible energy across cereals is due to differences in the cereal's chemical characteristics, but a part of the variation, although minor, is related to other factors, such as, methods of determining digestibility, feeding level, age, liveweight and pig genotype. Up until recently this non-feed variation, though recognized has been ignored in dietary formulation practice, but with the development of computerized mathematical pig growth models, the opportunity is presented to review traditional practices and consider the introduction of appropriate allowances for non-feed sources of variation.

Digestibility coefficients provide the main criterion for assessing the nutritive value of feeds for pigs. The non-feed sources of variation are believed to be influenced by four main factors namely: (1) method of determination, (2) feeding level, (3) liveweight and (4) genotype. The central theme of this thesis is to examine these factors in relation to the measurements of apparent digestibility of dietary energy (ADE) and the apparent digestibility of dietary organic matter (ADOM) as obtained using the growing pig.

There were three objectives. The first was to examine the influence of collection method and length of faecal collection period on ADE and ADOM for two cereal products (wheat and wheat by-products) using total faecal collection and indicator methods. The second was to assess the influence of feeding level and liveweight on ADE and ADOM using an indicator method. The third was to determine the effect of animal genotype on ADE and ADOM using the method of total collection.

Chapter 1

Review of Literature

1.1 Introduction

Digestibility coefficients are among the most important parameters in evaluation of the nutritive value of feeds for pigs. Several studies, on dietary energy digestibility in the growing pig, have emphasized the effect of different influencing factors. The present study begins with a review of the literature on the process of digestion and nutrient absorption in the pig and an examination of factors influencing the determination of digestible energy and digestible protein and amino acid values in feedstuffs for the pig.

1.2 Digestion and Absorption in the Pig

1.2.1 Morphology of the Digestive Tract

The digestive tract comprises the mouth cavity, oesophagus, stomach, small intestine and large intestine. There are two major glands, the liver and pancreas, connected to the digestive tract.

The mouth region consists of a tongue, lips, cheeks and teeth. It is adapted to obtaining feed, physically grinding the feed into smaller particles and secreting saliva via salivary glands. The teeth comprise incisors for cutting feed and molars which reduce feed size. Saliva is secreted by the three salivary glands, the parotid, submaxillary and sublingual glands under the control of the autonomic nervous system. It serves to moisten the feed and lubricate the oesophagus to ensure easy swallowing of the feed bolus. Saliva consists of water and mucus and has some digestive enzymes, notably salivary amylase. The consistency of mucus varies with the type of food. Feed stays in the mouth for a limited time before passing into the oesophagus which forms the first part of the tubular

digestive system. The lumenal boundary includes a stratified squamous epithelium, beneath which is numerous tubuloacinar mucus glands lubricating the feed bolus.

The stomach is classified into four functionally distinct regions: oesophageal, cardiac, gastric gland and pyloric. It is both a temporary storage organ and the first major organ for digestive activity. Apart from initiation of protein digestion, much of the physical structure of feeds is disrupted here. This makes the chemical structures more available for enzymatic hydrolysis in the small intestine.

The small intestine comprises three parts: duodenum, jejunum and ileum. The length of central jejunum accounts for 80-90% of the small intestinal length and the remainder is approximately equally divided into two regions, which are the cranial duodenum and the caudal ileum (Longland, 1991). The opening of the common bile duct is 2-5 cm from the pylorus and the pancreatic duct opens a further 10 cm beyond (Low and Zebrowska, 1989). The majority of food digestion by host enzymes and the absorption of end products occur in the small intestine. Nutrient absorption is facilitated by the greatly increased surface area provided by the villi, which occur along the length of the small intestine. Microflora are present throughout the small intestine, and become progressively more profuse towards the large intestine.

The large intestine starts at the caecum and connects with the ileum at the caeco-colic junction by way of an ileal-caecal valve. It comprises a short, blind-ended caecum, that connects with the colon which opens to the exterior at the anus. The large intestine of the mature pig is 4-4.5 m long and has a much greater diameter than the small intestine (Low and Zebrowska, 1989). The rectum is a short-length of the terminal colon ending in the anus. The large intestine is separated from the small intestine by the one-way, ileo-caecal valve.

The large intestine contains a dense population of microorganisms (especially bacteria). The majority of microbial fermentation occurs in the large intestine, the end

products of which are absorbed across the mucosa. Thus the large intestine partly removes nutrients flowing from the small intestine before the undigested residue is excreted via the rectum and anus.

1.2.2 Digestion of Carbohydrate

Carbohydrates in animal feeds can generally be classified as storage carbohydrates (e.g. starch (or glycogen) and certain disaccharides), which can be hydrolyzed by the host enzymes to their constituent monomers, and the non-starch polysaccharides (NSP) of plant cell walls (e.g. cellulose, hemicellulose and pectins), which cannot be digested by the host enzymes but must be fermented by the microflora, yielding volatile fatty acids (VFA) and gases.

Starch consists of varying proportions of amylose and amylopectin. Amylose consists of long, linear chains of α 1-4 linked glucose residues, whereas amylopectin generally has shorter linear chains of α 1-4 linked glucose units which are branched by α 1-6 linkages. The disaccharide sucrose, the storage carbohydrate of some plants (e.g. sugar cane and sugar beet), contains glucose and fructose.

The first starch-degrading enzyme encountered by the ingested feed in pigs is α-amylase secreted by the submaxillary and sublingual salivary glands. It breaks down starch to a mixture of maltose, maltotriose and various dextrins and it requires the presence of chloride for full activity. It is active over the pH range 3.8-9.4 with an optimum, in the presence of chloride, of 6.9.

The linear chains of amylopectin and glycogen are hydrolyzed by α-amylase, but the α 1-4 links near branch points and the α 1-6 links themselves are not degraded. These unhydrolyzed portions of one or more α 1-6 linkages are the α-limit dextrins (Roberts and Whelan, 1960).

The α-amylase digestion of starch continues during its passage to and residence in the oesophageal region of the stomach, until mixing with HCL-containing gastric juice reduces the pH to less than 3.5 – the lower pH limit of α-amylase activity. Some starch, hemicellulose and sugar breakdown may also occur in the upper regions of the stomach, due to the fermentation activity of the gastric microflora, the main end product being lactic acid (Friend et al., 1963).

The main site for the digestion of storage carbohydrates in pigs is the small intestine. The pH of the gastric digesta flowing into the duodenum is gradually raised to a level suitable for carbohydrase activity by the secretion of alkaline pancreatic juice, bile and the products of Brunner's glands. These secretions are largely stimulated by the presence of feed of low pH in the small intestine. Starch hydrolysis continues as a result of secretion of α-amylase by the pancreas. The action of the pig pancreatic α-amylase differs from that of salivary amylase by its unequal action on susceptible bonds in the early stages of hydrolysis, producing relatively large amounts of reducing sugars as opposed to products of longer chain lengths (Banks et al., 1970).

The dextrins and sugars from the starch, together with those present in the diet, are exposed to the action of the carbohydrases on the surface of the small intestinal mucosa, and are largely split to monosaccharides. These are actively absorbed by the mucosa and pass into the capillaries leading to the portal vein. Intestinal maltase digests maltose produced by the action of amylase; sucrase splits sucrose to glucose and fructose.

Passage of feed down the small intestine is speeded by dietary fibre, but although fibre reduces the time for intestinal digestion, only energy yield appears to be affected (Lawrence, 1984).

Storage carbohydrates reach the large intestine, together with the majority of the ingested fibre. The large intestine accounts for about 40% of gastrointestinal volume. Transit through the large colon takes some 35 hours and accounts for 80% of the time

feed particles spend in the gut. Transit of large particles is slower than that of small particles. The large intestine accounts for about 15% of the dry matter digestion in pigs fed a high-grain diet.

1.2.3 Digestion of Protein

Digestion of protein starts in the stomach as a result of the action of the pepsins of the gastric juice. Like other proteolytic enzymes throughout the body, they are formed as inactive precursors which are activated by hydrolytic removal of a peptide from the N-terminal end of the molecule. The precursors of the pepsins are pesinogens and are secreted by the mucosa of the fundal and pyloric regions of the stomach.

Pig pesinogens are hydrolyzed to pepsins in acid conditions, slowly at pH 4 and rapidly at pH 2 (Ryle, 1960), the pepsin produced catalyzing the activation, so that the process is autocatalytic. At pH 4 the intramolecular activation due to acid activity alone is slow, but below pH 3 the intramolecular reaction is rapid and predominant.

The pepsins each have two pH optima, one near pH 2.0 and the other about 3.5 (Taylor, 1959), the pepsins from the pyloric region having slightly lower pH optima than those from the fundal region. The pH in the pyloric region is normally lower than that in the fundus. The way in which activity falls off with increasing pH above 3.5 depends on substrate. The rate of hydrolysis decreases for bonds involving glutamic acid and cystine and activity is low on bonds between valine and glycine, tyrosine and cystine and tyrosine and serine.

As the contents of the stomach pass through the duodenum they are mixed together with pancreatic and duodenal secretions and bile. These secretions are alkaline, with the result that pH rises progressively reaching nearly 7 by the end of the small intestine. This takes the pH digesta from the range of the pepsins into the optimal range for the pancreatic proteolytic enzymes. The amino acids formed by protein breakdown

are absorbed and the peptides further broken down by the intestinal mucosal enzymes before or after absorption. The proteolytic enzymes involved in initial digestion can be classified into three groups. These are: (1) endopeptidases which act on a susceptible peptide link wherever it occurs in the protein chain, provided it is accessible to the enzyme, (2) carboxypeptidases, which remove an amino acid residue from the carboxyl end of the chain and (3) aminopeptidases, which remove an amino acid residue from the amino acid end of the chain.

The endopeptidases and carboxypeptidases are secreted by the pancreas as inactive precursors called zymogens, while the aminopeptidases are associated with the intestinal mucosa, being present either on the brush border or within the mucosal cell. The zymogens of all the pancreatic proteases are activated by trypsin which splits an arginyl or lysyl bond to release a small peptide from the N-terminal end of the molecule, the rest of the molecule forming the active enzyme. Initially trypsinogen is activated by enterokinase (enteropeptidase), an enzyme located on the brush border of the duodenal mucosa. The trypsin produced can activate more trysinogen as well as activating the other proenzymes. The aminopeptidases of the intestinal mucosa complete protein digestion, by removing a single amino acid residue from the amino acid end of a peptide chain. The aminopeptidases located in the brush border hydrolyze peptides in the intestinal lumen whereas those in the cytoplasm obviously only hydrolyze peptides which have been absorbed.

The absorption of the digestion products of proteins by the mucosal cells involves five processes: (1) hydrolysis of peptides in the lumen by aminopeptidases on the luminal surface of the mucosal brush border, (2) absorption of free amino acids from the lumen, (3) absorption of small peptides, (4) hydrolysis of absorbed small peptides by aminopeptidases within the mucosal cell and (5) metabolism of some amino acids including some resynthesis of protein.

Unabsorbed amino acids are utilized by large intestinal microbes, and some are deaminated to ammonia and amines, which are absorbed. This absorbed ammonia is not normally available to the pig for protein synthesis. The disappearance of dietary nitrogen in the large intestine falsely inflates protein digestibility figures when measurement is based on faeces collection. For this reason, the most meaningful measurements of protein and amino acid digestibilities are made with cannulated pigs where digesta can be collected directly from the terminal ileum. Only the protein that disappears in the small intestine is absorbed as amino acids and is directly nutritionally available to the pig (Tanksley and Knabe, 1984).

1.2.4 Digestion of Fat

The hydrolysis of fat is initiated in the stomach by gastric lipase. However, dietary fat is not digested to any significant extent before the small intestine and leaves the stomach of the pig in relatively large globules. Dietary fats consist mainly of triglycerides with some phospholipids, sterols and sterol esters. Endogenous phospholipids are also added to the gut contents, mainly in the bile.

The hydrolysis of fats in the small intestine is catalyzed by at least three different enzymes and one coenzyme (colipase) from pancreatic juice. These enzymes are (1) pancreatic lipase, which is rather non-specific and breaks down triacylglycerides into monoacylglycerides and fatty acids. (2) Pancreatic cholesterol esterase, which in the presence of bile salts, hydrolyses sterol esters to free sterol and free fatty acids (Swell et al., 1953). (3) Pancreatic phospholipase which hydrolyses dietary phospholipid by removing the fatty acid in the 2 position of the glycerol, yielding lysolecithin (De Haas et al., 1965). The intestinal mucosa secrets alkaline phosphatase, which could hydrolyse lysolecithin further, but it is thought that lysolecithin is absorbed directly by the mucosa.

In the presence of bile salts, the end products of fat digestion (i.e. the monoglycerides, free fatty acids, lysolecithin, free sterols and free esters) pass into

micellar solution and are then absorbed. Fat is absorbed along the entire small intestine, but not uniformly. Fat absorption occurs mainly in the jejunum. The digestion and absorption of fats depends on their ability to form micelles. The micellar lipid in the small intestinal lumen of the pig contains about 45% fatty acids, 25% monoglyceride, 10% diglyceride and 5% each of triglyceride, cholesterol, cholesterol esters and phospholipid. The monoglycerides and fatty acids are taken up by the mucosa from this micellar solution, the shorter chain acids transported through the mucosa to the portal blood. The monoglycerides and the longer chain fatty acids are synthesized within the mucosal cells to triglycerides and phospholipids which accumulate as lipoprotein-covered droplets (chylomicrons) which are taken up by the intestinal lymph.

This process in turn is affected by a number of factors such as chain length of the fatty acids, the degree of the unsaturation, the positioning of fatty acids in the triglyceride molecule, the relative concentrations of free and esterfied fatty acids, rate of passage of digesta, age of animal and feeding method.

1.3 Energy Evaluation in Feedstuffs for the Pig

1.3.1 Energy Values Concepts

1.3.1.1 Gross Energy (GE)

Gross energy is defined as the amount of heat released (i.e. the heat of combustion of a unit weight of material (feed, excreta etc.)) as determined by bomb calorimetry when a substance (feedstuff, diet, faeces, urine, animal body) is completely oxidized under 25 to 30 atmospheres of oxygen. Gross energy in a feed is not totally available to the animal because some energy is lost in faeces, in urine, as gases of fermentation (methane, hydrogen) and as heat (Noblet, 1996).

1.3.1.2 Digestible Energy (DE)

The apparent digestible energy content of a feed corresponds to its gross energy content less energy losses in the resultant faeces. The gross energy and the faecal energy can be both measured directly by bomb calorimetry. Digestible energy is simple to determine and to apply, but it overestimates the energy value of crude protein (Batterham, 1990) and it also overestimates the energetic value of feedstuffs or diets of low energy concentration relative to that of feedstuffs or diets of high energy concentration, due mainly to the fact that the amount of endogenous protein and fat secreted into the alimentary canal varies in proportion to the amount of dietary dry matter (Just, 1982c).

Digestible energy is not a true measure of the feed energy absorbed from the digestive tract since faeces contain endogenous losses (digestive secretions and intestinal cell debris). Furthermore, small amounts of various gases are produced. The energy in these gases is lost from the pig and not measured. Quantification of endogenous loss and gas losses is difficult (Noblet and Henry, 1991).

True digestible energy is the intake of gross energy minus faecal energy of food origin corrected for that of endogenous origin. The measure of digestible energy used in practice is the apparent digestible energy measure because gas and endogenous energy losses are difficult to measure routinely.

The traditional methods for determining digestible energy are time-consuming and expensive. Despite these shortcomings the digestible energy system is the most commonly used measure for the determination of the energy value of pig feeds (National Research Council, 1988) and has the perceived advantages of being considered largely independent of genotype and environment when similar feeding levels are used (Noblet et al., 1985).

1.3.1.3 Metabolizable Energy (ME)

The metabolizable energy content of a feed is the digestible energy content less the energy losses in the urine. The urinary energy loss, mostly in the form of nitrogen, is closely dependent on the dietary protein level, and especially the amino acid balance. Therefore, in the metabolizable energy system of measurement the values are frequently adjusted to a standard level of body nitrogen retention or to zero nitrogen balance (Henry et al., 1988).

Metabolizable energy is a measure of the amount of energy which is available for metabolic processes. Most of the energy lost in gases is due to methane production. Whilst the energy content of feed, faeces and urine can be measured with pigs kept in metabolism crates, the measurement of methane production necessitates the pig to be housed in a respiration chamber (Noblet, 1996). Generally, the loss of energy in methane is small (0.5-1.0% of gross energy) and is unaffected by the composition of balanced diets (Batterham, 1990). Just (1982c) claims it can be neglected for practical purposes and hence the determination and application of metabolizable energy is almost as simple as for digestible energy. Metabolizable energy is strictly a non-additive measure (Batterham, 1990).

1.3.1.4 Net Energy (NE)

Net energy is defined as the metabolizable energy content of a feed minus the heat increment (HI) associated with the metabolic utilization of metabolizable energy and the energy cost of ingestion and digestion of the feed. Nutrients such as fats are absorbed in the small intestine and deposited with little modification, generating less heat than fibre (Batterham, 1990), which is fermented in the hindgut and absorbed as volatile fatty acids. The losses of energy as thermal energy may be expected to vary with diet composition and type of production or biochemical process (Just, 1982c).

Net energy is the energy available for maintenance (NEm), for growth (NEg) and production (NEp). Theoretically, net energy represents the best estimate of a feedstuff's energy availability to the animal for purposes of maintenance tissue replacement and for growth and production. Net energy value can be measured by feeding a particular diet and determining the energy lost as the heat increment, either by calorimetry methods (direct measurement of retained energy) or by comparative slaughter techniques (measurements of production) (Just, 1982c).

The maintenance requirement for energy is defined as that amount of energy that can maintain a zero energy balance in the pig (Just, 1982c). The maintenance requirement is influenced by a number of factors such as genotype, sex, age and weight, gut contents, management and the degree of adaptation of the pigs to their holding quarters.

For pigs on a positive energy balance, the net energy value of the diet is the sum of estimated net energy for maintenance (NEm), net energy for growth (NEg) and net energy for production (NEp). Net energy is a difficult measurement to determine in terms of time and resources. Determinations require sophisticated equipment (respiration chambers or calorimeters) or complex methods (comparative slaughter techniques). They should be accomplished with balanced diets at constant levels (Noblet and Henry, 1991). The NE value of a given feed is related to the heat increment associated with utilization or its final utilization as maintenance, growth and production. NE values are often not additive (Batterham, 1990).

1.3.2 Determination and Prediction of Energy Values in Feedstuffs by *In Vivo* Methods

1.3.2.1 Digestible Energy

The digestible energy value of feeds for pigs can be obtained directly for pigs kept in metabolism crates from determination of the amounts of dietary and faecal energy. An advantage of the digestible energy system is its additivity (i.e. the digestible energy

contributions of the ingredients of the diet are not influenced by the mix of ingredients comprising the diet). This assertion is supported by the studies of Whittemore and Moffat (1976) and Young et al. (1977).

The most commonly used method for estimating digestibility, relies on a quantitative collection of faeces over several consecutive days for animals kept in metabolism cages (classical method). Correction of the total amount of the component ingested by the amount excreted in the faeces provides an assessment of digestibility.

The use of indigestible markers to determined nutrient and energy digestibility is an attractive alternative technique as it dispenses with the need to measure feed intake and faeces output. In the pig, chromic oxide has been the most frequently used faecal marker. However, the indicator method may be limited in that recoveries of chromic oxide of between only 75-98% have been reported (McCarthy et al., 1977).

Frape et al. (1976) detected a small departure from linearity associated with substitution methodology, and the significance of this to composite diets should be established. Additionally, digestible energy may be different for a very young pig which has not developed the appropriate digestive enzymes to deal with the feed, or for the older animal with active fermentative bacteria in its caecum (Morgan and Whittemore, 1982). The total collection method has been widely used for measurements of digestible energy of raw materials reported in feeding tables (National Research Council, 1988; IRNA, 1989; Standing Committee Agriculture, 1987). The indicator method is suitable for purposes of undertaking spot checks and for routinely assessing feedstuffs.

However, the total collection method is lengthy and costly and the value derived is specific to that batch of feed. The establishment of a quantitative relationship between chemical composition and digestible energy would allow feeds to be assessed more quickly and cheaply. Consequently, alternative indirect approaches have been proposed.

1.3.2.1.1 Feedstuffs

For raw materials there have been two main indirect approaches adopted. One approach is to relate the digestible energy of a feed to its content of digestible nutrients based on measurement of gross chemical composition and estimation of the digestibility coefficient (DC) of nutrients. The digestible energy of a feed is predicted from regression equations (e.g. DE=0.0239DCP+0.0363DF+0.0210DCF, Just, 1982c). Tabulated digestibility coefficients (DC) of nutrients determined according to the Weende procedure (crude protein, crude fat and nitrogen free extract) are available (Deutsche Landwirtschafts-Gesellschaft, 1984; Central Veevoeder Bureau, 1986). In comparison with mean tabulated values for raw materials, this method takes into account the variations in chemical composition of some ingredients (Noblet, 1996). However, by doing so, the DC is assumed to be constant irrespective of other nutrient levels. In fact, interaction between digestibility coefficients and fibre levels are likely to occur in pigs. Therefore they may not be applicable for those determined directly (Noblet and Henry, 1991).

A second approach is to use individual equations based on chemical analyses for prediction of the DE value of a feed (Noblet and Henry, 1993). Whether these equations (e.g. wheat, DE=-4.35+1.17xGE-0.052xCF, Batterham et al., 1980) have advantages over tabulated values is doubtful. However, for many ingredients no suitable predictive equations have been developed.

1.3.2.1.2 Diet

The digestible energy content of compound feeds can be obtained by adding the DE contributed by individual ingredients and assuming no interaction. When the actual composition of the feed is unknown, the only possibility is to use prediction equations based on chemical criteria. Fibre has an important effect on the accuracy of the prediction (Noblet, 1996).

Numerous investigations (Wiseman and Cole, 1980; Just et al., 1984; Morgan et al., 1987) have shown that (1) crude protein, fat, and nitrogen-free extract (or sugar and starch) contribute positively to the DE value of diets; (2) ash tends to act as an energy diluent and thus has a negative influence; (3) fibre contributes in a negative manner.

Generally, there are two main types of prediction equation; (1) those that account for all the chemical fractions that contribute towards the energy content of a diet (e.g. DE=17.37-0.051xAsh+0.010xCP+0.016xEE-0.027CF, Noblet and Perez, 1993); and (2) those that have a constant term and include one or more modifiers of this term (e.g. DE=5.62-0.040xCP+0.69xGE-0.016xNDF-0.0233Ash, Morgan et al., 1987). The former is normally based on equations involving crude protein, fat, crude fibre, and nitrogen-free extract. These equations are relatively easy to apply as they are based on the principal components of the proximate analysis system of the diets or feeds and are routinely conducted in many laboratories. The latter may be simple equations with a constant term, gross energy, to represent the energy components and a chemical constituent (normally an estimator of fibre content) to act as a modifier to gross energy.

Depending on the fibre source, the digestible energy value of the diet when predicted from such an equation will be either overestimated when fibre is poorly digestible (e.g. from wheat straw) or underestimated when fibre is highly digestible (e.g. sugar beet pulp or soy bean hulls). Ash content has a significant and negative contribution to digestible energy. However, the coefficient assigned to ash is usually higher than what would be expected from the dilution effect of ash (Just et al., 1984; Morgan et al., 1987; Noblet and Perez, 1993).

1.3.2.2 Metabolizable Energy

Theoretically the metabolizable energy content corresponds to the digestible energy content less the energy lost in the urine and gas. Most of the energy in gas is due

to methane production. The accurate measurement of metabolizable energy requires methane losses to be taken into account in addition to urine, but this is only possible in a respiration chamber. The metabolizable energy content of feed is usually determined in feeding trials similar to digestibility trials, but in which urine and faeces, are collected. Losses of energy through methane in pigs are very low, especially in growing animals (they usually represent less than 0.5% of gross energy, Henry et al., 1988). Although methane losses are likely to be higher in older animals, especially with diets containing a high level of fermentable carbohydrates, in practical conditions this source of energy loss is neglected, so that metabolizable energy is simply determined by subtracting from digestible energy the energy loss in urine.

Energy lost in urine represents a variable percentage of digestible energy since urinary energy is highly dependent on the amount of nitrogen in the urine. The urinary energy loss, mostly in the form of nitrogen, is closely dependent on the dietary protein level, and especially the amino acid balance (i.e. the level of the limiting essential amino acid) (Henry et al., 1988). Therefore, for metabolizable energy determination, it is necessary to standardize the level of nitrogen retention, to that at optimum protein utilization or to zero nitrogen balance. The ME: DE ratio is linearly related to dietary protein content (Noblet, 1996).

Metabolizable energy is preferably measured under conditions of optimum protein and amino acid balance, in order to obtain a ratio between nitrogen retention and nitrogen intake of 0.50 or more according to apparently digested nitrogen in growing animals (Henry et al., 1988). With balanced diets metabolizable energy represents a high and rather fixed proportion of digestible energy around 0.95 (Henry et al., 1988). But for a single feedstuff the ME: DE ratio is inversely related to protein level. In most situations, the ME: DE ratio is considered relatively constant and equivalent to about 0.96 (Noblet, 1996). However, this constant ratio is subject to change when dietary crude protein content and protein retention are either high or low. Additionally, this mean value cannot be applied to single feed ingredients. For instance, ME: DE ratios (methane energy loss included) ranged from 100% for animal fat to 97-98% for cereals, 93 to 96% for protein

sources (soybean meal, peas) and 90 to 92% for fibrous protein sources (rapeseed meal, sunflower meal) (Noblet et al., 1993b).

The approaches for predicting the metabolizable energy value of pig feeds are similar to those described for digestible energy. But since direct metabolizable energy measurements are not carried out routinely, values have usually been calculated from digestible energy values with a ME: DE ratio either constant or preferably, related to the protein content of the diet (IRNA, 1989; National Research Council, 1988). Metabolizable energy is also predicted from equations relating metabolizable energy to digestible nutrients content (Just, 1982c). Like digestible energy, the metabolizable energy content of mixed diets can be estimated from chemical composition (Noblet and Perez, 1993). In this case, the main difference between the corresponding equations for digestible energy and metabolizable energy concern the coefficient obtained for crude protein, which is lower in the metabolizable energy equations. The limitations of using a digestible energy equation also apply to metabolizable energy equations.

Finally, metabolizable energy values can be corrected to a zero or constant body N balance (De Goey and Ewan, 1975) or for the amount of fermented carbohydrates (Deutsche Landwirtschafts-Gesellschaft, 1984). These corrections represent attempts to standardize evaluation conditions or to get closer to the estimation of net energy. However, these correction factors are questionable, not always relevant and are insufficient to estimate the theoretical metabolizable energy value of the diet (Noblet and Henry, 1991).

1.3.2.3 Net Energy

Net energy is metabolizable energy minus the loss of energy during the digestion, absorption and metabolic assimilation of nutrients (the heat increment of feeding). The amount of heat evolved depends on whether the feedstuff is digested by mammalian enzymes or fermented by the gut microflora (Longland and Low, 1995), the latter

pathway generating more heat (Longland and Low, 1995). The classical method for determining net energy is that of comparative slaughter (Batterham, 1990). This involves the slaughter of a pre-experimental group of pigs, followed by the measurement of gross energy intake of the experimental diet by their littermates over a period of at least one-month, whereupon the experimental group is slaughtered. The gut contents are removed and all parts of the pigs are recovered, minced and analyzed for gross energy. The difference between the energy content of the pre-experimental pigs and those receiving the test diet is the net energy value of the ingested feed.

Although this method yields accurate results, it is a destructive and expensive technique (Kotarbinska and Kielanowski, 1969). For kinetic studies, calorimetry, either direct (involving measurements of heat loss) or indirect (involving measurement of inspired and expired gases is employed. Detailed accounts of the use of calorimetry in providing net energy values for practical feeding systems have been described by Nehring (1969), Nehring et al. (1965) and Nehring et al. (1969).

However, determination of the net energy value of a feed is time-consuming, labour intensive and costly in terms of feed, animals and equipment and it would be highly impractical to determine the energy value of every feed or diet in this way. The efficiency ratio between net energy and metabolizable energy (k) varies initially according to the final utilization of energy (Noblet, 1996).

The efficiencies of metabolizable energy conversion to net energy vary according to the chemical characteristics of the feed, since nutrients (carbohydrates, amino acids, long-chain fatty acids or volatile fatty acids) are not used with similar biochemical efficiencies. The ratio between Net energy for maintenance (NE_m) and metabolizable energy for maintenance (ME_m) corresponds to the efficiency of utilization of metabolizable energy to net energy for maintenance. When metabolizable energy intake is higher than metabolizable energy requirement for maintenance, a proportion of the additional energy supply (metabolizable energy for production: ME_p) is retained in the

body as protein or fat or exported as milk (NE_p) in lactating females; the ratio NE_p: MEp corresponds to the efficiency of utilization of metabolizable energy for growth (k_g) or milk production (k_l). During growth, energy gain includes protein and fat energy; the efficiency of utilization of metabolizable energy for energy gain as protein or as fat are defined as k_p and k_f, respectively. Metabolizable energy originates from different nutrients (carbohydrates, amino acids, long-chain fatty acids or volatile fatty acids).

With standard cereal-soyabean meal diets, the following k-values are found; 72% for milk energy in sows (k_l) (Noblet et al., 1990), 75% for energy storage on growing pigs (k_g), 80% and 60% for energy deposition as fat (k_f) and as protein (k_p), respectively (Noblet et al., 1991) and 77% for meeting maintenance requirements (Noblet et al., 1993b).

The main objective in the net energy evaluation of feeds will be to partition the efficiencies of utilization of diets to net energy for maintenance or body energy storage and milk production. It is apparent that the same feed will have different net energy values depending on its final utilization. Moreover, the k value for a particular process will differ depending on what form the energy is supplied in. Efficiency of protein deposition is 85 to 90% while efficiency of fat deposition ranges from 70 to 85% and 98% when metabolizable energy is provided by protein, carbohydrate (glucose) and fat, respectively (Armstrong, 1969).

Finally, the efficiency of utilization of metabolizable energy to net energy will be affected by the climatic environment since the heat increment of feeding is partly used for thermoregulatory purposes. Heat increment and its variation with diet composition will be decreased under sub-optimal or cold climatic conditions (Noblet et al., 1985 and 1989). The practical consequence of this effect of environmental temperature on k is that net energy measurements of feeds should be carried out under thermoneutral conditions.

The net energy value of feeds is usually calculated from prediction equations. There have been two types of prediction equations developed for assessing the net energy content of diets. They are based on the prediction of net energy for fattening or for growth and maintenance (Just, 1982c; Noblet et al., 1994a and 1994b).

1.3.3 Prediction of Energy Value in Feedstuffs by *In Vitro* Methods

Much interest has been focused on the development of rapid, feasible and accurate methods for feed evaluation to replace traditional *in vivo* studies. This has primarily been for reasons that traditional feed evaluation and the determination of digestibility is performed in time-consuming and costly experiments, requiring animals and facilities. Therefore, from the earliest times of research in animal nutrition, efforts have been made to develop simpler and quicker laboratory methods as alternatives to *in vivo* trials.

Rapid techniques, including *in vitro* and *in sacco* methods, have long been routinely used for the evaluation of feeds for ruminants. *In vitro* techniques with rumen fluid or semi-purified enzyme preparation, or both have been widely used for the evaluation of ruminant feeds (Osbourn and Terry, 1977), but there has apparently been less work to use intestinal fluid for digestibility studies in simple-stomached animals. In recent years, such methods have been proposed for the nutritive evaluation of compound feeds and ingredients for monogastric animals. *In vitro* methods for the evaluation of feeds for simple-stomached animals have been developed using either contents of the pig stomach and different parts of the small and large intestine (Vervaeke et al., 1979; Holzgraefe et al., 1985), blends of pepsin and hydrochloric acid in combination with pig intestinal fluid (Furuya et al., 1979; Clunies and Leeson, 1984) as inocula for incubations and relationships between *in vitro* solubilization and meabolizable energy and digestible energy content of feeds or the apparent digestibility of components have been determined (Furuya et al., 1979; Clunies et al., 1984; Babinszky et al., 1990; Graham et al., 1989b).

Furuya et al. (1979) used a two-stage *in vitro* method using pepsin and pig intestinal fluid to estimate the digestibility of dry matter and crude protein in pig diets. The system attempts to stimulate gastric and intestinal digestion in the pig or other monogastric animals. Samples of diets, ground to pass through a 1mm screen, were incubated for 4 hr with a commercial pepsin preparation in acid solution followed by intestinal fluid obtained from a fistulated pig. There was a high correlation (r=0.98) between digestibility measured *in vitro* and the standard procedure (*in vivo*) for typical pig diets.

There were some limitations to the *in vitro* method. First, the efficiency of the intestinal fluid in digesting diets might be influenced by the diet of the host animal. In the *in vitro* method pancreatic enzymes contained in the intestinal fluid were considered to play an important role in digestion, since the major part of intestinal digestion in the lumen is the result of pancreatic enzymes activity (Rerat et al., 1976).

Secondly, any digestion occurring in the large intestine, such as that of fibrous material, is excluded from measurement by this method. It is probable then, that if samples containing a higher crude fibre content such as roughage had been examined, the correlation for dry matter digestibility with *in vivo* digestibility would have been lower.

Since this method only assesses stomach and small intestinal digestion, a further modification might be required for fibrous foods. Clunies et al. (1984) and Löwgren et al. (1989) examined the method and predicted digestion in the small intestine and large intestine of pigs, respectively. The result indicated that the correlation between *in vitro* and *in vivo* were high (r=0.99, 0.93 for dry matter and crude protein digestibility, respectively). Dierick et al. (1985) developed a two step pepsin-jejunal method. This method used pepsin/pancreatin or pronase. The results from this study indicated that jejunal fluid could replace an appropriate pancreatin solution without reducing accuracy.

Babinszky et al. (1990) investigated seven single feeds and sixteen mixed feeds for pigs using the pepsin-pancreatin method and found an improved correlation to faecal digestible crude protein in fattening pigs by the pre-extraction of lipids with solvent rather that the addition of lipase and bile salt to the incubation mixture with pancreatin. Using the linear regression of *in vitro* on *in vivo*, digestible crude protein in dry matter could be predicted. The result indicated that the correlation coefficient for feedstuffs and diets were 0.99 and 0.95, respectively.

The trouble with many of these assays is that they do not stand up to independent scrutiny, particularly when applied to one ingredient. Boisen (1991) developed an *in vitro* method, having considerable promise, which could be used to predict the energy digestibility for individual feedstuffs based on the *in vitro* dry matter or organic matter digestibility.

Boisen and Fernandez (1991) have demonstrated that a general relationship between *in vitro* digestibility of organic matter and faecal digestibility of energy in a great variety of single feedsstuffs and mixtures was obtained after a three-step incubation, with a sequence of pepsin, pancreatin and a mixture of fibre-degrading enzymes.

In a further study, Boisen (1995) showed that the relationship between predicted and determined values of energy digestibility was high when the method was applied to individual feedstuffs (R^2 = 0.92 and 0.97, RSD = 0.7 and 1.5 for barley and sunflower meal, respectively).

An alternative approach to estimating the digestible energy value of pig feeds is to introduce nylon bags containing the test feed into the gut via a duodenal cannula, and then remove them either via cannulae or in the faeces, and then chemically analyze the resultant undigested residue. Graham et al. (1985) introduced bags via duodenal cannulae and reported a close correlation (R^2 = 0.95) between *in sacco* and *in vivo* organic matter

disappearance for 11 samples of feedstuff including cereals and alternative feeds such as distillers grains, repaseed meal, peas, clover and grass.

1.3.4 Application of Energy Values in Diet Formulation for the Pig

The quality of an energy system will be appreciated through its ability to predict animal performance within a satisfactory degree of error and the energy value of both raw materials and compound feeds. There are several comprehensive reviews where methodological aspects on this topic have been presented (Morgan and Whittemore, 1982; Wiseman and Cole, 1983; Morgan et al., 1987; Henry et al., 1988; Batterham, 1990; Noblet and Henry, 1991; Noblet, 1996).

In practical conditions, the NE value of raw materials can be calculated either from the DE (or ME) values given in feeding tables (IRNA, 1989; Standing Committee Agriculture, 1987; National Research Council, 1988) or from the digestible nutrients content estimated from tables (Deutsche Landwirtschafts-Gesellschaft, 1984; Central Veevoeder Bureau, 1986), where digestible nitrogen-free extract is divided into starch (assumed or be 100% digestible) and digestible residue.

If there is to be a method for the simple and routine determination of the DE of a mixed compounded diet on the basis of chemical analysis it is essential to start by examining the relationships between as wide as possible a range of chemical parameters and realized DE. This needs to be done with simple mixtures, to test for interactions (particularly with regard to proteins, fats and fibres) before examining complex mixtures. Ultimately regression equations relating chemical composition to DE should be sought, but it is possible that different regressions would be needed for diets of different chemical character and for pigs of different physiological state (piglet, growing, adult).

The DE does not provide a true measure of the energy value of the absorbed nutrients from the digestive tract, since faeces contain endogenous losses (digestive

secretions, intestinal cell debris) and gas and heat from hindgut fermentation are produced. In addition, the DE system has a limitation regarding the under- and over-estimation of some ingredients (i.e. it overestimates the energy value of protein rich feeds, and fibrous feeds to some extent, while the value of fat is underestimated). Noblet et al. (1990) compared the energy value of animal fat and soyabean on a DE basis. Animal fat energy content was equivalent to 1.8 the value of soyabean meal; on a NE basis, the ratio is 3.5.

The second limitation for correctly predicting the energy value of a diet, remains the estimation of its DE or digestible nutrient content. Variation of digestibility coefficients with feeding level or physiological stage and negative interactive effects have been indicated. For instance, the digestibility coefficient for energy is not constant for a given feed at all stages of pig production. Therefore, even if the efficiency of utilization of ME for NE can be considered as constant at all stages of pig production, different NE values should be used due to large differences in DE or digestible nutrient contents between stages. Therefore, two model stages are recommended: adult sows (whatever their feeding level) and 60kg pigs as representative of the 30kg and 100kg body weight growing period.

Consequently, the commonly accepted concept that the DE content of a diet is only related to its chemical composition cannot be further accepted since animal and other non-dietary factors modify the hierarchy between diets and ingredients. For some ingredients, a range for most probable DE (and ME and NE) values could be then suggested. These observations and the tendency for preparing more complex pig diets (including more by-products) emphasize the importance of future studies on digestive interactions.

Obviously the hierarchy between feeds obtained in the DE or ME systems will vary in the NE system according to their specific chemical composition. Results in the least-cost formulation will therefore depend on the energy system. Relative to the DE

estimate, NE is reduced when protein and fibre contents are higher while it is increased when more energy is provided by fat or starch.

Another advantage of the NE system is that the energy requirements for animals can be expressed on the same basis as energy values of feeds. Two alternatives can be used to transform energy requirements expressed on a DE basis to NE requirements. In the study of Noblet et al. (1989), the ratio NE: DE (mean 71%) varied between 64 and 76%, the extreme values being obtained in unusual pig diets. With more realistic diets, the range would be smaller (from 68 to 73%). In other words, the advantage and the consequences of a NE system are much more important for the choice of ingredients (least-cost formulation) than for evaluating conventional pig diets. However, economical or technical considerations are likely to produce diets of extreme composition. Finally, with regard to the comparison of ME and NE systems, the conclusions are similar to those given for DE and NE with the bias due to protein content being slightly attenuated.

1.4 Factors Influencing the Determination of Digestible Energy

1.4.1 Effect of Animal Factors

1.4.1.1 Age & Liveweight

Change in the digestive capacity of the pig with age and liveweight have been demonstrated by several workers. The effect of age and liveweight on the digestibility of energy is controversial.

A positive effect of bodyweight on dietary digestible energy has been found in growing pigs by Whiting and Bezeau (1957), Cunningham et al. (1962), Roth and Kirchgessner (1984), Everts et al. (1986), Noblet et al. (1993c), Noblet and Shi (1994), Noblet et al. (1994b) and Bakker et al. (1995).

Higher digestibilities of energy for adult sows compared to growing pigs were observed by Fernandez et al. (1979), Fernandez and Jorgensen (1986), Fernandez et al. (1986) and Noblet and Shi (1993).

In contrast, Kass et al. (1980) found that liveweight has a negative effect on the digestive ability. A higher digestibility of energy for growing pigs than adult sows was observed by Rundgren, (1983), Jongbloed and Smits (1984) and Everts and Smits (1987).

Cunningham et al. (1962) found that the digestibility of crude fibre and crude protein in a wood cellulose decreased in the late growing period, but still remained higher than in the early growing period. Digestibility was influenced more by the level of feeding and changes in body weight than by age.

Similar effects of body weight on digestive utilization of dietary energy or nutrients have been reported by Roth and Kirchgessner (1984) and Everts et al. (1986) in growing pigs. Noblet et al. (1993c) showed that the digestibility coefficient of energy increased by 0.30 and 0.45 unit for each 10kg increase in body weight for diets containing 4 and 6% crude fibre over the 30kg to 100kg body weight period, respectively.

Noblet et al. (1994b) observed that stage of growth affected the energy value of diets. Except for cornstarch, digestible energy content was increased in heavier pigs; the largest difference was found for fibre (+. 6 Mcal/kg of dry matter between 45kg live weight and 150kg live weight pigs). In growing pigs, the digestibility coefficient of energy increased with body weight, with larger differences with high fibre diets or raw materials.

Similar interactions between body weight of pigs and diet characteristics were observed over a wider body weight range by Noblet and Shi (1994). The digestibility coefficient of energy was higher in heavier pigs, with a tendency ($P = .06$) for a more accentuated variation with low digestibility diets or with high-fibre ingredients. Bakker et

al. (1995) found that at 60kg liveweight the apparent digestibility coefficients were lower than at 90kg liveweight. Effects of source of carbohydrates, amount of fat or the interaction were also less significant at 60kg than at 90kg.

Fernandez et al. (1979) showed a higher digestibility in pigs with a live weight of 180 and 200kg respectively. Differences in feeding level might be an influencing factor in their comparison. Fernandez and Jorgensen (1986) found a significantly higher digestibility of nutrients in sows than in small pigs; the largest differences were found with normal grinding and high fibre content in the diet. Increasing the amount of fibre in the diets depressed the digestibility significantly, while increasing the live weight of the pigs improved digestibility at all levels of crude fibre. There was a trend towards greater differences between live weight groups receiving the highest amount of crude fibre in the case of gross energy digestibility.

Similar effects of body weight on digestive utilization of dietary energy or nutrients have been observed by Noblet and Shi (1993) when adult sows were compared with growing pigs. However, Kass et al. (1980) found a higher digestibility for lucerne meal in pigs of 48kg than in pigs of 89kg live weight.

Rundgren (1983) showed that there were no differences between the digestibility of energy in large sows and growing pigs given ensiled peas. Jongbloed and Smits (1984) studied results from investigations concerning the food value of ensiled maize for growing pigs and adult sows. The results indicated a proportionately higher energy value of 0.05 to 0.06 for sows compared with growing pigs.

Everts and Smits (1987) compared growing pigs (70kg) and adult sows (180-200kg) at the same feeding level and observed no significant differences in energy digestibility. In summary and with growing pigs many authors observed a positive effect of body weight on digestibility. However, the differences between growing pigs and adult sows may have been confounded with feeding level.

1.4.1.2 Sex

There has been limited research on the effect of gender of animal on differences in the digestibility of energy. Wenk and Morel (1985) reported that there was no significant difference in the digestibility of energy between castrates and females.

1.4.1.3 Genotype

The effect of genotype on the digestibility of dietary energy is controversial. It is assumed that lean pigs have a higher loss of energy during metabolism than fat pigs because of the greater mass of active body tissue. On an energy basis body protein synthesis is more expensive than fat synthesis. A higher digestibility of dietary energy for lean selection lines has been observed by Wenk (1982) and Wenk and Morel (1985). However Sundstøl et al. (1979) and Hofstetter and Wenk (1985) found no significant difference in the digestibility of dietary energy between a lean and a fat selection line.

The results of Sundstøl et al. (1979) showed that the digestibility of energy tended to be higher for fat pigs than lean counterparts, but this was partly compensated for by greater energy losses in the urine. No significant differences in digestibility were observed between type of pig or feeding level.

Wenk and Morel (1985) showed that their +line (fast growing animals with low back-fat thickness, around 60% halothane positive) showed a slightly but statistically significantly higher digestibility of energy than their -line (the slow growing animals with high back-fat thickness and mainly halothane negative). Wenk (1982) made the same observation in other experiments with the same selection lines.

In contrast Hofstetter and Wenk (1985) reported no statistically significant difference in the digestibility of the energy between these two selection lines.

1.4.2 Effect of Dietary Factors

1.4.2.1 Chemical Composition

The chemical characteristics of a feedstuff have an effect on energy digestibility in pigs. The digestibility coefficients for energy commonly range between 0.70 and 0.90 and a greater variation is observed for raw materials (0 to 100%). With regard to crude protein and crude fat, their digestibility coefficients vary between 0.60 and 0.95 according to their characteristics and their origin, while soluble carbohydrates (starch and sugars) are highly digestible (95 to 100%). In fact, most of the variation in the digestibility of energy is associated with the presence of fibre (defined as the sum of non-starch polysaccharides (NSP) and lignin) which is less digestible (below 50%). The digestive utilization of fibre is variable: for instance, Chabeauti et al. (1991) found a digestibility coefficient for energy in total non-starch polysaccharides in wheat straw, wheat bran, sugar beet pulp and soyabean hulls equivalent to 16, 46, 69, and 79%, respectively.

The major single chemical fraction affecting the digestibility of a feed by pigs is, therefore, its fibre content. The negative effect of dietary fibre on the digestibility coefficient of energy for pigs has been researched by King and Taverner (1975), Just (1982a), Fernandez and Jorgensen (1986) and Morgan et al. (1987). Most studies show that neutral detergent fibre acts as a diluent, while its digestibility averages 45% (about 1% decrease in the digestibility of energy per 1% increase in neutral detergent fibre in the diet). Therefore, the negative effect of fibre on diet energy digestibility is due not only to its lower degradation but also to modifications in the apparent digestibility of the other chemical constituents of the diet.

Several workers (Noblet and Shi, 1993; Noblet and Perez, 1993; Noblet et al., 1993a) have demonstrated that the presence of fibre in the diet affects the apparent digestibilities of crude protein and lipid. Noblet and Shi (1993) showed that the amount of digestible crude protein was negatively affected by amounts of neutral detergent fibre.

Noblet and Shi (1993) and Noblet and Perez (1993) indicated that the apparent digestibility of fat was reduced when the dietary fibre content was increased. Noblet et al. (1993a) observed that crude protein digestibility decreased when the neutral detergent fibre content was increased.

A curvilinear response of the apparent digestibility coefficient of ether extract to ether extract content in diets has been observed by Bayley and Lewis (1965), Just (1982b), Dierick et al. (1990), Noblet et al. (1993a) and Noblet and Shi (1993). The low digestibility coefficient for fat at low dietary fat contents was due to the high proportion of fat as endogenous fat in the faeces. However, the literature is contentious. Values for the digestibility coefficient of ether extract or the digestibility coefficient of energy in animal fat were about 0.80 in the study of Just (1982b) and 0.76 in the study of Noblet et al. (1993a). In most feeding tables or publications (Wiseman et al., 1990), higher values (>0.90) were reported. Differences in analytical procedures or quality of the fat can partly explain these discrepancies. But they are also undoubtedly due to differences in feeding levels and subsequent rates of passage in the digestive tract, and the possible interactions between fat and other chemical constituents (fibre) of the diet (Noblet and Shi, 1993).

Noblet and Perez (1993) found that the digestibility coefficient of crude protein varies between 0.64 and 0.97 (0.79 average). It was positively affected by the crude protein content of the diet and was reduced when more ash and fibre were present in the diet. The high protein content of some diets resulted from the addition of highly digestible protein sources such as soyabean meal, soyabean proteins, corn gluten meal and peas.

1.4.2.2 Processing

The processing of feedstuffs may have an effect on the digestibility of the gross energy component of pig diets. Common processing techniques such as grinding, heat treatment and pelleting can modify the nutritive value of diets (Just, 1978). The results of

a digestibility experiment in which the effect of the fineness of grinding of a balanced diet was studied in relation to crude fibre content and live weight of pigs, showed that a fine grind was more beneficial to small pigs than to adult animals. This influence was more pronounced with increased fibre in the diet. In the case of gross energy, the finer grinding had a negative influence in 225kg pigs.

Pelleting is carried out for several reasons, such as ease of handling, reduction of salmonella and possible improvement in the feed conversion ratio and digestibility (Skoch et al., 1983a,b; Patterson, 1989). Seerley et al. (1962), Lawrence (1971), Lawrence (1983), Skoch et al. (1983a,b), Nuzback et al. (1984), and Smits et al. (1994) found a positive effect of pelleting on nutrient digestibility. However, some researchers have found no effect or even negative effects (Bayley and Thomson, 1969; Baird, 1973; Nuzback et al., 1984; Rowan and Lawrence, 1986; Graham et al., 1989a; Patterson 1989).

Seerley et al. (1962) found a higher apparent digestibility of the gross energy for a corn-soyabean meal diet when it was pelleted. The increase in the digestible energy content of the diet was similar to the result presented by Lawrence (1971) who found strong evidence that cubing significantly improved digestibility. Lawrence (1971) showed that cold pelleting resulted in slightly higher energy values than steam pelleting and postulated that the dry pelleting could be superior to the moist pelleting because of a lower temperature in the dry pelleting process. While no heat was used in making the pellets used in these trials, there was considerable heat generated by extrusion through the die. This heating could explain the slightly higher dry matter content of the pelleted diet.

Lawrence (1983) reported that pelleting or particle size of the diet had no significant effects on the apparent digestibility of dry matter, modified acid detergent fibre, gross energy, digestible and metabolizable energy contents or nitrogen retention. Although pelleting gave slightly higher digestible energy and metabolizable energy contents, the only significant difference was in the apparent digestibility of the ether extract fraction of the diet.

Skoch et al. (1983a) observed that there were no statistically significant differences between treatments in dry matter and crude protein digestibility. The digestion trial showed that pelleting had an effect on energy digestibility. Skoch et al. (1983b) showed that Steam pelleting and extrusion cooking increased dry matter and energy digestibilities. Dry matter and energy digestibilities were higher with the dry-pelleted diets than with the mash and steam-pelleted diets. Steam pelleting increased these digestibilities over those observed with the mash diet.

Nuzback et al. (1984) compared the effect of grinding and pelleting in four treatments (6.25mm meal, 6.25mm pelleted, 12.5mm meal and 12.5mm pelleted). The grinding alfalfa through a 6.25mm particle size increased utilization of dry matter, gross energy and fibre as compared with that ground to 12.5mm. Cellulose digestibility increased when alfalfa was pelleted but other digestibility coefficients were not affected. Smits et al. (1994) showed that pelleting had no influence on the proximate analysis of the diet, but in general increased the digestibility of nutrients.

Bayley and Thomson (1969) found no differences in the digestible energy contents of diets as a result of steam pelleting. Baird (1973) reported that pelleting had no significant effect upon apparent digestibility of dry matter, crude protein, crude fibre or gross energy.

Rowan and Lawrence (1986) showed, in a metabolism experiment, that the changes in composition from pelleting were without significant effect on the apparent digestibility of dry matter, nitrogen and gross energy.

Graham et al. (1989a) investigated the influences of pelleting and β-glucanase supplementation on the digestibility of dietary components in a barely-based diet in pigs fitted with cannulas at the terminal ileum. There were no between-diet differences in faecal apparent digestibility of energy. Patterson (1989) reported that the physical form of the diet had only small non-significant effects on the digestibility of energy.

1.4.2.3 Feeding Level

Increasing the level of feeding may diminish digestion efficiency, as it can be expected to increase the rate of passage (Roth and Kirchgessner, 1985). In particular hindgut digestion is sensitive to the time digesta are subjected to fermentation.

The effect of feeding level on the digestibility of energy in pigs is controversial. A decrease in digestibility when feeding level increases has been observed by Parker and Clawson (1967) and Everts and Smits (1987) in sows and by Cunningham et al. (1962), Roth and Kirchgessner (1985), Sugimoto (1985), Everts and Smits (1987) and Smits et al. (1994) in growing pigs.

In contrast, no effect or even a positive effect of increased feeding levels on energy digestibility has been reported by Mitchell and Hamilton (1929), Gregory and Dickerson (1952), Zivkovic and Bowland (1963), Dammers (1964b), Peers et al. (1977) and Fernandez et al. (1986).

Just et al. (1983) found that decreasing feed intake tended to improve the digestibility of crude fat, crude fibre, energy and metabolizable energy kg^{-1} dry matter. Tollet et al. (1961), on the contrary, observed higher digestibilities of energy and protein with increasing feeding levels in growing pigs. Duée et al. (1983) also found a higher digestibility of crude protein in sows with high feeding level on a ration of pure barley.

In an experiment where wood cellulose was added to a balanced diet to increase the crude fibre levels from 40 to 200 g/kg, Cunningham et al. (1962) found that level of feeding had a considerable effect on the apparent digestibility of dry matter, crude fibre and N. Roth and Kirchgessner (1985) showed a decreased digestibility of diets containing cellulose and oat hulls when fed at 2.5 times maintenance compared to feeding at maintenance levels.

Everts and Smits (1987) found that the feeding level affected digestibility in a non linear way, the effect of increasing feeding level from 1.2 x M to 2.4 x M had a stronger negative effect than the increase from 2.4 x M to 3.6 x M. When sows and growing pigs are fed at similar levels (x maintenance), no significant difference in digestibility coefficients was observed.

The responses in digestibility to changes in body weight or feeding level may vary according to the composition of the diets with greater differences between feeding levels or body weights for low digestibility diets (Everts and Smits, 1987; Fernandez et al., 1986; Fernandez and Jorgensen, 1986). These observations suggest the existence of digestive interactions between diet composition and feeding level and body weight on the digestibility of nutrients (Noblet and Henry, 1991).

Sugimoto (1985) concluded from the results of his experiment that the relationship between feeding level and digestibility depended upon the quality of the feed (i.e. rations containing less digestible nutrients could be influenced to a larger extent by feeding level than could rations which contained more digestible nutrients). The effect of feeding level on digestibility, according to Van Es (1982), depended on several factors such as the level and source of crude fibre in the feed, the digestibility of the feed and of crude fibre, the feeding level and the age or live weight of the pig.

The effect of feeding level on digestibility is a non-linear effect (Parker and Clawson, 1967; Van Es, 1982; Everts and Smits, 1987) and differs between nutrients (Roth and Kirchgessner, 1985).

Smits et al. (1994) showed variable effects of the feeding level on digestibility and feeding value. The strongest effect on digestibility was found for the cereal-based diets and for the by-product-based diet. In contrast, hardly any effect was observed for by-product based and legume-seed-based diets.

Mitchell and Hamilton (1929) and Dammers (1964b) found that the apparent digestibility of energy in balanced diets given to growing pigs was not affected by level of feeding from maintenance to 3 x maintenance. Similarly, Peers et al. (1977) did not find differences in energy digestibility between pigs fed at maintenance level and three times maintenance. The experiments were conducted with growing pigs fed a diet comprising barely, maize and soya bean meal. The result of the experiment was that the apparent digestibility of ash and ether extract were significantly greater at the higher level of intake, but coefficients for dry matter, gross energy and crude fibre were not affected. Similar observations were made by Gregory and Dickerson (1952) and Zivkovic and Bowland (1963), using a much narrower range of feeding level.

Digestibility trials with adult sows fed 26 different feedstuffs varying widely in chemical composition did not show digestibility differences when comparing different feeding levels (maintenance, 80% and 120% of maintenance levels) (Fernandez et al., 1986). The data from Fernandez et al. (1986), indicated that despite the large variations in the chemical composition of the feedstuffs used, the differences between feeding levels were not big enough to influence digestibility to a measurable degree.

In contrast, Tollet et al. (1961) reported an improvement in energy and N digestibility with a higher level of feeding. Morgan et al. (1975) found a small but significant decrease in digestibility with increasing level of feeding.

Noblet and Shi (1993) and Shi and Noblet (1993) compared the digestibility of energy and nutrients in growing pigs fed *ad libitum* and adults sows fed at maintenance. Noblet and Shi (1993) reported that the sows fed at maintenance level had a superior capacity to digest all dietary nutrients. The digestibility coefficient of energy was 11.5% higher in the sows (84.7 vs. 75.8%). Consequently, the digestible energy contents differed by about 1.6 (range: 0.7 to 2.4) MJ per kg dry matter. Similarly to energy, the digestibility coefficients for organic matter, crude protein, ether extract or fibre fractions

were higher in sows. Compared to the growing pig, the corresponding digestibility coefficients were increased by 11, 14, 25, 70, 40, 70 and 20%, respectively.

Shi and Noblet (1993) showed that the digestible values of ingredients were higher in sows than in growing pigs, the difference being greater for high fibre diets. Apart from wheat straw, which contributed negatively to digestible energy in growing pigs, the greatest difference was obtained for sugar beet pulp, for which the digestible energy value in growing pigs represented 60% of the value obtained with sows. No difference was observed for wheat and cornstarch.

Similarly to data obtained for mixed diets (Noblet and Shi, 1993), the difference in digestible energy and metabolizable energy values of ingredients between sows and growing pigs was negligible for low fibre ingredients (wheat and maize starch). Higher differences between growing pigs and sows were observed for high fibre ingredients, such as sugar beet pulp, corn gluten feed, wheat bran and wheat millings. Everts et al. (1986) observed a depressive effect of higher feeding levels in sows on digestive utilization of the diets.

1.4.2.4 Anti Nutritional Factors

There is only limited research on the effect of anti-nutritional factors on the digestibility of energy in pigs. The effect of mycotoxins and β-glucans on the digestibility of energy in pigs has been researched by Williams and Blaney (1994), Taylor et al. (1985) and Miller et al. (1994). Williams and Blaney (1994) showed that there was no effect on the apparent digestibility of energy due to an increasing level of inclusion of infected maize in a diet fed to pigs. There was no statistically significant difference in apparent digestibility between the infected maize diet and the control diet.

Total β-glucans have also been found to be negatively correlated with the digestible energy content of barley for swine (Taylor et al., 1985). Miller et al. (1994)

reported that increasing total β-glucans from 3.4 to 6.8% decreased digestible energy from 3679 to 3542 kcal kg^{-1}. The mixed-linked β-glucans (β-glucanase, xylanase and amylase) in barley resulted in improved nutrient digestibility (Graham et al., 1989a; Inborr et al., 1991). Inborr et al. (1993) found that dry matter and starch digestibilities in the fourth quarter of the small intestine were improved by premix enzyme (β-glucanase, xylanase and α-amylase). Both enzyme supplements increased β-glucan digestibility in the third and fourth quarter.

1.4.3 Effect of Environmental Factors

1.4.3.1 Temperature

The effect of temperature on the digestibility of dietary energy in pigs is a controversial subject. Pigs react to temperature extremes by adjusting their feed intake and heat exchange with the environment. Hot environmental conditions, lead to a reduced feed intake, while cold conditions stimulate feed intake.

Fialho and Cline (1991) found that the apparent digestibility of protein was significantly higher for pigs at 25°C than at the upper critical temperature (35°C). This was expected, since at high ambient temperatures the animal may have to activate heat loss mechanisms, which might result in a lowering of the efficiency of energy utilization. Digestible energy decreased as environmental temperature was increased from 25 to 35°C.

A relatively larger part of the digestible energy of fibrous feedstuffs is lost as heat during the digestive and metabolic processes than from feedstuffs higher in starch and fat. The energy value of a fibrous diet may be influenced by the thermal environment in which the animals are maintained (Noblet et al., 1985).

However Noblet et al. (1989) did not find an effect of temperature on the digestibility of dietary energy.

Vajrabukka et al. (1985) showed that mean apparent dry matter digestibility was higher for pigs kept in a hot room. Digestibility increased for pigs given four diets varying in energy and protein, after about 5 weeks. The improved apparent digestibility of dry matter and energy might be explained by a gradual decline in endogenous faecal output.

Noblet et al. (1989) reported that digestibility coefficients of energy, organic matter and nutrients were significantly reduced when straw meal or alfalfa meal were added to the basal diet, but none of these coefficients was affected by environmental temperature.

1.4.3.2 Housing System

The housing system may also have an effect on the digestibility of energy. Several workers have shown differences in digestibility between housing systems (McCarthy et al., 1977; Mateos and Sell, 1981; Metz and Dekker, 1985; Bakker and Jongbloed, 1994).

The classical method has been compared with the marker method, using pigs in metabolism cages. The marker method, however, can also be applied with pigs in pens. McCarthy et al. (1977) observed lower digestibility coefficients obtained with a marker for pigs in pens than those obtained in cages.

Metz and Dekker (1985) found that due to housing in pens, retention time of digesta in the gut decreased, which resulted in on average 1.8% lower digestibility for the dietary dry matter. This effect was found to be enhanced by feeding a fibrous feed (46% hominy feed, 29% coconut expeller, 10% dried potato pulp) compared to a cereal feed (54% barely, 30% wheat). So, not only the housing system, but the fibrous feed itself also can have a depressing effect on retention time (Kass et al., 1980) and on apparent digestibility.

McCarthy et al. (1977) reported that total collection gave higher digestible energy and digestible nitrogen coefficients than the HCl insoluble ash method using 5.6kg pigs housed in wire rabbit cages and 22.4kg pigs housed in metabolism cages. The differences were only statistically significant for the 20kg pigs. A possible explanation as to why significance was detected at only the 20kg liveweight could be that the 5kg pigs were kept in modified rabbit cages, which allowed for very accurate records of actual feed intake and faecal output. In contrast, 20kg pigs were placed in larger metabolism cages, in which they could move around more freely, which possibly resulted in less accurate measurement of faeces output or feed intake.

Bakker and Jongbloed (1994) reported that when Cr_2O_3 was used as a marker, pen-housing resulted in a faecal digestibility of organic matter which was on average 2.5 (1.7-4.5) units lower and a faecal digestibility of crude protein averaging 4.5 (3.0-5.8) units lower than when measured in the metabolism cages. No statistically significant interactions were demonstrated between housing system and dietary composition. In contrast, with acid-insoluble ash used as a marker, significantly higher digestibility values were observed for pigs in pens, when fed a cellulose containing diet. With this diet, the faecal digestibility of organic matter was on average 14.7 units higher and the faecal digestibility of crude protein was on average 10.9 units higher for the penned pigs. When evaluated with Cr_2O_3 as marker, the digestibility was lower in pigs housed in groups in pens compared to pigs housed individually in metabolism cages. A larger effect was observed in the faecal digestibility of crude protein than in the faecal digestibility of organic matter. The results obtained with acid-insoluble ash as the marker varied considerably.

1.4.3.3 Humidity

There does not appear to be an effect of air humidity on the digestibility of dietary energy.

1.5 Evaluation of Protein and Amino Acid Digestibility Values in Feedstuffs for Pigs

1.5.1 Protein and Amino Acid Digestibility Values

1.5.1.1 Digestibility vs Availability

Feed ingredients vary not only in their gross amino acid composition, but also in the digestibility and availability of the amino acids. With the high cost and general world-wide shortage of protein-rich feedstuffs, it is becoming increasingly important that the proportion of each amino acid digested and absorbed for useful metabolism, is known rather than relying on gross amino acid content. Accurate information on the digestibility of amino acids in feeds is needed, therefore, to accurately formulate diets to meet the animal's requirement. Minimization of wastage of dietary amino acids should be an aim both to enhance biological efficiency and to minimize environmental contamination. Furthermore, the need for a rapid, simple and economical method to assess protein quality is highlighted by the range and quantity of materials and the effects of processing and storage on amino acid digestibility. In addition, new protein sources are continuously being sourced and these need to be evaluated for protein quality.

It is important when discussing protein digestion to distinguish between the concepts of digestibility and availability. The two terms are often used differently. For some, digestibility and availability are synonymous. Digestibility refers to the breakdown of protein and the uptake of an amino acid from the gut whereas availability is usually defined as the degree of the uptake and subsequent utilization of the amino acid for protein synthesis and other anabolic processes. The term: digestibility was defined by several workers (McNab, 1976; Agricultural Research Council, 1981; Low, 1982; Tanksley and Knabe, 1984; Sauer et al., 1989) and the term: availability (Erbersdobler, 1976; Low, 1982; Tanksley and Knabe, 1984; Sauer and Ozimek, 1986).

The term digestibility refers to the combined effects of digestion and absorption but provides no information as to the extent of utilization of the absorbed nutrients (McNab, 1976). Digestibility was defined as the difference between the amount of the amino acid in the diet and that in the ileal digesta or faeces, divided by the amount in the diet (Agricultural Research Council, 1981; Low, 1982; Tanksley and Knabe, 1984; Sauer et al., 1989). Thus, the fates of the amino acids that disappear from the gastrointestinal tract are not considered in the estimation of the digestibility of amino acids (Butts et al., 1991).

The amino acid levels of feedstuffs, determined after chemical analysis, predict their potential value for satisfying the amino acid requirements of an animal. All the determined amino acids in a feedstuff, however, might not be biologically available to the animal because of factors such as incomplete protein hydrolysis resulting from inaccessibility of the protein to proteolytic enzymes or inhibition of enzymes (e.g., trypsin inhibitor in raw soyabeans) (Tanksley and Knabe, 1984; Zebrowska, 1978).

Erbersdobler (1976) defined an amino acid as being available if it was digested and absorbed and not excreted without being utilized. Availability should refer strictly to the digestion, absorption and subsequent metabolism of a nutrient (Low, 1982). The availability of an amino acid in individual feesdstuffs was defined as the proportion of the dietary amino acid that was absorbed from the diet in a form suitable for utilization by the animal (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). The majority of amino acids were utilized for protein synthesis, but varying proportions were catabolized. The concept of availability refers to the proportion of total amino acids that is digested and absorbed in a form suitable for protein synthesis.

Digestibility values for amino acids may overestimate their availability, especially in materials damaged by excess heat during processing (Carpenter, 1973). Carpenter (1973) has provided the example of heated milk powders, but argues that in materials which do not contain reducing sugars, digestibility measurements may closely equate

with estimates of availability. The absorption of non-utilizable forms of amino acids, moreover, is not a major limitation of the digestibility assay (Austic, 1983) as urinary losses represent only a small fraction of amino acid intake in feedstuffs which have been damaged by heat treatment.

Amino acid availability is a complex phenomenon affected by many interacting factors (Moughan, 1991). For most amino acids in most feedstuffs, availability should equal digestibility, but there is likely to be a discrepancy between digestibility and availability, particularly for the amino acid lysine because of its free ε-amino acid group, in heated-treated foods (Moughan, 1989).

Chemically unavailable lysine might be absorbed and then not utilized. Therefore, lysine digestibility coefficients themselves are likely to be inaccurate, at least for some processed foods. With the early stage of the Maillard reaction, for example, which is predominant under the normal conditions of food processing, the deoxyketosyl lysine derivative (Amadori compound) formed is hydrolyzed back to lysine in the presence of strong acids. Thus conventional amino acid analysis leads to overprediction of the actual lysine content in foods or ileal digesta for an animal fed the processed feedstuff.

Consequently, with heat processed feedstuffs ileal lysine digestibility coefficients are likely to be biased, and to an unknown degree. Also and for feedstuffs generally, it is to be expected as noted by Batterham et al. (1990a) that digestibility values would overestimate availability. A proportion of the absorbed amino acids, including the first-limiting amino acid would be inevitably catabolized by the animal with the degree of such catabolism possibly varying with the level of uptake (Moughan, 1991). For this reason, absolute values for body lysine retention cannot be used to accurately assess the adequacy of ileal lysine digestibility coefficients (Batterham et al., 1990b).

1.5.1.2 Faecal vs Ileal Digestibility

The estimation of amino acid digestibility in dietary protein sources has often been made following the method outlined by Kuiken and Lyman (1948). In the traditional approach, the amount of an amino acid truly absorbed from the alimentary canal is estimated as the difference between the amount of the amino acid consumed in the diet and that voided in the faeces, after correction of the latter for the amount presumed to be excreted in the metabolic faecal nitrogen. Because of difficulties in measuring the excretion of metabolic faecal nitrogen (Mason, 1980), apparent faecal digestibility has frequently been measured.

The most commonly used procedure for determining amino acid digestibility has been the faecal analysis method (Kuiken and Lyman, 1948; Dammers, 1964a; Eggum, 1973; Poppe, 1976; Zebrowska, 1978; Raharjo and Farrell, 1981; Tanksley and Knabe, 1984). Using this procedure, digestible amino acid values represent the amount of amino acids in the feedstuff that disappear over the total digestive tract. In this method, apparent amino acid digestibilities are calculated as the difference between the amount of amino acid consumed minus that excreted in the faeces, divided by the amount consumed.

While the overall apparent digestibility measurement (i.e. using faecal collection) is not technically difficult, there are basic objections to this approach because of the presence of undigested and unabsorbed endogenous protein in the faeces (Just, 1980), and because of possible microbial alterations of undigested and unabsorbed endogenous and exogenous nitrogen residues, in the large intestine (Payne et al., 1968; Holmes et al., 1974; Raharjo and Farrell, 1981).

Although the faecal method is highly reproducible (Eggum, 1977), its accuracy has been criticised due to the modification of nitrogenous compounds by the hindgut microflora. Taverner (1979) conjectured that the inability of faecal analysis to account for differences in hindgut microbial activity might explain the difficulties experienced in

reconciling pig growth data with faecal nitrogen digestibility data, in experiments with various processing techniques, with oats (Crampton and Bell, 1946), wheat (Lawrence, 1967) and barley (Cole et al., 1970).

The hindgut microflora hydrolyse the nitrogenous compounds and most of the nitrogen is absorbed as amines (amides) and ammonia (Michel, 1966) instead of amino acids and is excreted in the urine. Further evidence of the intestinal fermentation that occurs in the large intestine of the pig is the predominance of bacterial nitrogen (62-76% bacterial nitrogen as a percentage of total nitrogen) in the faeces (Mason, 1984). As a result, the amino acid composition of faeces in pigs fed diets that differ widely in amino acid composition and digestibility is rather similar (Mason et al., 1976).

Although the effect of hindgut microbial metabolism on protein digestion appears to be a rather general phenomenon across animals species (Moughan and Donkoh, 1991), it is not necessarily of practical significance in all cases. The extent of microbial activity depends on the type and numbers of micro-organism present, the type of feedstuff and the time of residence of material in the hindgut. It is thus a function of both animal species and diet.

It is generally considered that the ileal method to determine dietary amino acid digestibility is theoretically better than the traditional faecal approach (Payne et al., 1968; Terpstra, 1977; Low, 1980a; Rerat, 1981; Sauer and Ozimek, 1986; Moughan and Smith, 1987; Sauer et al., 1989).

Ileal amino acid digestibilities are not influenced by the microbial activity in the large intestine but are confounded by the residue of non-absorbed endogenous protein excretion (Austic, 1983).

It was argued by Payne et al. (1968) that ileal analysis should provide a better index of amino acid digestibility. In accordance with the conclusion of Terpstra (1977)

that bacterial amino acid breakdown occurs at a greater rate than synthesis, most workers have found that amino acid digestibility estimated on ileal analysis is lower than comparable faecal based values.

The ileal digestibilities of most amino acids are lower than corresponding digestibilities determined over the entire digestive tract (Zebrowska, 1978; Sauer et al., 1980; Tanksley and Knabe, 1980). According to Zebrowska (1978) the amount of amino acids disappearing in the large intestine usually varies from 5 to 35% of total amino acids ingested. It appeared that the lower the ileal digestibilities of nitrogen and amino acids, the greater are the difference between ileal and faecal digestibilities. This is understandable, as with diets containing highly digestible protein, much material is absorbed before the digesta enter the large intestine whereas with protein sources of lower quality there are larger residues to allow a disappearance of amino acids between the terminal ileum and rectum.

Large differences between ileal and faecal apparent digestibilities have typically been found for proteins of low digestibility (Zebrowska and Buraczewski, 1977; Jorgensen and Sauer, 1982). Differences were lower for high quality proteins, such as, milk power or casein. The study of Jorgensen and Sauer (1982) is of particular interest in that the digestibilities of amino acids in protein sources commonly used in pig diets were investigated. Large differences were found between ileal and faecal digestibilities of amino acids in soyabean-, sunflower-, fish- and meat- and bone-meal, differences being greatest for threonine, phenyalanine and lysine and least for arginine and methionine. These workers also demonstrated considerable variation among the ileal digestibilities of amino acids within a protein source and concluded that in correcting gross to digestible amino acid levels, the use of a common correction factor for amino acids (e.g. protein digestibility) might not be appropriate.

From several studies, involving the determination of ileal and faecal nitrogen and amino acid digestibilities in pigs and employing a variety of methods for collecting ileal

digesta, some general findings have merged, indicating the superior predictive accuracy of ileal digestibility values. The differences between ileal digestibility and apparent faeces digestibility do not appear to be constant. Depending on the amino acid and on the feedstuff considered, the digestibilities obtained with the faecal method overestimated (Low, 1982; Sauer et al., 1982; Tanksley and Knabe, 1984) or underestimated those obtained by the ileal method. For example, Tavernier (1984) compared a diet based on soyabean meal with one based on meat and bone meal and found that discrepancies between faecal digestibility and ileal amino acid digestibility were much greater for the meat and bone meal.

Sauer and Ozimek (1986) summarised ileal amino acid digestibility for several proteins across feedstuffs. Knabe et al. (1989) presented the results of eight trials that were conducted to determine the apparent digestibility of amino acid in 30 samples of protein feedstuffs using standardised procedure. Variation in digestibilities among samples of the same feedsfuff was greatest for the meat and bone meals. Regression of ileal essential amino acid digestibilities on ileal and faecal nitrogen digestibility indicated that amino acid digestibilities could be predicted more precisely from ileal nitrogen digestibility than from faecal nitrogen digestibility.

Further justification for assessing the amounts of nitrogen and amino acid absorbed at the terminal ileum came from studies where proteins were infused into the large intestine and were largely degraded with a low utilisation, compared to oral administration (Zebrowska, 1973). Reports by other researchers (Zebrowska, 1975; Sauer 1976; Hodgdon et al., 1977; Gargallo and Zimmerman, 1981; Just et al., 1981) clearly confirmed that protein and amino acids infused in the large intestine made little or no contribution to the protein status of the pig. In addition, other studies (Low and Partridge, 1984; Just et al., 1985; Leibholz, 1985; Moughan and Smith, 1985; Dierick et al., 1987) indicated close correlation between the apparent ileal digestibility of amino acids and animal performance.

Apparent ileal digestibility coefficients have been shown to be sensitive in detecting small differences in protein digestibility due to the processing of foods (Rudolph et al., 1983; Vandergrift et al., 1983; Van Weerden et al., 1985; Sauer and Ozimek, 1986; Knabe et al., 1989). Several studies (Tanksley and Knabe, 1982; Low et al., 1982; Just et al., 1985; Moughan and Smith, 1985; Laplace et al., 1989) have demonstrated that apparent ileal digestibility coefficients accurately describe the extent of amino acid uptake from the gut, at least for a range of commonly used feedstuffs which have not been subjected to high temperature during their processing. In this case, the ileal digestibility of amino acids is a good indicator of amino acid availability (Laplace et al, 1985; Leibholz, 1985; Dierick et al., 1987; Green and Kiener, 1988; Laplace et al., 1989). Amino acid digestibilities could be predicted more precisely from ileal nitrogen digestibility than from faecal nitrogen digestibility.

Due to heat processing, a considerable proportion of the ileal digestible lysine is probably absorbed in a form that is inefficiently utilised, resulting in an overestimation of lysine absorption from the gut (Batterham et al., 1990a,b; Wiseman et al., 1991). Wiseman et al. (1991) found that for a diet containing overheated fish meal, there was no advantage in using ileal rather than faecal apparent digestible amino acid values, in terms of accuracy of the diet formulation. Batterham et al. (1990a,b), using a diet containing meat and bone meal and fish meal, reported that apparent lysine digestibility values (when lysine was the first limiting amino acid) overestimated lysine retention and pig performance.

1.5.1.3 Apparent, True and Real Digestibility

Low (1982) distinguished between three types of digestibility measurement. Apparent digestibility may be defined as the difference between the amount of an amino acid in the diet, and in ileal digesta or faeces as a proportion of that in the diet. True digestibility is defined similarity except that the amounts of endogenous amino acids in faeces or ileal digesta are subtracted from the total amount of amino acids in the faeces or ileal digesta. Real digestibility is calculated in the same manner as apparent digestibility

but applies when the difference between the intake and output of faecal or ileal amino acids is measured using nitrogen isotopes.

Taverner (1979), in a study with growing pigs, found that apparent ileal amino acid digestibility was influenced by the protein level of the diet but not the true ileal digestibility. Apparent digestibility increased curvilinearly with increasing dietary protein concentration (Eggum, 1977). Taverner (1979) and Sauer et al. (1980) also noted that the effect of level of endogenous protein on the apparent digestibility of protein was greater at lower dietary protein levels. In fact, Haydon et al. (1980) found no effect of the level of crude protein on apparent ileal nitrogen and amino acid digestibility when a diet containing 160g/kg crude protein was fed to growing pigs at daily intakes exceeding 0.03 of bodyweight.

The apparent digestibility of relatively low-protein feedstuffs (cereals with a protein content of 10-13%) was generally determined by feeding the animal the feedstuff alone. Thus, the endogenous ileal flow was directly related to that food material alone, and therefore the digestibility value related directly to that feedstuff. However, the apparent digestibility of protein for protein-rich feedstuffs (soyabean meal) is generally determined after dilution with an N-free mixture, to give a diet with a protein content of about 18%. Therefore, the endogenous ileal protein loss and, consequently, the determined values for apparent digestibility are influenced not only by the composition of the protein-rich feedstuff itself, but also by the extent of dilution with the N-free mixture, as well as the composition of the protein-free mixture.

Owing to the influence of dietary content on values of apparent digestibility, it has been argued that the digestibility of protein and amino acids from cereals and other low-protein feedsfuffs are underestimated when using apparent digestibility (Batterham, 1994). To avoid this, Sauer et al. (1989) recommended that analyses should be performed on diets that contain at least 150-160g crude protein per kg feed. Determined values for apparent digestibility in protein-rich feedsfuffs were, therefore, not directly useful for

practical purposes. However, values of net digestibility could be calculated from these apparent digestibilities (Boisen and Moughan, 1996).

Ileal digesta contain appreciable quantities of non-dietary amino acids from sources such as digestive secretions, mucus and cells, hair and bacteria, and to obtain a true estimate of digestibility, correction should be made for this non-dietary (mainly endogenous) component. True as opposed to apparent estimates of digestibility should more clearly describe the amino acids absorbed from the diet.

True digestibility has the advantage over apparent digestibility in that it is a fundamental property of a feed ingredient regardless of the dietary conditions under which that ingredient was fed to the animal (McNab, 1976). True digestibilities of dietary amino acids have been shown to be directly related to the amino acid digestibilities of the individual protein components of the mixed diet (Eggum and Jacobsen, 1976). This additivity seemed unlikely to apply, however, with apparent amino acid digestibilities which would only be meaningful under strictly standardized conditions (Eggum, 1977).

For a given amino acid, the apparent digestibility increases exponentially with the ingested quantity because endogenous excretion, as a percent of total excretion, decreases proportionally (Sauer et al., 1980; Bell et al., 1983; Furuya and Kaji, 1989; Keith and Bell, 1991). By contrast, several studies (Taverner, 1979; Hopkins, 1981; Sarwar and Peace, 1986; Green, 1987; McNab, 1989; Furuya and Kaji, 1989; Zuprizal et al., 1991) indicate that true amino acid digestibility is not affected by the level of dietary protein intake.

Despite the possible limitations of using estimates of apparent amino acid digestibility and due to the difficulties in determining endogenous excretion (Kidder and Manners, 1978), it seems that estimates of the apparent ileal amino acid digestion and absorption are useful in practical dietary formulation (Low and Partridge, 1980; Low, 1982; Austic, 1983; Sauer et al., 1983). Nevertheless, experimental information

concerning the validity of apparent ileal digestibility coefficients for predicting the level of digestion and absorption of amino acids in the pig is greatly needed (Braude, 1980; Just, 1980; Agricultural Research Council, 1981). Although some validation trials have been undertaken (Tanksley and Knabe, 1980,1982; Fuller et al., 1981; Low et al., 1982), findings have been contradictory.

It has sometimes been argued (Just Nielsen, 1968; Low, 1982) that on a practical basis, apparent digestibility coefficients are more relevant than true digestibility coefficients since both undigested dietary and endogenous amino acids are lost to the animal and thus have to be accounted for in diet formulation. Low (1980a) claimed that for practical purposes, apparent digestibility coefficients are more meaningful than true digestibility coefficients because the former represent the net loss which results from feeding a test diet, without taking into account the origin of the amino acids. This might be important for estimating the digestibility of complete feeds formulated to satisfy requirements which do not include the endogenous amino acid excretion from the digestive tract. However, for the purpose of evaluating individual feedstuffs for diet formulations, the endogenous excretion of an amino acid is accounted for in the estimation of the requirement for that amino acid and there is also a trend (Agricultural Research Council, 1981) towards expressing amino acid requirements for pig growth, in units of truly absorbed amino acids.

Apparent amino acid digestibility coefficients are influenced by the dietary crude protein content of the test diet (Furuya and Kaji, 1989) and apparent digestibility values are thus comparable only under standardized conditions. True rather than apparent digestibility values are thus considered more suitable for diet formulation.

The argument as to whether apparent or true digestibility values are preferred for practical formulation is also inextricably linked to the approaches adopted in estimating amino acid requirements for growth. In the formulation of diets for pigs, it is assumed that the supply of digestible amino acids in a mixture of feedstuffs is equal to the sum of

the supply based on the digestibility values determined for the single ingredients. For feedstuffs with a lower level in one amino acid, their ileal digestibility would be reduced by the influence of the endogenous ileal contributions (Sauer and Ozimek, 1986; Furuya and Kaji, 1989). As true ileal amino acids digestibility is corrected for endogenous ileal amino acids, true rather than apparent digestibility values would be expected to be more additive (Tavemer et al., 1981).

1.5.2 Determination of Protein and Amino Acid Digestibility Values in Feedstuffs by *In Vivo* Methods

1.5.2.1 Digesta Collection Method with Pig

Considerable work has been done on ileal collection methods in pigs. There are several methods of digesta collection with pigs such as the slaughter technique, cannulation methods and ileo-rectal anastomosis. The different methodologies for the measurement of digestion have been discussed in reviews (Sauer et al., 1989; Low, 1990; Fuller, 1991). In addition, the different approaches to digesta collection with pigs have practical advantages and disadvantages (Table 1.1).

1.5.2.1.1 Cannulation Methods

Simple T-Cannulation

This method involves the surgical implantation of a simple T-cannula, 5-15cm anterior to the ileo-caecal valve. With this technique, digesta can be collected by spot-sampling. This requires the use of an indigestible marker such as chromic oxide, titanium dioxide or barium sulphate (Kotb and Luckey, 1972). With regard to this technique, several concerns have been reported. There was some concern with regard to obtaining representative samples in T-cannulated pigs (Zebrowska, 1978; Sauer and Ozimek, 1986; Den Hartog et al., 1988a; Schroder et al., 1989; Kohler et al., 1990; Leterme et al., 1990; Potkins et al., 1991) and comparison between simple T-cannulated pigs and intact pigs

using digesta collection methods have been made (Furuya et al., 1974; Livingstone, 1982; Jorgensen et al., 1985; Livingstone and McWilliam, 1985; Moughan and Smith, 1987; Donkoh et al., 1994). A modification to improve digesta sampling using a suction tube has been described by Dierick et al. (1983). However, the use of this technique which requires additional devices (e.g. peristaltic pumps) complicates the collection of digesta.

Simple T-cannulation has been widely accepted by workers as a means of sampling ileal digesta in pigs and has been shown to be an acceptable technique for digesta collection, at least when non-bulky diets are used (Den Hartog et al., 1988a; Kohler et al., 1990). When simple T-cannulation of the ileum is adopted, the surgery is less invasive than with other cannulation approaches and the procedure has fewer adverse effects on the physiology of the alimentary tract, although Livingstone (1982) reported reduced food intake and rate and efficiency of growth in pigs with simple cannulae in the terminal ileum. Furuya et al. (1974) detected no significant differences in growth rate or faecal digestibility between non-fistulated pigs and those prepared with T-piece cannulae. Jorgensen et al. (1985) found that simple T-cannulated pigs grew at a similar rate to intact pigs and Livingstone and McWilliam (1985) reported similar voluntary food intakes between T-cannulated and normal pigs but a slightly lower (7%) mean growth rate with the cannulated animals. Donkoh et al. (1994) reported no statistically significant differences in apparent faecal or ileal amino acid digestibility between T-cannulated or intact pigs. They indicated that the simple T-cannula implanted in the terminal ileum caused minimal disturbance to protein digestion over the entire digestive tract.

The surgery required for simple T-cannula implantation is costly, and with high fibre diets cannulas are susceptible to blockage (Potkins et al., 1991). Further, cannulation might lead to discomfort in the animal and physiological effects, due to the cannula, might become important as the cannulated animals age. For these reasons, it would be useful to develop an alternative method of digesta collection and the slaughter technique might be a satisfactory alternative. Moughan and Smith (1987) found similar ileal digestibilities of the amino acids in ground barley as determined with intact and cannulated pigs. The apparent ileal amino acid digestibility values for meat and bone

meal determined by the slaughter method were comparable to those obtained by simple T-cannulation. Moreover, the similar magnitude of the coefficients of variation of the apparent ileal N and amino acid digestibility values for both methods demonstrated that digestibility was not consistently more variable with the slaughter method in comparison with cannulation (Donkoh et al., 1994).

Post-Valve T-Caecum Cannulation

A modification of the simple T-cannulation has been developed by Van Leeuwen et al. (1988). This technique which uses only one large T-cannula involves the anatomy of the transition from the ileum into the caecum-colon. After removal of the caecum the cannula is jointed with the remnants of the caecum directly opposite to the ileo-caecal valve. When the cannula is opened the ileo-caecal valve, which normally protrudes into the caecum, would protrude into the cannula. The intestinal and external diameter of 25 and 30mm, respectively guarantees a passage capacity that should be sufficient to collect digesta almost quantitatively.

Van Leeuwen et al. (1991) reported that the post-valve T-caecum cannulation (PVTC) technique was supposed to enable almost complete collection of digesta from pigs and allowed normal functioning of the ileocaecal valve and colon. The PVTC technique has the advantage that, during collection, most of the digesta pass through the cannula because the ileocaecal valve protrudes directly into the cannulas.

Den Hartog et al. (1988a) showed that the apparent ileal digestibilities of dry matter and nitrogen determined in pigs fitted with a PVTC-cannula were comparable with results determined in pigs fitted with simple or re-entrant cannulas fed different diets.

Table 1.1 Practical advantages and disadvantages of different digesta collection methods with pigs

Digesta collection method	Advantages	Disadvantages
Slaughter technique	-Quick to conduct (completion within 7 days) -No disruption of intestinal wall -More acceptable on ethical grounds -Determine apparent ileal digestibility of diets at the completion of growth experiments	-Only one sample of ileal digesta collected -Difficulty in collecting adequate samples from highly digestible diets -Impossible to get adequate sample of faeces for digestible energy studies -Requires more pigs than cannulation experiments
Simple T-piece cannulae	-Surgery relatively simple -Collections can be made over a longer period of time -Faecal sampling can be undertaken -Requires one set of pigs for a number of collections	-Less acceptable on animal ethics grounds -Leakage may occur at the base of cannulae (causing discomfort to the pig and results in termination of the collections) -Requiring labour and expertise minimizing irritation from this leakage
Post-valvular T-caecum Cannulae (PVTC)	-No interference with intestinal wall, ileo-caecal valve, colon -Replace cannulae as the pig develops -Complete collection of digesta -Recover nylon-bags used in digestibility studies	
Re-entrant cannulae	-Possible to collect total digesta	-Severing of small intestine caused by blockage of cannula with fibrous or ground materials
Ileo-rectal anastomosis	-Complete collection of ileal digesta over growth span of the pig	-Unacceptable on animal ethics grounds -Ileal contents contaminated by material from the large intestine

Comparative studies in the pig with simple T-cannulas and post-valve caecal simple cannulas (Den Hartog et al., 1988a; Kohler et al., 1990) showed that the digestibilities of dry matter and nitrogen were comparable with the two methods. It was concluded that, at least for refined diets and with frequent spot-sampling of digesta, T-cannulated pigs should give reliable observations on the digestibility of dietary nitrogen. The post-valve T-caecum technique has been described as a technique for cannulation of animals in order to determine ileal digestibility.

Steered Ileo-Caecal Valve Cannulation

As a modification of the PVTC cannulation the so-called ileo-caecal valve (SCIV) cannulation has been reported by Mroz et al. (1991). In contrast to the PVTC cannulation the caecectomy was omitted since the ileo-caecal valve could be steered into the T-shaped cannula, using two rings placed proximal to the ileo-caecal valve. One ring was fitted around the terminal ileum, close to the caecal wall, and another ring was introduced into the ileum proximal to the outer ring. Using a thread, that was connected with the inner ring, the ileo-caecal valve could be steered into the cannula. Post slaughter examination showed anatomical-pathological changes that were related to proliferation of fibrous tissues between the two rings and dilation of the distal ileum by muscular hypertrophy anterior to the ileo-caecal valve. Therefore Mroz et al. (1991) suggested a limited application of this technique of about 6-8 weeks.

Re-Entrant Cannulation

Ileo-Ileal and Ileo-Caecal Re-Entrant Cannulation

The ileo-ileal and ileo-caecal re-entrant cannulation procedures have been described by Cunningham et al. (1962) and Hazem and Drochner (1976). This technique allows complete sampling of digesta but requires complete transsection of the small intestine, thus interrupting the transmission of the normal migrating myo-electric complex which is necessary for normal digesta passage. There are difficulties in the use

of fibrous diets, such as blockages of the cannula proximal to the ileum (Zebrowska, 1978; Sauer and Ozimek, 1986; Oslage et al., 1987; Schroder et al., 1989). In addition leakages around the cannulas have been reported.

Studies with pigs fitted with re-entrant cannulas are often hampered by problems that result from blockage of digesta. The pigs would go off feed abruptly as a result. Although frequent inspection and cleaning of the cannulas reduces the incidence of blockage, it remained a major problem. The extent to which blockage occurs depends on various factors: the fibre content of the diet, the fineness of grinding of the diet, the viscosity of the digesta and the amount of digesta that have to pass via the cannula. To minimize these problems, in many studies the diets were finely ground and feed intake was restricted (Sauer et al., 1977; Tavemer and Farrell, 1981).

Both re-entrant and T-cannulation caused a direct disturbance of the small intestine. This might have an influence on the processes of digestion and absorption. Studies in pigs (Buraczewska et al., 1975; Huisman et al., 1984; Metz et al., 1985) showed no effect of cannulation on faecal digestibility. Results reported by Sauer et al. (1977) and Sauer et al. (1979), however, indicated that the insertion of ileo-caecal re-entrant cannulas resulted in higher faecal digestibility of amino acids than compared to intact animals. In agreement with these studies Jorgensen et al. (1985) also reported higher faecal digestibility of dry matter, nitrogen and lysine for cannulated pigs compared with intact pigs.

Ileo-Colic Post-Valve Cannulation

To prevent problems associated with a direct manipulation of the small intestine a modification of the re-entrant cannulation has been developed by Darcy et al. (1980a,b). This technique preserves the functional role of the ileo-caecal valve since the proximal part of the re-entrant cannulation was formed into the remnants of the caecum. Using this technique, disorders reported for the cannulation of the ileum (e.g. blockages proximal to

the cannula and separation of digesta components) were avoided. Nevertheless using fibrous diet blockages of the proximal cannula still occurred. Darcy et al. (1980a,b) reported that the preservation of the functional role of the ileo-caecal sphincter prolonged the retention time of digesta in the small intestine by 60 to 90 min, which might benefit the digestion of certain feedstuffs. These might include feedstuffs that contain protein of low or medium digestibility and that respond to prolonged enzymatic hydrolysis. The apparent ileal nitrogen digestibility of a diet with 8% crude protein was higher in pigs prepared with the IPV (Ileo-Colic Post-Valve)-procedure than pigs fitted with ileo-caecal re-entrant cannulas, 83.7 and 81.7%, respectively. In addition the surgical procedure was very complex and handling was too time-consuming for routine measurements to be feasible (Darcy-Vrillon and Laplace, 1990).

Slaughter Technique

Digestibility measurement at the distal ileum requires collection of ileal digesta. The simplest method for the assessment of amino acid digestibility up to the terminal ileum involved collecting the digesta from the ileum under anaesthesia before sacrifice of the animal (Payne et al., 1968; Kies et al., 1986; Moughan and Smith, 1987; George et al., 1988; Van Barneveld et al., 1991). The digestibility of amino acids could then be measured with reference to an indigestible marker given with the test feedstuff. The slaughter technique has the distinct advantage of involving minimal disruption to normal digestive function in the animal and allows samples of digesta to be taken from several parts of the digestive tract. The main technical criticism of this method concerns the potential difficulty of obtaining representative samples of digesta, thereby the variability of digestibility estimates. However, digestibility data obtained using this technique (coupled with a frequent feeding regime or sampling at a predetermined optimal time) are not necessarily any more variable than those obtained from cannulated animals (Moughan, 1991).

Another factor considered to possibly influence the accuracy of digestibility estimates determined with the slaughter technique is the sloughing of epithelial cells into the gut lumen, with effect on the nitrogen content of the digesta (Badawy et al., 1957,1958; Fell, 1961). Sampling can be done under anaesthesia or by euthanasia with a barbiturate such as sodium pentobartitone (Badawy, 1964). This approach is, however, expensive when applied to large species (Fuller, 1991). Thorpe and Thomlinson (1967) found that epithelial cell shedding in the pig increased with the time after death. In their work, no macroscopic lesions were found in any of the animals at the initial sampling within 5 and 10 min after death. However, gastrointestinal tympany developed in all pigs commencing with moderate gaseous distention of the stomach and small intestine from 90 min after death. Thorpe and Thomlinson (1967) also found that cell shedding commenced later at the ileum than at the duodenum. Donkoh et al. (1994) reported relatively low inter-animal variation for apparent ileal amino acid digestibility suggesting that epithelial cell shedding did not occur to any significant in their study.

Ileo-Rectal Anastomosis

Another technique, which allows for routine total collection of ileal digesta, is ileo-rectal anastomosis, whereby digesta pass directly from the ileum to the rectum bypassing the large intestine (Fuller and Livingstone, 1982; Picard et al., 1984; Darcy-Vrillon and Laplace, 1985; Souffrant et al., 1985; Hennig et al., 1986; Green et al., 1987; Green and Kiener, 1989). There are several variations of this method, which are also referred to as the ileo-rectal shunt (IRS) procedure involving complete transection of the distal ileum to the ileo-caecal valve. Pigs prepared with the IRS require much less time and effort to maintain than pigs fitted with re-entrant cannulas. Food intake can be maintained at normal levels and diets relatively high in fibre, which included many of the by-products, can be tested. However, there are serious doubts concerning the physiological normality of anatomized animals (Picard et al., 1984; Moughan, 1991).

As an alternative to the different cannulation techniques the ileo-rectal anastomosis has been proposed initially by Fuller and Livingstone (1982). These workers fitted the ileum as a simple end-to-end anastomosis to the rectum, allowing residual digesta of the colon to be evacuated via the anus. Using this technique reflux of ileal digesta into the distal colon was still possible. Picard et al. (1984) modified this technique by separating the large intestine completely from the small intestine. This modification, that has been further adapted by Green et al. (1987) and Laplace et al. (1989) required the insertion of a cannula for evacuation of residual digesta and gases out of the colon. Since the ileo-caecal valve has been suspected to restrict the passage of digesta and therefore this be a possible influence on digestibility (Laplace and Borgida, 1976). Souffrant et al. (1985) recommended a modification maintaining the integrity of the ileum and ileo-caecal valve by fitting the remaining part of the caecum immediately posterior to the ileo-caecal valve with the rectum. This technique carried out as end-to-side ileo-rectal anastomosis maintains an intact junction between the colon and the rectum.

Green (1988) reported no differences between the two methods of anastomosis. Maintaining an intact ileum and ileo-caecal valve had no influence on the digestibility values. The extra time and effort necessary for the post-, rather than pre-, valve ileo-rectal anastomosis operation were not rewarded by any improvement in digestibility measurements. In general agreement with the results, Laplace et al. (1985) has reported a similarity between amino acid digestibility values determined in pigs with pre-valve ileo-rectal anastomosis and those values determined with post-valve ileo-colic re-entrant cannulas, when assaying a standard diet and a semi-purified diet, although differences statistically significant between the two methods were observed when assaying a beet-pulp diet.

1.5.2.2 Determination of Endogenous Protein and Amino Acid Loss

Endogenous ileal and faecal amino acid excretions have traditionally been determined following the feeding of a protein-free diet to pigs. The use of protein-free diets has been criticized because of the different amounts of endogenous secretions they

induce compared to protein-containing diets (Corring and Saucier, 1972). They might create a physiologically abnormal state (Low, 1980b).

The other commonly used approach is the regression method, whereby the amino acid flow at the terminal ileum is measured at increasing levels of inclusion of the feedstuff in the diet and endogenous loss is determined by extrapolation. This method assumes that the increase in ileal amino acid flow is attributed entirely to an increase in undigested food protein and that there is no change in the amount of the endogenous secretion.

An alternative approach which allows measurement of endogenous amino acid excretion under physiological conditions has been developed (Moughan et al., 1990; Butts et al., 1993). An animal is fed an enzyme hydrolyzed casein based diet and the digesta collected from the terminal ileum are ultrafiltered to separate dietary and endogenous amino acids.

Another approach is the homoarginine method, in which the lysine in dietary protein is guanidinated to form homoarginine, and the protein is fed to the test animal (Hagemeister and Erbersdobler, 1985; Moughan and Rutherfurd, 1990). The homoarginine is digested and absorbed but not used for protein synthesis, so therefore does not reappear in endogenous secretions although it is eventually transformed back to lysine. Moughan and Rutherfurd (1990) have demonstrated that endogenous flow of lysine at the terminal ileum of rats fed a diet containing guanidinated protein was substantially higher than that of rats given a protein-free diet. This method, however, provides data for lysine only.

The relatively expensive procedure of ^{15}N labelling of either the dietary protein or the whole animal has been used (De Lange et al., 1990). This method assumes a constant amino acid composition of endogenous protein and that all nitrogen-containing

substances are labelled uniformly. The results achieved might be dependent upon the choice of precursor pool.

Endogenous protein loss at the terminal ileum of the pig is primarily dependent on dry matter intake but may be significantly influenced by the chemical composition of the feed. Semi-synthetic protein-free diets with a low content of dietary fibre and without anti-nutritional compounds result in minimal endogenous ileal protein losses. Protein, naturally occurring dietary fibre and anti-nutritional factors in the diet all increase the endogenous protein loss above that level found in a protein-free diet. Determined values of endogenous protein loss obtained for protein-rich feedstuffs that have been diluted with an N-free mixture are underestimated. The effect on the endogenous protein loss of specific inducing factors in the feed could generally be considered to be proportional to the amount of factor in the feed.

1.5.2.3 Determination of Amino Acid Availability in Feedstuffs

Availability of amino acids *in vivo* could be determined by the measurement of protein deposition, in nitrogen balance study though this had its limitations (Duncan, 1966; Just et al., 1982). A superior approach is to determine the retention of amino acids in the whole body after slaughter.

Availability can also be determined from blood amino acid levels (Morrison et al., 1961; Zimmerman and Scott, 1965) or from blood urea (Munchow and Bergner, 1967; Eggum, 1970, 1973). Blood amino acid concentrations, however, are influenced by physiological and pathological as well as dietary factors (Feigin et al., 1967; Boomgardt and MacDonald, 1969; Ishibashi and Kametaka, 1974). The plasma free amino acid assay has been used to assess the effects of heat processing on the availabilities of some amino acids (Smith and Scott, 1965; Erbersdobler et al., 1972).

Measures of amino acid availability can be based on urinary urea output (Brown and Cline, 1974) or free amino acid levels in muscle (Pion, 1973). The sensitivity of these tests as predictors of amino acids availability, needs to be investigated.

The growth assay provides a combined measure of digestibility and post-absorptive utilization of the amino acids. A refinement of the growth assay is the slope-ratio procedure which has been described by Batterham et al. (1979). Considerable differences in the estimates of lysine availability, as determined by the slope-ratio assay with weaner and grower pigs given the same sample of cottonseed meal, were obtained. The results indicated much higher lysine availability (0.69) for weaner pigs (Leibholz, 1986) compared to growing pigs (0.27) (Leibholz, 1985).

Differences in feeding method may also influence the estimate obtained by the dose/response assay. The pigs of Leibholz (1986) had unrestricted assess to food, while in the experiments of Batterham et al. (1984) the level of feed intake was the same for all pigs. Under controlled feeding, all animals received similar quantities of test proteins (on a liveweight basis), whereas with full feeding, there were differences in intake and this made it more difficult to relate the responses to the tested amino acid alone.

The use of microbiological assays to estimate amino acid availability and protein quality has been applied. The principle of the assay is that the amount of the amino acid in the protein under investigation which becomes available to the micro-organism, and thereby influences its growth, corresponds to the amount which would become available to the animal after digestion and metabolism.

1.5.3 Application of Protein and Amino Acids Digestibility Values for Diet Formulation in the Pig

Ileal digestibility values offer a rapid and convenient method for improving the precision of diet formulation. Ileal digestibility values are not equivalent to amino acid availability. In the formulation of diets both ileal digestibility and availability values have

application, depending on the protein sources in the diet. Ileal digestibility is a concept that has been used to increase the accuracy of ration formulation in meeting the amino acid requirements of monogastric animals. This system relates to a specific component of the digestive process.

Studies are required to accurately relate the improved sensitivity of the ileal analysis method (as compared to the faecal analysis method) to a biological change, e.g. increased growth rate or nitrogen retention. Factors which influence ileal digestibility include level of feeding, associative effects between feeds such that values are non additive, particle size, starch type, non-starch polysaccharides and the effects of feed processing (Low, 1990).

The advantage of ileal digestibility values over total amino acids values was demonstrated by Tanksley and Knabe (1984). Tanksley and Knabe (1984) concluded that there were important performance benefits in formulating diets on the basis of their apparent ileal digestibility values for amino acids when they contained unusual protein sources, but not for diets based on maize and soya. Similar conclusions were drawn in a review by Sauer and Ozimek (1986). Better correlations between protein deposited in female pigs and ileal rather than faecal digestible amino acids values were found by Just et al. (1985). Dierick et al. (1987) correlated daily gain and feed conversion in pigs with apparently digested crude protein measured in ileal digesta of faeces and found the former provided a better prediction of performance. Low and Partridge (1984) found a close correlation between ileal apparent digestibility of lysine and lysine deposition in the whole body but only when this amino acid was limiting. This illustrates the important point that it is only information about the first, second or perhaps third limiting amino acid in the diet which is of importance.

Use of ileal digestibility values in diet formulation increases the range of ingredients that can be employed in the formulation and improves the accuracy with which they can be used. It permits increased accuracy in predicting animal performance.

It also has direct benefits in economic terms by reducing margins included for safety, which by definition are additional to requirements. A major effort is required to demonstrate these advantages in practice.

When deciding upon which type of digestibility measure to use it is important to establish the objective for acquiring such information. For example, if the objective is to obtain an accurate estimate of the uptake of amino acids from the digestive tract of an animal, and the animal is fed a diet that does not contain either fibre or ANFs (anti-nutritional factors), then either true or real digestibility would be appropriate. If the diet contains fibre or ANFs, however, real digestibility would need to be used.

Estimates of the amino acid requirements of pigs, determined either empirically or by theoretical models, have usually taken into consideration the endogenous loss of amino acids of the animal. In most instances, however, this endogenous loss of amino acid (EAAL) will pertain to the basal level, rather than the increased EAAL associated with specific ingredients. This latter fact has implications for choice of digestibility value used. If, apparent digestibility values are chosen then EAAL in the pig is costed against the feed.

The subsequent use of apparent digestibility values in conjunction with estimates of a pig's amino acid requirement which have already accounted for EAAL will result in a double penalty against the feed. Obviously, this is unacceptable. Using the true digestibility values, however, would result in a fair representation of the protein source, particularly if it dose not contain fibre or ANFs, as the basal EAAL used to obtain the true values will in most cases be similar to the basal EAAL pertaining to estimation of the pig's amino acid requirements.

1.7 Conclusion

The apparent digestibility of diets may be affected by many factors such as the chemical composition of the feedstuffs, methods of assessment and chemical analyses,

and characteristics of the animals (animal bodyweight, age and genotype). Identifying and quantifying their effects on digestibility, whilst requiring considerable developmental work may ultimately benefit feed formulation practices and lead to an improvement in the efficiency with which feedstuffs fed to animals are utilized.

In the current studies a selection of factors were investigated. In the first part of the study (chapter 2), the digestibilities of apparent faecal digestible energy (ADE) and apparent faecal digestible organic matter (ADOM) were examined using two contrasting methodologies, total faecal collection and chromic oxide procedures, as assessed over different collection periods in a factorial study involving diets of predominately two feed materials differing widely in dietary fibre, wheat and wheat by-product (bran and broll). In the second part of the study (chapter 3), methodology was further examined by investigating the influence of feeding level (6 or 11% of metabolic liveweight) and liveweight (25 or 90 kg) on ADE and ADOM in the two cereal based diets. The third part of the study (chapter 4) evaluated the effect of genotype on ADE and ADOM for the two cereal based diets. A discussion of the findings and their implications with reference to the responses obtained by others investigating this field are presented in chapter 5.

Chapter 2

Effect of Faeces Collection Method and the Length of the Faeces Collection Period on the Apparent Faecal Digestibility of Energy (ADE) and Organic Matter (ADOM) in Wheat and Wheat by-Products (Broll and Bran) in the Growing Pig

2.1 Introduction

The apparent digestibility of feeds can be affected by numerous factors. Pfirter (1983) distinguished effects associated with the animals, the environment, the feed and the method of feeding. In evaluating feeds and establishing feed recommendations it would be useful to understand how digestibility is influenced by factors such as the chemical composition of the diet, the feeding level, and the body weight of the animals. However, in some cases it is difficult to estimate the effect of any one single factor.

Digestibility coefficients are among the most important parameters in evaluating the nutritive value of feeds for pigs. The most commonly used method for estimating digestibility, relies on a quantitative collection of faeces over several consecutive days from animals kept in metabolism cages (classical method). Theoretically, determination of the total amount of the component of interest ingested corrected for the amount subsequently excreted in the faeces should provide an unbiased estimate of digestibility.

The use of indigestible markers to determine nutrient digestibility is an attractive alternative technique as it removes the need to measure total food intake and total faeces output. In the pig, chromic oxide has been the most frequently used faecal marker (McCarthy et al., 1974). However, problems with incomplete recoveries of chromic oxide, (usually between 75-80%), have been encountered by several researchers (Moore, 1957; McCarthy et al., 1974,1977; Moughan et al., 1991; Bakker and Jongbloed, 1994).

The optimal length of the faeces collection period in digestibility trials has proved to be controversial. The collection period should not be less than 10 days for swine and

horses, and not less than 12 to 15 days for ruminants (Grindley, 1917). Several researchers have demonstrated that the time elapsed between feeding of the test ingredient and the start of collection as well as the length of the collection period have an effect on the determined digestibility coefficient (Clawson et al., 1955; Den Hartog et al., 1988b).

The objective of the present study was to determine the influence of the two factors, collection method and duration of collection period, on the apparent faecal digestibility of energy and organic matter in two cereal products, wheat and wheat by-product (a bran and broll mixture) fed to the growing pig. Wheat (low fibre content) and wheat by-product (high fibre content) were chosen to characterize the effect of fibre on digestibility. The collection methods were those of total collection and faecal output as measured by chromic oxide. For the "duration of faeces collection" distinction was made between daily faeces collection and three collection periods (days 1 to 5); (days 6 to 10); day 11 to 12 (Den Hartog et al., 1988b).

2.2 Materials and Methods

2.2.1 Animals and Housing

Twelve male Large White X Landrace pigs of 30kg liveweight (± 2.42 kg) were randomly selected from a weaner pool at the Pig Research Unit, Massey University. The pigs were kept in individual smooth-walled steel metabolism crates at the University's Animal Physiology Unit. The metabolism crates were designed to allow for separate and complete faeces collection. The crates were housed in a controlled-environment room, and the ambient temperature was maintained at 23°C ± 0.4°C. Ethics approval for the study was granted by the Massey University Animal Ethics Committee.

Table 2.1 Ingredient composition (g Kg^{-1} air dry weight) of the experimental diet

Ingredient	Wheat diet	Wheat by-product diet
Wheat	993.5	
Wheat bran		745.1
Wheat broll		248.4
Chromic oxide	4.0	4.0
Vitamin + mineral mix[1]	2.5	2.5

1. Pig grower/finisher vitamin and mineral premix, Danmix, Nutritech International Ltd., Auckland, New Zealand. Composition and stated allowances (mg/kg diet) were as follows: Vitamin A 4.00, Vitamin D_3 0.80, Vitamin E 0.12, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Vitamin K_3, Biotin, Folic acid, Nicotinic acid, Panthothenic acid, Choline Chloride: Cobalt, Copper, Iodine, Iron, Manganese, Selenium (120ppm), Zinc

2.2.2 Diets and Feeding

The ingredient compositions of the experimental diets (containing wheat or wheat by-products) are given in Table 2.1. The determined nutrient compositions of the diets are given in Table 2.2. Each ingredient was ground through a hammer mill at the University's Feed Processing Unit using the screen size (4mm sieve) normally used for pig feeds. Each of the two diets, wheat diet and wheat by-product diet (broll and bran) contained a commercial pig grower/finisher vitamin and mineral supplement (Danmix, Nutritech International Ltd. Auckland, New Zealand) included at a level of 2.5g/kg of diet and chromic oxide included at 4g/kg diet.

2.2.3 Experimental

Pigs were allocated randomly to the metabolic crates and the diets allocated randomly to the pigs such that there were six pigs per treatment diet. The trial comprised a seven-day adaptation period, through which a commercial pig grower mash diet was provided, followed by a twelve-day faeces collection period over which the treatment

Table 2.2 Chemical composition of the experimental diet

Component	Wheat diet	Wheat by-product diet
Dry matter (%)	86.25	87.73
[1]Gross energy (MJ/kg)	15.75	16.38
[1]Crude protein (%)	13.23	14.28
[1]Neutral detergent fibre (%)	12.38	31.32
[1]Lignin (%)	1.03	2.77
[1]Acid detergent fibre (%)	2.78	9.39
[1]Hemicellulose (%)	9.60	21.93
[1]Cellulose (%)	1.75	6.62
[1]Fat (%)	1.27	3.14

[1] as fed basis

diets were fed. Throughout, the daily dry feed allowance was fixed at 10% of metabolic body weight ($W^{0.75}$) and supplied in two equal amounts at 09:00 h and 16:00 h at a feed: water mixture of 2:1 (w (kg)/v (l)). The pigs had free access to fresh water at all times. They were weighed prior to the commencement of the adaptation period and at the beginning and the end of the collection period.

At the start of the collection period, the crates were set up so that the faeces could be collected free of urine. All spillage of feed or feed refusals were weighed and recorded on a daily basis. Each day the faecal output of each pig was collected onto a clean plastic sheet. The pig's faeces were emptied into a clean tared bucket, the bucket and contents weighed, the material then thoroughly mixed and a sample of approximately 60g drawn and placed into a tared plastic cup. The cup and contents were immediately weighed and the cup, fitted with a lid, stored at -20°C until at a convenient time the contents were freeze-dried, weighed and chemically analyzed.

For the determination of ADE and ADOM by the method of total collection, the wet weight of the daily faecal samples as a fraction of the wet weight of the daily faecal matter voided was used with the corresponding analyzed energy and organic matter production to calculated the daily ADE and ADOM. Period ADE and ADOM values were calculated the mean of the daily values for the period. For the determinations involving chromic oxide, the ratio of the chromic oxide in the daily test feed to that in the sample of collected daily faeces provided the basis on which daily faecal output and daily ADE and ADOM were calculated. Again, as for the method of total collection, period ADE and ADOM were calculated as the mean of the daily values in the period. Samples of each diet were taken at each feeding time over the final twelve days of the trial.

2.2.4 Chemical Analysis

Prior to chemical analysis both diets and freeze-dried faecal samples were ground in a laboratory mill (1 mm mesh diameter sieve, Wiley mill. USA).

Duplicate determinations of dry matter were performed on all feed and faeces samples drawn (AOAC, 1984). Dry matter content was expressed as a proportion of the weight of each sample. Gross energy content was determined on duplicate sub-samples of each feed and faeces sample by the method of AOAC (1984) using an adiabatic bomb calorimeter (Gallenkamp and Co. Ltd., London). Total nitrogen content was determined on duplicate samples of both diet samples using the macro Kjeldahl procedure (Kjeltec Auto 1030 analyser, Tecator, Sweden). Crude protein was calculated as total N x 6.25 according to AOAC (1990). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin contents were determined in duplicate samples of both diets using the method described by Robertson and Van Soest (1981). Duplicate determinations of organic matter were made on oven-dried samples of diets and faeces. Organic matter was calculated as the difference in weight between the initial sample and the ash remaining after combustion, and was expressed as a proportion of the weight of the original sample. Duplicate estimates for the chromium contents of diet and faecal samples were determined using the method of Costigan and Ellis (1987). The chromium content was

measured at 357.7 nm in a NO_2-acetylene flame using a GBC 904 Atomic Absorption Spectrophotometer (GBC Scientific Equipment Pty Ltd. Australia). Duplicate estimates for the crude fat content of diet samples were determined using the method of soxhlet extraction (Hexane extract). Crude fat was calculated as the difference in weight between the initial flask and the flask remaining after extraction, and was expressed as a proportion of the weight of the original sample.

2.2.5 Statistical Analysis

The apparent digestibility coefficients were calculated for the total collection methods as follow. Energy is used as the example.

$$\text{Apparent digestible energy (\%)} = \frac{\text{(GE content consumed–GE content voided in faeces)} \times 100}{\text{(GE content consumed)} \times 1}$$

GE content consumed = Feed intake (Kg DM) x GE of feed (MJ/kg DM) – Feed refusal (Kg DM) x GE of feed refusal (MJ/kg DM)

GE content voided in faeces = Faeces output (Kg DM) x GE of faeces (MJ/kg DM)

The corresponding expression using the indicator method is as follows:

$$\text{Apparent digestibility (\%)} = 100-\left(100 \times \frac{\text{\% Cr in feed (Cr \% / DM)}}{\text{\% Cr in faeces (Cr \% / DM)}} \times \frac{\text{Energy content in faeces (MJ/kg DM)}}{\text{Energy content in feed (MJ/kg DM)}}\right)$$

Chromium recovery was calculated on the basis of total chromium in the feed intake and total chromium in the faecal output as: $\frac{\text{Cr eliminated}}{\text{Cr ingested}} \times \frac{100}{1}$

The effects of the length of the faeces collection period, collection method and pig within diet on the apparent faecal digestibility coefficients of dietary energy and organic matter were statistically analyzed using a General Linear Model Procedure (SAS Institute Inc., U.S.A). Two models were investigated. In model 1 data was analyzed to take into account the effect of days of collection. For model 2, the collection period was divided into three periods according to the approach adopted in the "classical" total collection procedure. Data of day 2 to 5, 6 to 10 and 11 and 12 were pooled together, and a period effect (1,2 or 3) was used in place of the day effect (Model 2). These three periods correspond to the "classical" adaptation period, collection period and post collection period (Den Hartog et al., 1988b).

Model 1 takes the form,

$$\mathbf{Y_{ijkl} = \mu + T_i + A(T)_{ij} + M_k + D_l + (T_i \times M_k) + (T_i \times D_l) + (M_k \times D_l) + (T_i \times M_k \times D_l) + e_{ijkl}}$$

where: Y_{ijkl}= Dependent variable (apparent faecal digestibility coefficients for organic matter or energy); μ = Overall mean; T_i = Diet (i =wheat, wheat by-product); $A(T)_{ij}$ = Pig within diet (j = 1,~,6); M_k = Method (k = total collection, indicator method); D_l = Days of collection (l =2,~,12); $(T_i \times M_k)$ = Interaction between diet and method; $(T_i \times D_l)$ = Interaction between diet and days of collection; $(M_k \times D_l)$ = Interaction between method and days of collection; $(T_i \times M_k \times D_l)$ = Interaction diet and method and day of collection; e_{ijkl} = Residual error.

Model 2 has the form,

$$Y_{ijkl} = \mu + T_i + A(T)_{ij} + M_k + P_l + (T_i \times M_k) + (T_i \times P_l) + (M_k \times P_l) + (T_i \times M_k \times P_l) + e_{ijkl}$$

where: Y_{ijkl}= Dependent variable (apparent faecal digestibility coefficients for organic matter or energy); μ = Overall mean; T_i = Diet (i = wheat, wheat by-product); $A(T)_{ij}$ = Pig within diet (j = 1,~,6); M_k = Method (k = total collection, indicator method); P_l = Period of collection (l =1,2,3), (T_i x M_k)=Interaction between diet and method; (T_i x P_l) = Interaction between diet and period of collection; (M_k x P_l) = Interaction between method and period of collection; (T_i x M_k x P_l) = Interaction diet and method and period of collection; e_{ijkl} = Residual error.

2.3 Results

The pigs readily consumed the wheat based diet and remained healthy throughout the 14-day study and gained in weight (7.7 ± 0.98 kg, mean ± SD). There were no food refusals and the overall average daily food intake (± SD) was 1.262 ± 0.07 kg per day for pigs given wheat based diet. However, the pigs fed the wheat by-product based diet exhibited some food refusals (0.102 ± 0.07 kg per day). They gained weight (7.8 ± 1.06kg, mean ± SD) and their average daily food intake (± SD) was 1.167 ± 0.05 kg per day.

The results for the effect of diet, method of collection and day (period) of collection are summarized in Tables 2.4 (ADOM) and 2.5 (ADE) and a significance summary for main class and interaction effects is given in Table 2.3.

For model 1, the diet, pig within diet, method of faecal collection and day of collection exerted significant effects on ADE and ADOM at $P<0.001$ in each case except ADE for days of collection for which significance was attained at $P<0.01$. The ADOM and ADE were greater for wheat 88.8% and 85.4% respectively versus 66.8% and 62.1%

Table 2.3 Statistical analysis of the effect of duration of collection period (days), collection method (total vs indicator), diet and their interactions on the apparent faecal digestibility of energy and organic matter[2]

	Digestibility			
	Organic Matter		Gross Energy	
Statistical	Model 1	Model 2	Model 1	Model 2
Diet	***	***	***	***
Method	***	***	***	***
Diet x Method	***	***	***	***
Pig(diet)	***	***	***	***
Day (or P[1])	***	NS	**	NS
Day (P[1]) x Method	***	NS	***	NS
Day (P[1]) x Diet	***	*	***	*
Day (P[1])x Diet x Method	**	NS	*	NS

1. Period 1, day 2-5; period 2, day 6-10; period 3, day 11-12.

2. NS= non significant, P>0.05; * = P<0.05; ** = P<0.01;*** = P<0.001

for wheat by-product and total collection resulted in greater digestibility than the indicator procedure for both organic matter (79.6% versus 76.0%) and energy (75.9% versus 71.6%). On days 2 through 12 digestibility remained relatively constant ranging for determined organic matter between 75.4% and 79.0% and for energy 71.3% and 75.4%.

The interactions, day x method, day x diet, and diet x method were significant at P<0.001 for ADE and ADOM and diet x method x day was significant at P<0.01 for ADOM and at P<0.05 for ADE. For day x method for both ADE and ADOM, total collection resulted in significantly greater digestibilities on 2 and 5 days at P<0.001, on 3 days at P<0.05 and on 4 days the differences were not significant (P>0.05). For day x diet on each day digestibilities associated with energy and organic matter were significantly greater (P<0.001) for wheat. For diet x method for both ADE and ADOM differences

Table 2.4 Least square means for the diet (wheat, W or wheat by-product, WB), method (total or indicator) and day or periods of collection on apparent faecal digestibility of organic matter (%) (±SE)[2]

	Diet			Method			Diet x Method					
Day	WB[4]	W[4]	Sig.[35]	Total	I	Sig.[35]	WB[4]T	WB[4]I	Sig.[35]	W[4]T	W[4]I	Sig.[35]
2	68.3	85.4	***	80.9	72.8	***	72.0	64.7	***	89.8	80.9	***
			(0.76)			(0.76)			(1.08)			(1.08)
3	68.0	90.1	***	80.6	77.5	*	69.5	66.5	NS	91.7	88.5	NS
			(0.76)			(0.76)			(1.08)			(1.08)
4	61.8	88.9	***	74.4	76.4	NS	60.2	63.4	NS	88.6	89.3	NS
			(0.76)			(0.76)			(1.08)			(1.08)
5	67.0	91.0	***	81.6	76.4	***	70.9	63.2	***	92.2	89.7	NS
			(0.76)			(0.76)			(1.08)			(1.08)
P1[1]	67.4	88.4	***	79.8	75.9	***	70.4	64.5	***	89.4	87.3	*
			(0.42)			(0.42)			(0.59)			(0.59)
6	66.2	89.2	***	80.3	75.0	***	70.8	61.6	***	89.8	88.5	NS
			(0.76)			(0.76)			(1.08)			(1.08)
7	66.6	88.7	***	80.7	74.6	***	71.5	61.7	***	89.9	87.5	NS
			(0.76)			(0.76)			(1.08)			(1.08)
8	66.2	88.7	***	79.4	75.5	**	69.7	62.8	***	89.2	88.1	NS
			(0.76)			(0.76)			(1.08)			(1.08)
9	67.1	88.8	***	79.8	78.0	**	70.2	64.0	***	89.3	88.3	NS
			(0.76)			(0.76)			(1.08)			(1.08)
10	68.5	89.3	***	79.8	78.0	NS	69.9	67.1	NS	89.7	88.9	NS
			(0.76)			(0.76)			(1.08)			(1.08)
P2[1]	66.9	88.9	***	80.0	75.8	***	70.4	63.4	***	89.6	88.3	NS
			(0.42)			(0.42)			(0.59)			(0.59)
11	68.0	88.2	***	78.7	77.4	NS	69.5	66.4	NS	87.9	88.4	NS
			(0.76)			(0.76)			(1.08)			(1.08)
12	66.7	88.7	***	79.1	76.3	*	68.4	65.1	NS	89.8	87.5	NS
			(0.76)			(0.76)			(1.08)			(1.08)
P3[1]	67.3	88.4	***	78.9	76.9	NS	68.9	65.7	*	88.8	88.0	NS
			(0.42)			(0.42)			(0.59)			(0.59)

1. Period 1, day 2-5; period 2, day 6-10; period 3, day 11-12.

2. Pooled standard error; n=6

3. NS = non significant, P>0.05; * = P<0.05; ** = P<0.01;*** = P<0.001

4. WB = wheat by-product, W = wheat; T =total collection method, I = indicator method (chromic oxide)

5. Differences tested by T-test

Table 2.5 Least square means for the diet (wheat, W or wheat by-product, WB), method (total or indicator) and day or periods of collection on apparent faecal digestibility of energy (%) (±SE)[2]

	Diet			Method			Diet x Method					
Day	WB[4]	W[4]	Sig.[35]	Total	I	Sig.[35]	WB[4]T	WB[4]I	Sig.[35]	W[4]T	W[4]I	Sig.[35]
2	63.0	80.9	***	77.0	67.0	***	67.2	59.0	***	86.8	75.1	***
			(0.93)			(0.93)			(1.31)			(1.31)
3	63.7	87.1	***	77.2	73.6	*	65.2	62.2	NS	89.3	85.0	NS
			(0.93)			(0.93)			(1.31)			(1.31)
4	57.1	85.5	***	70.6	72.0	NS	56.2	58.1	NS	85.0	85.3	NS
			(0.93)			(0.93)			(1.31)			(1.31)
5	62.1	88.2	***	78.1	72.2	***	66.4	57.8	***	89.3	86.5	NS
			(0.93)			(0.93)			(1.31)			(1.31)
P1[1]	62.4	85.2	***	76.2	71.4	***	65.5	59.2	***	86.9	83.5	***
			(0.48)			(0.48)			(0.68)			(0.68)
6	61.4	85.8	***	76.7	70.6	***	66.6	56.2	***	86.7	84.9	NS
			(0.93)			(0.93)			(1.31)			(1.31)
7	62.5	85.4	***	77.4	70.4	***	67.9	57.0	***	86.9	83.8	NS
			(0.93)			(0.93)			(1.31)			(1.31)
8	61.6	85.4	***	75.7	71.2	**	65.5	57.7	***	86.0	84.7	NS
			(0.93)			(0.93)			(1.31)			(1.31)
9	62.6	85.3	***	76.0	71.9	**	66.0	59.2	**	86.0	84.7	NS
			(0.93)			(0.93)			(1.31)			(1.31)
10	64.0	86.2	***	76.1	74.1	NS	65.5	62.5	NS	86.3	85.7	NS
			(0.93)			(0.93)			(1.31)			(1.31)
P2[1]	62.4	85.6	***	76.4	71.6	***	66.3	58.5	***	86.5	84.8	NS
			(0.48)			(0.48)			(0.68)			(0.68)
11	63.4	84.2	***	74.5	73.1	NS	65.1	61.6	NS	83.9	84.6	NS
			(0.93)			(0.93)			(1.31)			(1.31)
12	61.8	85.2	***	75.2	71.8	*	63.7	60.0	NS	86.7	83.7	NS
			(0.93)			(0.93)			(1.31)			(1.31)
P3[1]	62.6	84.7	***	74.8	72.5	NS	64.4	60.8	*	85.3	84.2	NS
			(0.48)			(0.48)			(0.68)			(0.68)

1. Period 1, day 2-5; period 2, day 6-10; period 3, day 11-12.

2. Pooled standard error; n=6

3. NS = non significant, P>0.05; * = P<0.05; ** = P<0.01;*** = P<0.001

4. WB = wheat by-product, W = wheat; T =total collection method, I = indicator method (chromic oxide)

5. Differences tested by T-test

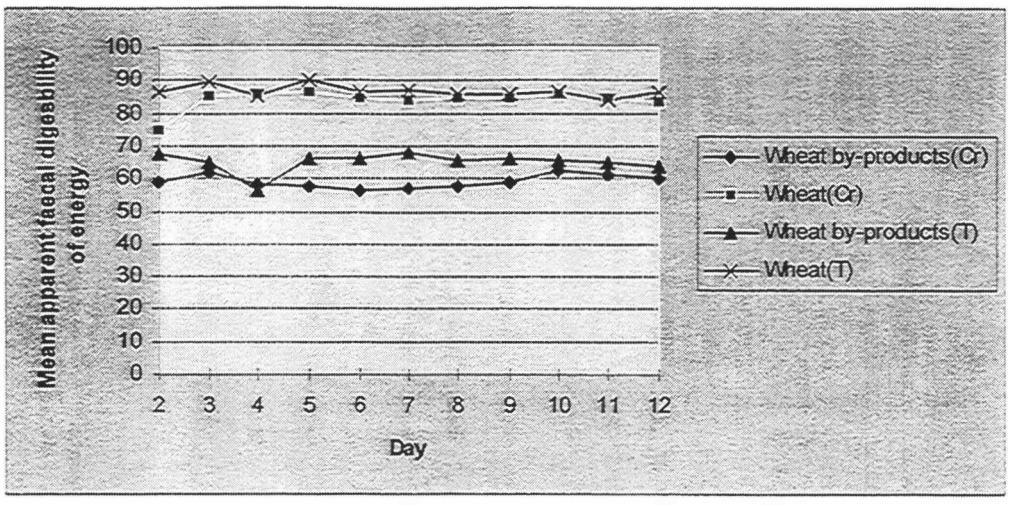


Figure 2.1 Effect of the length of faeces collection days for growing pigs given wheat or wheat by-product based diets on mean apparent faecal digestibility (%) of energy using the total collection or indicator methods

between WT (wheat with total collection combination) and WI (wheat with indicator method combination) were not significant but WBT (wheat by-product with total collection combination) was larger than WBI (wheat by-product with indicator method combination) ($P<0.001$) and both WT (ADE 86.7%, ADOM 89.8%) and WI (ADE 84.0%, ADOM 87.8%) were significantly greater than WBT (ADE 65.0%, ADOM 69.3%) and WBI (ADE 59.2%, ADOM 64.2%) ($P<0.001$). For the three way interaction, TWB resulted in significantly greater digestibilities ($P<0.001$) than IWB on collection days 2,5,6,7,8 and 9 for both energy and organic matter, but except for collection days 2 the WT and WI treatments were not significantly different.

The size of the disparity in digestibility relationships between WT, WI, WBT and WBI over days is demonstrated schematically in Figure 2.1 (ADE) and Figure 2.2 (ADOM). The figures illustrate that from day 2 in the case of WBI and WBT and day 3 in the case of WI and WT, digestibilities of organic matter and energy were consistent in magnitude over days.

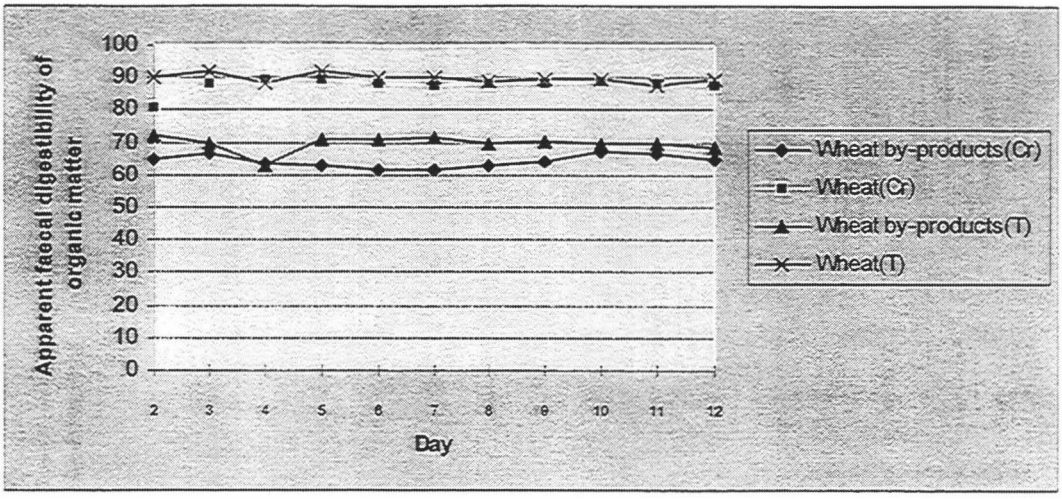


Figure 2.2 Effect of the length of faeces collection days for growing pigs given wheat or wheat by-product based diets on mean apparent faecal digestibility (%) of organic matter using the total collection or indicator methods

For model 2, the main class effects (diet, pig within diet and method of faecal collection) were compatible with those of model 1. Differences in digestibilities for both organic matter and energy were not significant at $P>0.05$ across periods of collection. Of the interactions, diet x method resulted in significant digestibility differences at $P<0.001$ for both organic matter and energy. Total collection gave greater digestibility than the indicator method in periods 1 and 2 but not in period 3. The interactions period x method and period x diet x method differences in both ADE and ADOM were not significant ($P>0.05$). The interaction period x diet was significant at $P<0.05$ for both ADE and ADOM. In each period the digestibility of organic matter and energy was greater for wheat than for wheat by-product. For ADE WT gave greater digestibility than WI in period 1 ($P<0.001$) but not in period 2 and 3, but in each period the digestibility of WBT was greater than WBI.

Correlation coefficients were obtained for the relationships ADE versus ADOM as assessed by a) total collection and b) chromic oxide and for the relationships c) ADE by total collection versus ADE by chromic oxide and d) ADOM by total collection versus ADOM by chromic oxide, for each of the diets, wheat and wheat by-product and for each of the periods 1 through 3 using for each period the average daily digestibilities obtained

Table 2.6 Correlation coefficients for ADE (total collection) versus ADOM (total collection); ADE (chromic oxide) versus ADOM (chromic oxide); ADE (total collection) versus ADE (chromic oxide) and ADOM (total collection) versus ADOM (chromic oxide) for wheat and wheat by-product for period[3] 1 through 3 employing ADE and ADOM pig daily determinations[4]

	Wheat			Wheat by-product			Overall		
Period	1	2	3	1	2	3	1	2	3
Total[1]DE[2]-Total[1]DOM[2]	0.88[d]	0.99[d]	0.96[c]	0.99[d]	0.99[d]	0.98[d]	0.99[d]	0.99[d]	0.99[d]
Ind.[1]DE[2]-Ind.[1]DOM[2]	0.99[d]	0.94[c]	0.98[d]	0.99[d]	0.99[d]	0.99[d]	0.99[d]	0.99[d]	0.99[d]
Total[1]DE[2]-Ind.[1]DE[2]	0.54[a]	0.53[a]	0.32[a]	0.75[a]	0.85[b]	0.41[a]	0.93[d]	0.99[d]	0.96[d]
Total[1]DOM[2]-Ind.[1]DOM[2]	0.12[a]	0.30[a]	0.31[a]	0.81[b]	0.88[b]	0.38[a]	0.94[d]	0.99[d]	0.97[d]

1. Total=Total collection method, Ind.=Indicator method
2. DE= Digestibility coefficients of energy
   DOM= Digestibility coefficients of organic matter
3. Period=1, day 2-day 5; 2, day 6-day 10; 3, day 11-day 12
4. a = non significant, P>0.05; b = P<0.05; c = P<0.01; d = P<0.001

by pig (Table 2.6). The relationships between ADE and ADOM whether involving total collection or chromic oxide were highly significantly correlated (P<0.001) for each period for each diet. For those involving total collection, except for period 1 for wheat (0.88), coefficients were ≥0.96 and for those involving digestibility determination by chromic oxide coefficients were ≥0.94 (for either wheat or wheat by-product).

The correlation coefficients describing the association between ADE or ADOM by total collection versus chromic oxide, were significant (P<0.001), but the variables were only moderately associated. For total collections ADE, versus chromic oxide ADE the coefficients for wheat ranged between 0.32 (period 3) and 0.54 (period 1) and for wheat by-product they were spread over 0.41 (period 3) and 0.85 (period 2). Somewhat similar values and variation were observed for total collection ADOM versus chromic oxide ADOM. The coefficients for wheat ranged between 0.12 (period 1) and 0.31

(period 3) and those for wheat by-product were spread from 0.88 (period 2) to 0.38 (period 3).

The effect of day of collection on chromium recovery (%) from wheat and wheat by-product diets is presented in Figure 2.3. The chromium recovery (%) measured from <10% on day 1 for both wheat and wheat by-product to 100% on day 4 then decreased on day 5 in the case of wheat to 73% after which it increased, somewhat erratically, to 100% on day 11 before decreasing to 80% on day 12. On day 5 wheat by-product fell to 79% and rose erratically over 7 days to 90% on day 12. The mean percentage (±SD) recovery rate of Cr from day 6 to day 12 was 84.76 ± 7.09 (%).

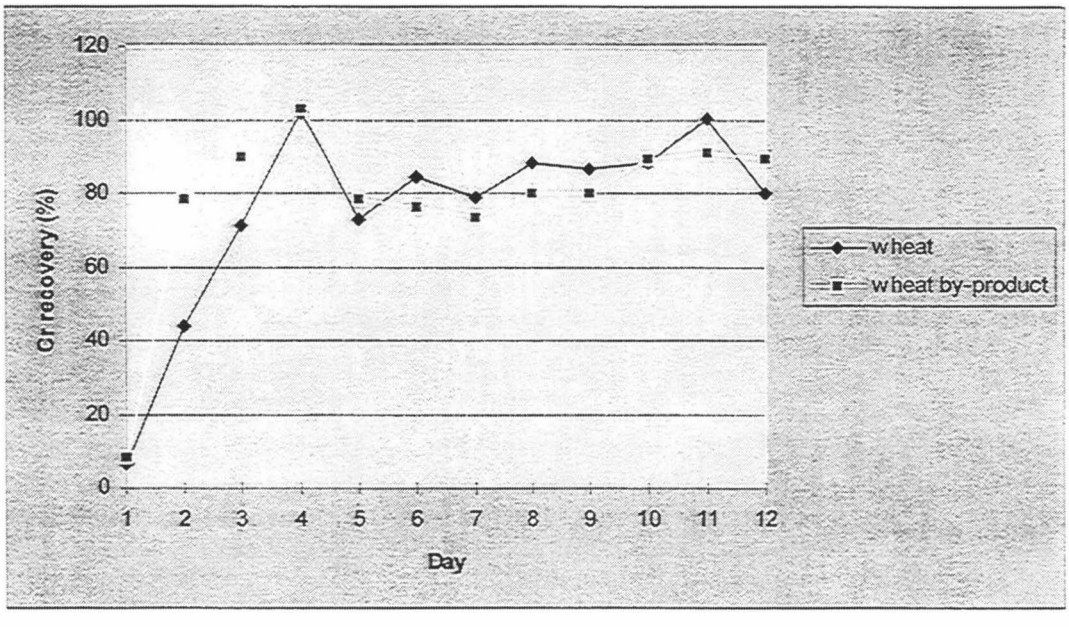


Figure 2.3 Effect of the duration of faeces collection (days) on chromium recovery (%) for wheat and wheat by-product diets in the pig

2.4 Discussion

Method of digestibility estimation had a significant (P<0.001) effect on energy and organic matter digestibility with total collection giving higher values in comparison with chromic oxide marker approach. Further analysis involving the interaction term, diet

x method, showed that the effect of method of collection on wheat was contributing little to the difference between methods and that the difference was mainly the result of significant ($P<0.05$) effects between methods when using wheat by-product. Significant differences between methods involving wheat by-product resulted on 6 of the 11 day collections for both ADE and ADOM and for each of the 3 periods.

The cause of the variation in significance between days (for wheat by-product versus method of collection) appears linked to changes in the recovery of chromic oxide. On days 3,4,10,11 and 12 when differences between methods were not significant, recoveries of chromic oxide were around 90% or better. On other days, when methodology differences affecting ADE and ADOM were significant, chromic oxide recoveries were markedly less than 90%. This explanation though still applicable is less compatible when assessed against the recoveries of chromic oxide from the faeces of pigs fed wheat. Recoveries were generally greater than those for wheat by-product and on days 1 and 2 when they were less than 60%, significant differences between methods of collection were obtained. However on days 3,5 and 7 recoveries were less than 80% and differences between methods were non significant.

The correlation coefficients between ADE x total collection and ADE as obtained by indicator, and for ADOM x total collection versus ADOM x indicator both suggest only moderately strong associations. For example, the correlation coefficients for wheat by-product for ADE between total collection and indicator methods ranged between 0.41 and 0.85 and for ADOM ranged between 0.38 and 0.88 over the periods. For wheat less variability was apparent but correlation coefficients were weaker. It is questionable whether linear prediction equations will permit sufficiently reliable estimations of total collection estimates of ADE and ADOM from indicator estimates or vice versa when the related correlation coefficients are only weak to moderately strong and quite variable. In contrast the correlation coefficients describing the strength of the linear relationships between ADE and ADOM both as ascertained by total collection procedures or as obtained using chromic oxide were high and relatively consistent within diets and across periods.

A comparison of apparent faecal digestibility coefficients obtained for methods involving either total collection or chromic oxide by different authors is given in Table 2.7. Whereas in the present study differences in ADE and ADOM for wheat between the two methods of collection were not significant (except in period 1, due to low value for day 2), but for wheat by-products they were. Everts and Smits (1987) reported that the total faecal collection method gives greater apparent digestible dry matter, organic matter and nitrogen than chromic oxide procedures in whole diets and they attributed the probable cause to lower than 100% recoveries of chromic oxide. Moughan et al. (1991) in their investigation of whole diets obtained chromic oxide recoveries of (85.3% ± 6.19) which they asserted probably accounted for the 3-4 percentage units higher ($P<0.05$) dry matter, organic matter and gross energy coefficients associated with total collection.

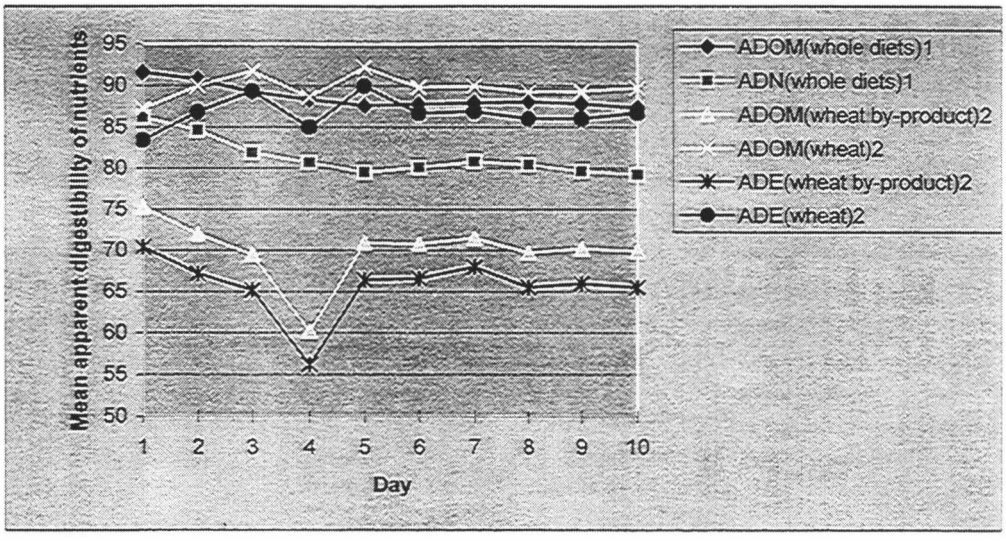
In contrast Bakker and Jongbloed (1994) obtained chromic oxide recoveries ranging from 99% to 106% with indicator methods giving slightly greater organic matter and nitrogen digestibility coefficients than with total collection. They attributed the high chromic oxide recoveries to the sampling faecal collection procedure employed which resulted in accumulated samples of 241g (as collected) drawn over 3 collection days. The faecal sampling procedure adopted for the current work followed the recommendations of Moughan et al. (1991) and involved sampling over 5 consecutive days minimum amounts of around 60g (as collected) per day.

In the present study, whereas differences in digestibility for both organic matter and energy were highly significant between days, under model 2 in which determinations were made over two 5 day periods and one final 2 day period, differences were small and non significant ($P>0.05$) between the latter two periods, but both digestibilities were greater than that of period 1. Figure 2.4 (also tables 2.4 and 2.5) shows that for the total collection method, organic matter and energy digestibility for both wheat by-product and wheat remained relatively uniform throughout although wheat by-product experienced high day 1 and low day 4 values. The day 4 values can only be attributed to a low day 4 output of faecal matter. However digestibility assessment by 5 day period was effective

Table 2.7 Comparison of the marker method versus total collection from results published in the literature

No of Pigs/diet	Feed	Nutrient	Total collection	Cr_2O_3	Level Significance[6]
8[1]	Barley	DM	74.0	71.0	
8[2]	Whole	DM	82.4	78.5	**
	diets	OM	83.9	81.0	***
		GE	81.4	78.2	***
8[3]	Whole	DM	80.2	79.0	NS
	diets	OM	83.8	82.9	NS
		N	83.1	82.4	NS
4[4]	Control	OM	90.7	90.7	NS
	Cellulose	OM	66.4	67.1	**
	Soybeanhull	OM	84.3	85.7	**
	Animal Fat	OM	88.2	88.9	**
	Control	N	87.9	87.9	NS
	Cellulose	N	76.9	75.2	**
	Soybeanhull	N	84.3	77.5	**
	Animal Fat	N	88.2	89.1	**
6[5]	Wheat	OM	89.3	87.9	NS
		GE	86.4	84.1	NS
	Wheat by	OM	70.1	64.4	***
	-products	GE	65.2	59.5	***

1. Clawson et al. (1955)
2. Moughan et al. (1991)
3. Everts and Smits (1987)
4. Bakker and Jongbloed (1994)
5. Present study
6. NS = non significant, $P>0.05$; ** = $P<0.01$;*** = $P<0.001$

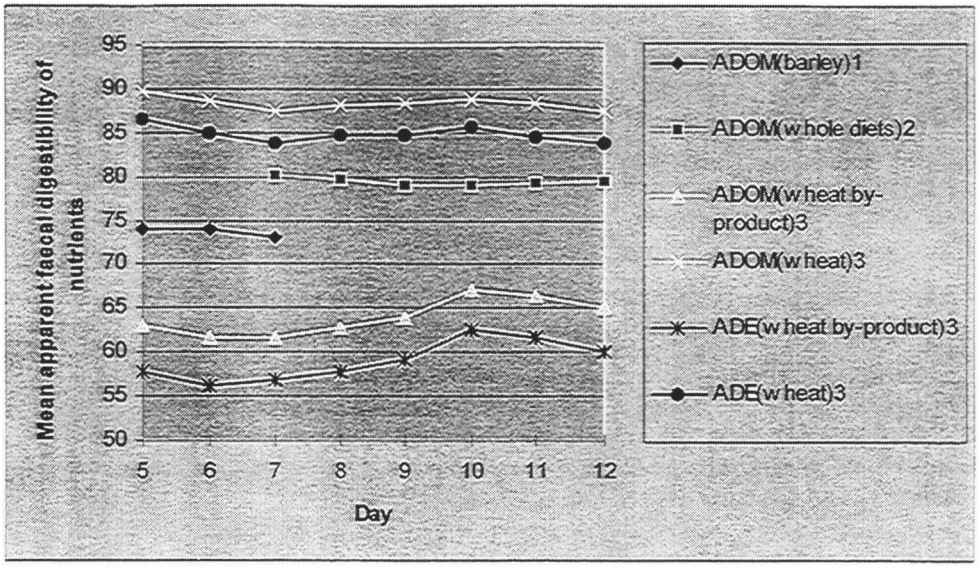


1. Den Hartog et al. (1988b)
2. Present study

Figure 2.4 Comparison between the present study and published data for the effect of length of collection period on apparent digestibility of nutrients in pigs using the total collection method

in balancing out these fluctuations and resulted in period means that were similar over each of the 3 periods. For the chromic oxide approach low digestibility was experienced for both organic matter and energy on day 1 for wheat and wheat by-product and on day 2 for wheat. These responses appear to have impacted strongly on the period 1 digestibilities for both organic matter and energy and are considered to be the major cause of the significant period effects. Figure 2.5 illustrates a visual picture of the uniformity over days in ADOM and ADE for wheat and wheat by-products over the final 7 days of collection using the indicator method. Again some daily movement in digestibility is apparent, but period means were effective in evening out daily differences.

The uniformity over time (days) in digestibilities obtained by Den Hartog et al. (1988b) for total collection methods and by Clawson et al. (1955) and Moughan et al. (1991) for chromic oxide measures are shown in Figure 2.4 and 2.5 respectively. Den Hartog et al. (1988b) investigating digestibility of protein (ADN) and organic matter



1. Clawson et al. (1955)
2. Moughan et al. (1991)
3. Present study

Figure 2.5 Comparison between the present study and published data for the effect of length of collection period on apparent digestibility of nutrients in pigs using the chromic oxide method

found that both decreased from day 1 to 4, but then remained stable through collection days 5 through 10. Clawson et al. (1955) reported that the concentration of chromic oxide in the faeces of pigs became relatively constant between 3 and 4 days after the start of feeding and found that the apparent digestibility of dry matter (ADDM) did not change from day 5 to 7. The work of Moughan et al. (1991) similarly demonstrated that the length of the collection period had no effect on ADOM when measured from day 7 to 12.

Lengthening the collection period increases the labour, time and expense and reduces the number of feeds that can be tested within a given period of time. Longer collection periods also increase the risk of some adverse event such as animal sickness or accident affecting results and necessitating repetition. On the other hand collection

periods that are too short may lead to insufficiently representative samples and greater variation.

The study described have showed that for two feedstuffs and using two collection methods, a collection interval of 5 days beginning from the start of feeding gave equivalent results to collection periods utilizing days 6 through 10 and days 11 through 12 following the start.

Of the collection methods, total collection has been widely accepted for digestibility estimations, but it has been criticized on a number of grounds. The quantitative collection of faeces is required to the made without loss or contamination with urine, hair, or sloughed skin. The faeces collected need to be of test feed origin. Faecal collection requires specialized equipment and is consuming of time and labour. Methods of containment reduce the animals freedom of movement and is subjected to criticism on animal welfare grounds. Many of these concerns are minimized by the use of an indigestible marker such as chromic oxide. Indicator methods do not require the measurement of feed intake and faecal output. Kotb and Luckey (1972) concluded that chromic oxide is not toxic and is quantitatively recoverable from the faeces of man and animals. However incomplete recovery of chromic oxide has been encountered in several studies (Moore, 1957; McCarthy et al., 1974,1977; Moughan et al., 1991; Bakker and Jongbloed, 1994).

In the present work there was incomplete recovery of chromic oxide on most days with recovery becoming rather more uniform from day 5 for both wheat and wheat by-product. The level of recovery appeared to influence the digestibility of wheat in period 1 but not thereafter and except for period 1 the digestibility coefficients of both the total collection and chromic oxide methods were alike for wheat, but total collection did result in significantly different coefficients compared to the chromic oxide procedure, for wheat by-product over each period.

Chapter 3

The Effect of Feeding Level and Liveweight on the Apparent Faecal Digestibility of Energy and Organic Matter in Wheat and Wheat by-Product (Broll and Bran) for the Growing Pig.

3.1 Introduction

Changes in the digestive capacity of the pig with age and liveweight have been described by several workers, but research results have not been altogether compatible. A positive effect of increasing body weight on energy digestibility has been found in growing pigs by Whiting and Bezeau (1957), Cunningham et al. (1962), Fernandez and Jorgensen (1986) and Noblet et al (1994b), but Kass et al. (1980) found digestive capacity was negatively related to live weight.

The effect of feeding level on the determined digestibility of energy in pigs has also been inconsistent. A decrease in energy apparent digestibility associated with an increase in feeding level has been observed by Parker and Clawson (1967) and Everts and Smits (1987) in sows and by Cunningham et al. (1962), Roth and Kirchgessner (1985), Everts and Smits (1987) and Smits et al. (1994) in growing pigs. In contrast, no effect or a positive effect has been reported by Mitchell and Hamilton (1929) and Peers et al. (1977).

The objective of the present study was to determine the influence of these two factors (feeding level and liveweight) on ADE and ADOM in two cereal products, wheat and wheat by-products, in the growing pig.

3.2 Materials and Methods

3.2.1 Animals and Housing

Forty eight Large White x Landrace entire male pigs of two different liveweights, 26kg (± 2.23 kg, n=24) and 92kg (± 4.31 kg, n=24) were held in individual pens in an environmental controlled area at the Pig Research Unit, Massey University. The ambient temperature was maintained at 23°C ± 0.4°C. Ethics approval for this study was given by the Massey University Animal Ethics Committee.

3.2.2 Diets and Feeding

The experimental diets had the same ingredient composition as those described in section 2.2.2. During the adaptation period the diets were given without chromic oxide, but during the experimental phase chromic oxide was added (4.0g/kg air-dry weight). The diets were fed daily in two equal portions at 09:00 and 16:00h. The diets were mixed with water (2:1 ratio w/v) and fed as a wet mash. Fresh water was available between meals.

3.2.3 Experimental

The study was conducted over two different time periods (replicates). Two different factors were investigated: liveweight, 25 vs. 90kg; feeding level, 0.06 ($W^{0.75}$) vs. 0.11 ($W^{0.75}$). In each replicate three pigs were randomly allocated to each of the diet x liveweight x feeding level combinations and each treatment combination was randomly assigned to a pen.

The pigs were on trial for 24 days. Each day the pen floors were hosed clean before feeding. From day 1 to 7 (the pretreatment period) the pigs were fed a commercial pig-grower diet supplemented with Tetramycin (0.3%) at 0.1 x $W^{0.75}$kg per day. From day 8 through 14 (the adaptation period) pigs allocated to the 6% of bodyweight$^{0.75}$ feeding level were fed the designated allowance, but for pigs destined to receive the

higher allowance the amount fed was raised by increments daily from an initial level of 6% $W^{0.75}$ to a final level of 11% $W^{0.75}$ on day 14. From day 15 through 24 (the experimental period) the pigs were fed the treatment diet (with chromic oxide) at the designated feeding levels. A fresh faecal sample from each pig was collected daily through days 20 to 24 and held in temporary storage in a closed plastic bag in a deep freeze (-20°C) until further sub-sampling following the end of the trial. The pigs were weighed prior to the commencement of the adaptation period and at the beginning and the end of the experimental period (day 15 and day 24).

At the end of the trial on days 25 and 26 the frozen samples for each pig were thawed and thoroughly mixed over days. A sub-sample of each pig's collection (approximately 60g) was placed in a tared plastic cup fitted with a lid and held in deep freeze (-20°C) and later freeze dried and weighed. Subsequent chromic oxide determinations on the freeze dried samples were used to estimate the dry matter of faeces voided per unit of test food fed.

3.2.4 Chemical Analysis

Feeds and faeces were analyzed in duplicate for dry matter (refer 2.2.4), gross energy (adiabatic bomb calorimeter) (refer 2.2.4), organic matter and chromium (Atomic Absorption Spectrophotometer) (refer 2.2.4). Both feed samples were analyzed in duplicate for nitrogen (macro Kjeldahl procedure) (refer 2.2.4), neutral detergent fibre (NDF) (refer 2.2.4), acid detergent fibre (ADF) (refer 2.2.4), lignin (Robertson and Van Soest, 1981) (refer 2.2.4) and crude fat (soxhlet extraction) (refer 2.2.4).

3.2.5 Statistical Analysis

The apparent digestibility coefficients for gross energy (GE) and organic matter (OM) were calculated using the expression:

$$\text{Apparent digestibility (\%)} = 100-\left(100 \times \frac{\text{\% Cr in feed}}{\text{\% Cr in faeces}} \times \frac{\text{GE/OM content of faeces}}{\text{GE/OM content of feed}}\right)$$

The effects of replicate diet, feeding level and liveweight and their interactions with aspect ADE and ADOM were statistically analyzed by analysis of variance using a factorial design 2x2x2x2 and the GLM Procedure of SAS Institute Inc., U.S.A. The following model was used.

$$\mathbf{Y_{ijkl} = \mu + T_i + F_j + L_k + R_l + (T_i \times F_j) + (T_i \times L_k) + (F_j \times L_k) + (T_i \times M_j \times D_k) + e_{ijkl}}$$

where: Y_{ijkl} = Dependent variable (apparent faecal digestibility coefficients of organic matter or energy); μ = Overall mean; T_i = Diet (i =wheat, wheat by-product); F_j = Feeding level (j =6%, 11%); L_k = Liveweight (k =light, heavy); R_l = Run (1 = first, second); (T_i x F_j) = Interaction between diet and feeding level; (T_i x L_k) = Interaction between diet and liveweight; (F_j x L_k) = Interaction between feeding level and liveweight; (T_i x M_j x D_k) = Interaction diet and feeding level and liveweight; e_{ijkl} = Residual error

3.3 Results

The pigs readily consumed the wheat based diet and the wheat by-products based diet, remained healthy and there were no food refusals throughout the 10-day study. The mean feed intakes and liveweight gains for pigs receiving the wheat and wheat by-product treatments are presented in Table 3.1.

The effect of diet, feeding level, liveweight and their interactions on mean ADE and ADOM (%) of the wheat and wheat by-product based diets are presented in Table 3.2. Differences in ADE and ADOM between runs 1 and 2, and between feeding at 6% versus 11% of metabolic liveweight ($W^{0.75}$) were small and statistically non significant. Differences due to liveweight were small, but significant ($P<0.05$) and those due to type

Table 3.1 Mean feed intakes and liveweight gains (± SD)[2] for light and heavy pigs fed wheat and wheat by-product based diets at 6% or 11% of metabolic liveweight ($W^{0.75}$)

	Wheat		Wheat by-product	
	6%	11%	6%	11%
25kg Liveweight				
Feed intake[1]	0.71 (0.03)	1.27 (0.06)	0.88 (0.06)	1.27 (0.08)
Liveweight gain[1]	0.36 (0.48)	4.06 (0.89)	0.0 (0.41)	2.20 (1.21)
90kg Liveweight				
Feed intake[1]	1.77 (0.06)	3.25 (0.12)	1.77 (0.05)	3.25 (0.15)
Liveweight gain[1]	1.83 (1.98)	1.21 (2.00)	0.0 (0.56)	8.35 (4.47)

1. Feed intake = kg / day, Liveweight gain = kg / experimental period

2. ± SD = Standard deviation

of feed, large and highly significant ($P<0.001$) for both ADE and ADOM. In the former case heavier pigs resulted in greater digestibility (ADE, 77.1% versus 76.0%; ADOM, 80.9% versus 80.2%) and in the latter case wheat gave rise to higher digestibilities than wheat by-product by about 12 and 13 percentage units for ADE and ADOM respectively. Interaction effects were non significant.

Correlations between ADE and ADOM for each feeding level x liveweight x diet combination are presented in Table 3.3. The linear association between DE and DOM for diets fed at 6% or at 11% of metabolic bodyweight to either 30kg or 100kg pigs was significant and strong with correlations of 0.98 to 0.99. Examination of the correlations by diet (wheat or wheat by-product) gave moderate to strong correlations that were quite variable ranging from 0.46 to 0.92 and except in two cases were significant at $P<0.05$ or $P<0.01$.

Table 3.2 Effect of diet, feeding level, liveweight and their interactions on mean apparent faecal digestibility coefficients for organic matter and energy (%) for wheat and wheat by-product based diets in the growing pig[1]

Diet	Wheat		Wheat by-product	
Feeding level ($W^{0.75}$)	6%	11%	6%	11%
25kg Liveweight				
Gross energy	83.0 (0.58)	82.2 (0.52)	70.2 (0.99)	68.8 (2.15)
Organic matter	87.0 (0.41)	86.3 (0.35)	73.9 (0.55)	73.7 (1.21)
90kg Liveweight				
Gross energy	83.8 (0.52)	83.6 (0.79)	71.0 (0.60)	70.1 (0.69)
Organic matter	87.7 (0.55)	87.4 (0.64)	74.6 (0.51)	73.9 (0.77)

	Level of significance				
Nutrient	Run	Diet	Feeding level	Liveweight	Interactions
Gross energy	NS	***	NS	*	NS
Organic matter	NS	***	NS	*	NS

1. NS = non significant, P>0.05; * = P<0.05; *** = P<0.001
   Mean values (±SE); n=6

3.4 Discussion

Feeding Level

The present study found that the apparent digestibility of feed energy and organic matter was unaffected by increasing the consumption of wheat and wheat by-product from a 6 to an 11% metabolic bodyweight basis. This response in growing pigs is supported by the published data of Peers et al. (1977) who assessed digestibility of dry matter, gross energy and nitrogen in barley and by some of the findings of Smits et al. (1994) who examined the digestibility of gross energy in legume seeds, maize by-product and wheat by-product and organic matter in wheat by-product and tapioca. Table 3.4

Table 3.3 Correlations between apparent faecal digestibility coefficients for organic matter and energy for each feeding level and liveweight within each diet[3]

	Wheat		Wheat by-product		Overall	
	25kg	90kg	25kg	90kg	25kg	90kg
6%[1]DE[2]-6%[1]DOM[2]	0.46[a]	0.92[d]	0.92[d]	0.58[b]	0.98[d]	0.99[d]
11%[1]DE[2]-11%[1]DOM[2]	0.89[d]	0.89[d]	0.77[c]	0.73[c]	0.98[d]	0.99[d]

1. 6%= 6% of metabolic bodyweight ($W^{0.75}$), 11%=11% of metabolic bodyweight ($W^{0.75}$)
2. DE= Digestibility coefficients of energy
   DOM= Digestibility coefficients of organic matter
3. a = non significant, P>0.1; b = P<0.1; c = P<0.05;d = P<0.01

provides a comparison of results between the present study and published data in growing pigs. The responses described above are in contrast to the observation of Parker and Clawson (1967) who found that dry matter, organic matter and nitrogen digestibilities of maize and barley diminished as feeding level increased. Everts and Smits (1987) also reported that an increase in feeding level had a significant negative effect on apparent faecal digestibility coefficients of nutrients and affected digestibility in a non linear way. Increasing feeding level from 1.2 times the maintenance requirement to 2.4 times maintenance had a stronger negative effect than the increase from 2.4 times maintenance to 3.6 times maintenance.

Fernandez et al. (1986) investigated nutrient digestibility in adult sows using 26 different feedstuffs varying widely in chemical composition. Digestibility differences were not apparent for feeding levels of maintenance and 80 and 120 percent of maintenance levels.

The relationship between feeding level and digestibility appears to depend on the quality of the feed. The digestibility of rations containing lower concentrations of digestible nutrients are influenced to a larger extent by feeding level than are rations

Table 3.4 Effect of feeding level on apparent faecal digestibility of nutrients

Feed	Nutrient	Feeding Level			Significance[8]
Barley[1]		M[5]		3M	
	DM	82.2		82.7	NS
	GE	83.3		83.5	NS
	N	83.4		83.9	NS
Maize[2]		L[6]	M[6]	H[6]	
	DM	88.6	86.9	85.4	*
	OM	91.7	89.9	88.4	*
	N	86.9	85.8	84.0	*
Barley[2]	DM	76.2	75.0	73.7	*
	OM	78.5	77.5	76.2	*
	N	81.8	80.2	78.5	*
Barley, wheat,		2.3M		2.8M	
Wheat by products[3]	OM	84.9		84.4	*
	GE	82.4		81.8	*
Maize, maize	GE	80.0		79.6	NS
by-product[3]					
Tapioca,	OM	85.0		81.6	NS
Legume seeds[3]	GE	82.4		81.6	NS
Wheat[4]		6%[7]		11%[7]	
	GE	83.4		82.8	NS
	OM	87.3		86.8	NS
Wheat by-product[4]	GE	70.5		69.4	NS
	OM	74.2		73.8	NS

1. Peers et al. (1977)
2. Parker and Clawson (1967)
3. Smits et al. (1994)
4. Present study
5. M= Maintenance
6. L= Low; M= Medium, H= High of feeding level
7. 6%= 6% of metabolic liveweight ($W^{0.75}$); 11%= 11% of metabolic liveweight ($W^{0.75}$)
8. NS = non significant, $P>0.05$; * = $P<0.05$

which contain higher amounts of digestible nutrients (Sugimoto, 1985). It is well known that crude fibre is a component of diets that affects digestibility. Cunningham et al. (1962) added wood cellulose to a balanced diet to increase the crude fibre levels from 40 to 200 g/kg and found that in doing so the level of feeding had a considerable effect on the apparent digestibility of dry matter, crude fibre and N.

Digestive efficiency may also be diminished by increasing the level of feeding as a result of the positive effect of the latter on rate of passage (Roth and Kirchgessner, 1985). In addition hindgut digestion is sensitive to the time that the digesta are subjected to fermentation and rapid passage of digesta may diminish the effectiveness of this mechanism. Further it should not be overlooked that apparent digestibility measures are subject to the influence of endogenous excretions whose contributions to the quantity of faecal output may be expected to vary according to both feeding level and the nature of material fed or the quantity of food residues produced as well as body size. In the present study the sum of the effects of all of these factors have resulted in no discernable affect on digestibility for the feeding levels examined. A tentative interpretation is that provision of wheat and wheat by-products at feeding levels based on a metabolic bodyweight basis overcome effects that endogenous excretions may have had on digestibility and that the pigs were able to digest the larger quantities of feed irrespective of type equally as well as the smaller amounts.

Liveweight

Differences in ADE and ADOM in the present study were small but significant ($P<0.05$) for larger pigs than smaller pigs. A summary of published data on the effect of liveweight on ADE and ADOM is presented in Table 3.5. The current results are consistent with those of Fernandez and Jorgensen (1986) and Noblet et al. (1994b). The former authors found that increasing the amount of fibre in the diets depressed the digestibility of gross energy significantly, whilst increasing the liveweight of the pigs raised digestibility (not significantly) at all levels of crude fibre. There was a trend towards larger differences between liveweight groups receiving the higher amounts of

Table 3.5 Comparison of the effect of liveweight on apparent faecal digestibility of nutrients obtained in the present study with published data

Feed	Nutrient	Liveweight			Significance[4]
Whole		25kg	46kg	78kg	
diet[1]	GE	90%	91%	92%	NS
Whole		45kg	100kg	150kg	
diet[2]	GE	88%	90.5%	91.0%	*
Wheat[3]		25kg		90kg	
	GE	82.5%		83.7%	*
	OM	86.6%		87.5%	*
Wheat	GE	69.5%		70.5%	*
By-product[3]	OM	73.8%		74.3%	*

1. Fernandez and Jorgensen (1986)
2. Noblet et al. (1994b)
3. Present study
4. * = $P<0.05$

crude fibre. Noblet et al. (1994b) investigated the digestibility of gross energy of whole diets over three liveweights (45, 100, and 150kg) and found small but significant increases ($P<0.05$) with increasing liveweight.

The positive effect of liveweight on digestibility has been attributed to the influence of the hindgut to the digestive process. Nielsen (1962) has described the development pattern of the digestive tract of pigs from 20kg to 150kg liveweight. The small intestine was nearly fully developed at 20kg, while the hindgut was still growing at 150kg. Noblet and Shi (1993) have reported that the difference in digestibility coefficients between growing pigs and sows was mainly due to a higher rate of degradation of fibre in the hindgut of the sows.

Chapter 4

Effect of Genotype on the Apparent Faecal Digestibility of Energy and Organic Matter in Wheat and Wheat by-Product (Broll and Bran) for the Growing Pig.

4.1 Introduction

Many factors influence the digestibility of dietary nutrients by the pig. They include genotype, environmental factors, methods of determination, feedstuffs, feeding methods and liveweight (Pfirter, 1983; Whiting and Bezeau, 1957; Noblet et al., 1994b; Clawson et al., 1955; Den Hartog et al., 1988b). An appreciation of the influence of these factors on digestibility coefficients may ultimately result in an improvement in dietary formulation practice and raise the efficiency of utilization of pig feeds.

The influence of genotype on the digestibility of energy is unclear. A higher digestibility of energy has been reported by Sundstøl et al. (1979) for obese pigs and Wenk (1982) and Wenk and Morel (1985) for lean genotypes, but Hofstetter and Wenk (1985) were unable to demonstrate a significant difference in the digestibility of energy between a lean and fat selected line.

The objective of this study was to determine the effect of genotype on ADE and ADOM of two cereal products (wheat and wheat by-products) in growing pigs using the total collection method.

4.2 Materials and Methods

4.2.1 Animals and Housing

Six, four-month old, Large White X Landrace entire male pigs of 54.0kg (± 3.1 kg) liveweight and six, three-month old, Kune-Kune entire male pigs of 19.0kg (± 3.9 kg) liveweight were kept in individual smooth-walled steel metabolism crates at the

University's Animal Physiology Unit. The metabolism crates were designed to permit the separate and complete collection of faeces and urine. The crates were installed in a controlled-environment room, the ambient temperature being maintained at 19°C ± 1.1°C. Ethics approval for the study was given by the Massey University Animal Ethics Committee.

Brief Description of Kune-Kune Pigs

The Kune-Kune is a domesticated pig found in the North Island of New Zealand that has been present for about 200 years. The Kune-Kune has been referred to as the "China pig", this term possibly reflecting either its origin or the people who brought it to New Zealand. In the early 1800s many Spanish and Portuguese mariners sailed to New Zealand by way of China and bordering countries (Clarke and Dzieciolowski, 1991) from where they may have acquired the ancestors of the Kune-Kune. Whatever its origins, it is believed to be of Asian or Chinese bloodstock. It is therefore likely to be a form of *Sus scrofa moupinensis* or a hybrid of this species and others. Thomson (1922) has described the breeds of pigs in New Zealand. It is evident that the indigenised pigs of the two islands have different origins. He refers to the compact, short legged and headed pig of the North Island and the long snouted Tamworth-type wild pigs of the South Island.

The Kune-Kune is a small fat pig with a short snout and short legs, possessing a pair of tassels which hang from the neck. The majority of Kune-Kune breeders free-range them on pasture. The height of the pasture varies from that of sheep grazed lands to an out of control type. Under either of these conditions most Kune-Kune perform well. The lower the pasture cover the lower the backfat in general. Some Kune-Kunes are run in orchards where they consume both the pasture vegetation and fallen fruit. In addition to pasture, many Kune-Kunes are fed on soaked and crushed grains, potatoes, fruit and vegetables, household scraps and silage.

4.2.2 Diets and Feeding

The ingredient composition of the experimental mash diet (containing either wheat or wheat by-product) is given in Table 4.1. The determined gross energy composition of the diet was 17.10 MJ/kg (as fed basis), or 18.90 MJ/kg (as dry matter basis) for the wheat by-product diet and 16.08 MJ/kg (as fed basis), or 18.11 MJ/kg (as dry matter basis) for the wheat based diet. The wheat and wheat by-product components were ground through a hammer mill using a 4mm screen size at the University's Feed Processing Unit. Both diets were supplemented with a commercial grower vitamin and mineral supplement (Danmix, Nutritech International Ltd. Auckland, New Zealand) at 0.25% of the diet.

Throughout the trial a pig grower commercial meal was fed during the adaptation period followed by treatment diets over the experimental (collection) period. The diets were presented daily as two equal portions at 9:00 and 16:00h. The diets were mixed with water in a 2:1 ratio w (kg)/v (l). The pigs had access to fresh water at all times. The level of dry feed fed daily to each pig was 10 percent of metabolic liveweight ($W^{0.75}$).

Table 4.1 Ingredient composition (g Kg^{-1} air dry weight) of the experimental diet

Ingredient	Wheat diet	Wheat by-product diet
Wheat	997.5	
Wheat bran		748.1
Wheat broll		249.4
Vitamin + mineral mix[1]	2.5	2.5

1. Pig grower/finisher vitamin and mineral premix, Danmix, Nutritech International Ltd., Auckland, New Zealand. Composition and stated allowances (mg/kg diet) were as follows: Vitamin A 4.00, Vitamin D_3 0.80, Vitamin E 0.12, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Vitamin K_3, Biotin, Folic acid, Nicotinic acid, Panthothenic acid, Choline Chloride: Cobalt, Copper, Iodine, Iron, Manganese, Selenium (120ppm), Zinc

4.2.3 Experimental

The trial involved two runs completed under identical conditions in time and in pigs, but the pigs were changed over so that those on the wheat diet in the first run became the pigs receiving the wheat by-product diet in the second run. In each run the pigs of each genotype were allocated to one of two treatment diets and the pigs were randomly assigned to the metabolic crates. The trial comprised a seven-day adaptation period, followed by a ten-day faeces collection period. All wastage of feed was weighed and recorded on a daily basis. Samples of the diets were taken at each feeding time over the final ten days of the trial. The pigs were weighed prior to the commencement of the adaptation period and at the beginning and the end of the collection period.

Before starting the collection period, the crates were set up so that the faeces were collected free of urine. Faecal outputs of each pig were collected daily on clean plastic sheets, weighed and stored in air tight plastic bags in a deep freeze (-20°C) until required for sampling. At a convenient time the pig faeces were thawed, the daily collections mixed thoroughly by pig, a representative sample taken (approximately 60g), weighed in tared cups, refrozen (-20°C) and later freeze-dried.

4.2.4 Chemical Analysis

Prior to chemical analysis the treatment diets and freeze-dried faecal samples were ground in a laboratory mill (1 mm mesh diameter sieve, Wiley mill. USA). Dry matter analyses were performed on the feeds and faeces in duplicate, as described earlier (AOAC, 1984) (refer section 2.2.4). Gross energy content was determined on duplicate samples of feed and faeces by conventional methods (AOAC, 1984) (refer section 2.2.4). Organic matter was determined on oven-dried samples of diets and faeces in duplicate (refer section 2.2.4).

4.2.5 Statistical Analysis

Apparent digestibility coefficients were calculated using the expression.

$$\text{Apparent digestibility (\%)} = \frac{\text{(GE/OM content in feed – GE/OM content in faeces) x 100}}{\text{(GE/OM content in feed) x 1}}$$

The effects of genotype and diet on apparent digestibility were determined by analysis of variance using the General Linear Model procedure of the statistical software program SAS (1985). The model employed was as follows.

$$\mathbf{Y_{ijk} = \mu + T_i + G_j + P(G)_{jk} + (T_i \times G_j) + e_{ijk}}$$

where Y_{ijk}= Dependent variable (apparent faecal digestibility coefficients of organic matter and energy); μ = Overall mean; T_i = Diet (i =wheat, wheat by-product); G_j = Genotype (j =Kune-Kune, Large White X Landrace); $P(G)_{jk}$ = Pig within genotype (k = 1,~,5); (T_i x G_j)= Interaction between diet and genotype; e_{ijk} = Residual error.

4.3 Results

The mean feed intakes and liveweight gains of Kune-Kune and Large White X Landrace pigs fed the wheat and wheat by-product diets are given in Table 4.2. The Kune-Kune and Large White X Landrace readily consumed the wheat based diet, gained in weight and remained healthy throughout the 10-day study. There were no food refusals within the wheat based diet. The pigs fed the wheat by-product based diet did refuse food (0.16 ± 0.10 kg per day for the Kune-Kune) and (0.38 ± 0.16 kg per day for the Large White X Landrace).

Table 4.2 Mean feed intakes and liveweight gains (± SD)[2] for Kune-Kune and Large White x Landrace pigs fed wheat and wheat by-product diets

	Wheat		Wheat by-product	
	Kune[1]	LW x LR[1]	Kune[1]	LW x LR[1]
Feed intake[3]	0.91 (0.11)	2.06 (0.25)	0.88 (0.12)	1.99 (0.09)
Livweight gain[3]	2.75 (0.45)	4.51 (0.59)	2.40 (0.59)	4.36 (2.20)

1. Kune=Kune Kune, LW x LR= Large White x Landrace
2. ± SD = Standard deviation
3. Feed intake = kg / day, Liveweight gain = kg / trial

The effect of diet and genotype on ADE and ADOM were analyzed using the data ten of pigs only, because two pigs, 1 LW x LR and 1 Kune-Kune, had large feed refusal during the experiment. Whereas the effect of diet and the interaction between diet and genotype were statistically significant ($P<0.001$) for both the ADE and ADOM the effect of genotype for both was non significant (refer Table 4.3). The effect of pig within genotype was not significant for ADE. With respect to the latter the digestibility of Kune Kune versus LW x LR for energy was 79.2 versus 78.7% and for organic matter was 82.8 versus 82.2%. Examination of the interaction, diet x genotype, shows that for both energy and organic matter no significant differences in digestibility were found between LW x LR and Kune Kune for wheat. However, for wheat by-product the Kune Kune pigs show a statistically significant better digestibility than the LW x LR ($P<0.001$).

The correlations between ADE and ADOM of each genotype within diet are presented in Table 4.4. The association between digestible energy and digestible organic matter was highly significant for both Kune-Kune and LW x LR (0.99, $P<0.001$) and for both genotypes within diets, in the case of Kune-Kune for wheat (0.94, $P<0.001$) and wheat by-product (0.94, $P<0.01$) and for LW x LR, 0.95, $P<0.001$ (wheat) and 0.98, $P<0.001$ (wheat by-product).

Table 4.3 Effect of genotype on mean apparent faecal digestibility of organic matter and energy (%) for wheat and wheat by-product diets in the pig[1]

	Wheat				-Wheat by-product			
Nutrient	Kune[2]	LWxLR[2]	SEM	Sig.	Kune[2]	LWxLR[2]	SEM	Sig.
Organic matter	90.3	90.9	0.24	NS	75.3	73.5	0.24	***
Gross energy	87.1	87.9	0.34	NS	71.3	69.4	0.34	**

	Level of significance			
Nutrient	Pig within genotype	Diet	Genotype	Diet x Genotype
Organic matter	*	***	NS	***
Gross energy	NS	***	NS	***

1. NS= non significant, P>0.05; * = P<0.05; ** = P<0.01;*** = P<0.001

2. Kune=Kune Kune, LW x LR= Large White x Landrace

4.4 Discussion

The intriguing finding of the present study was the interaction effect of diet and genotype on the digestibility of both organic matter and energy in which in the low fibre diet (wheat based), LW x LR achieved slightly greater digestibility, but in the high fibre diet (wheat by-product) coefficients for the Kune-Kune were greater. The responses achieved for wheat in this study are consistent with those of Wenk and Morel (1985) who fed barley and maize and Yen et al. (1983) who fed maize to lines designated as lean or obese and found greater digestibility associated with lean lines. On the other hand Sundstol et al. (1979) reported greater digestibility of organic matter associated with obese pigs on feeding diets based on barley, sorghum and oats which is not in keeping with the result of the present study involving wheat by-product. In contrast Hofstetter and Wenk (1985) were unable to demonstrate differences in the digestibility of energy over lean and obese lines when diets based on barley and maize were fed. A summary of published findings of apparent faecal digestibility coefficients of nutrients as recorded for obese and lean lines of pigs is given in Table 4.5.

Table 4.4 Correlations between apparent faecal digestibility coefficients of organic matter and energy as estimated within diet and genotype[3]

	Wheat	Wheat by-product	Overall
Kune[1]DE[2]-Kune[1]DOM[2]	0.94[b]	0.94[a]	0.99[b]
LWxLR[1]DE[2]-LWxLR[1]DOM[2]	0.95[b]	0.98[b]	0.99[b]

1. Kune=Kune Kune, LW x LR= Large White x Landrace
2. DE= Digestibility coefficients of energy
   DOM= Digestibility coefficients of organic matter
3. a = P<0.01; b = P<0.001

Breed differences in digestibility coefficients have been demonstrated by Kemp et al. (1991). They conducted a study comparing Meishan and Dutch Landrace gilts aged 12-13 weeks and weighing approximately 28 kg when fed either a standard diet based on maize, barley and wheat (2.3% crude fibre) or a high crude fibre diet (11%) in which oats replaced wheat at 90 g/kg $^{0.75}$. Digestibility coefficients for crude fibre and crude fat were greater (P<0.05) for the Meishan pigs.

The information in the foregoing suggests quite clearly that genotype differences may influence the digestibility of certain feeds. It is tempting to speculate that the genotype responses may be the result of digestive morphological and physiological differences. With respect to the current work longer transit times in the small intestine and/or greater small intestinal length and/or weight may combine to improve digestibility of low fibre feeds whereas genotypes of greater hind gut development may facilitate greater digestion of high fibre feeds (Pond et al., 1988). As was focussed in chapter 3, greater liveweight may positively influence digestibility and may have contributed to the improved digestibility of energy and organic matter in Large White x Landrace pigs fed the wheat based diet, but such effects, to the extent that they influence the digestibility of high fibre feeds, appear in the current work, to have been overshadowed by more profound influences related to the Kune Kune genotype.

Table 4.5 Comparison of the effect of genotype on the apparent faecal digestibility of nutrients obtained in the present study with that found in published studies

Feed	Nutrient	Genotype		Significance[6]
		Lean	Obese	
Barley Maize[1]	GE	83.8	82.3	NS
Maize[2]	GE	79.8	77.9	**
Barley, Oats Sorghum[3]	OM	81.9	85.8	*
Barley Maize[4]	GE	79.5	78.9	**
Wheat[5]	OM	90.9	90.3	NS
	GE	87.9	87.1	NS
Wheat[5]	OM	73.5	75.3	***
by-product	GE	69.4	71.3	**

1. Hofstetter and Wenk (1985)
2. Yen et al. (1983)
3. Sundstøl et al. (1979)
4. Wenk and Morel (1985)
5. Present study
6. NS= non significant, $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

Kune-Kune pigs have shown themselves to be quite capable of surviving and fattening well on pasture and high fibre foods. The ability to utilize pasture, household scraps and fibrous materials may lie in the structure of the digestive system and the gut microflora this lifestyle has evolved.

Chapter 5

Summary and General Conclusion

The apparent digestibility of feeds may be affected by many factors. Pfirter (1983) distinguished effects associated with the animals, the environment, the feed and the method of feeding. An appreciation of the influence of these factors on digestibility may ultimately improve feed formulation practice and raise the efficiency of utilization of feedstuffs for farm animals. However, identifying and quantifying the influence of such factors requires considerable development work and the literature contains many examples of relationships for which contrasting responses have been obtained. The studies reported in this thesis were conducted to clarify some of these issues. This section discusses these findings and their implications and the work of others in this field.

Digestibility coefficients are among the most important parameters available for the evaluation of the nutritive value of feeds for pigs. The evidence in the literature suggests however that coefficients may be affected by a number of factors, among them methodology and faecal collection interval. These factors were investigated in the first study (chapter 2) in which digestibility of energy and organic matter were contrasted across two methodologies (total faecal collection and chromic oxide) over different collection periods in a factorial study involving a wheat and a wheat by-product (bran and broll) based diet. The study showed that there were substantial differences in the quantity of energy and organic matter that pigs could extract from wheat and wheat by-products; that total collection evaluations resulted in greater coefficients than those involving chromic oxide and that the more fibrous material (wheat by-product) exacerbated the effect; but that period of collection (when over a number of days) did not greatly influence digestibility coefficients when obtained using total collection or chromic oxide.

The most appropriate length of the collection period raises a number of issues. Lengthening the collection period increases the labour, time and expense involved and limits the number of trials that may be conducted and rations that may be tested over a

given length of time. Longer collection periods increase the risk of accident, animal sickness, or other untoward events that may adversely affect responses and result in the need for a re-run. Collection periods that are too short may distort results because of a failure to achieve equilibrium status or because the period of collection accounts incompletely for the variation present. In the current work coefficients were unaffected by whether faecal collection took place over periods 1-5 days, 6-10 days or 11 to 12 days from the start of test food provision. However in the case of the chromic oxide procedure, chromium recovery of wheat and wheat by-product did not achieve relative stability until about day 5 and the lack of difference between the first period and later period coefficients seems somewhat fortuitous. Beyond day 4 of collection chromic oxide recovery was very much more stable and for this reason a stabilization interval to precede a collection interval may provide a preferred procedure for assessment using the chromic oxide method.

The present study showed that total collection gave greater digestibility coefficients than chromic oxide procedures and that this effect was more pronounced in the case of wheat by-product. The greater between method differences associated with wheat by-product were tentatively attributed to the smaller chromium recoveries generally associated with the more fibrous diet through the latter two thirds of the collection time. These results were consistent with those of Everts and Smits (1987) and Moughan et al. (1991) both of who worked with whole diets and attributed the responses to reduced chromium recoveries. Greater chromium recovery has been reported by Bakker and Jongbloed (1994) who recorded greater coefficients for chromium oxide procedures and who attributed the greater recoveries to larger faecal sampling. In the current study thorough mixing and sampling of the daily faecal collection should theoretically have precluded non representative effects arising from smaller faecal sample size. It is unclear why recoveries of chromic oxide of wheat by-product were less than those of wheat, but the recoveries obtained suggest that textural and component differences in the two feeds resulted in degree of separation of chromic oxide of the digesta in the gut that were greater for the more fibrous diet.

The total collection method involving faecal collection over a number of days has been widely employed by research workers on the grounds that it provides a direct and reliable estimate of digestibility. However, it does suffer disadvantages. Faecal collection needs to be made without loss or contamination with urine, hair, or sloughed skin. The faeces collected should originate from the test feed. The method involves a need for specialized equipment and is time consuming and laborious. The metabolic crates employed restrict the pig's movement and are open to criticism on animal welfare grounds. In contrast the chromic oxide procedure is much more economical of time and labour. It obviates the need to measure quantitatively the feed ingested and faeces voided. Kotb and Luckey (1972) have concluded it is not toxic. Its main disadvantages lie in incomplete chromium recoveries that appear to be affected by feed characteristics. This result in a misrepresentation of the true character of a feedsftuff in both absolute and relative terms.

In the second study (chapter 3) the effect of feeding level and liveweight on ADE and ADOM were investigated. The effect of ingesting test feed at 6% or 11% of metabolic liveweight ($W^{0.75}$) had no effect on the digestibility of energy and organic matter either as assessed for the different diets overall (effect of feeding level) or as assessed by type diet (wheat or wheat by-product) or as assessed on pigs of different liveweight (25kg or 90kg). The general nature of the finding is supported by the results of Peers et al. (1977) on barley and Smits et al. (1994) who investigated digestibility in legume seeds, maize by-product, wheat by-product and tapioca, but contrasts with those of Parker and Clawson (1967) and Everts and Smits (1987) who reported diminishing coefficients with increasing feed intake.

The work of Cunningham et al. (1962) suggests that increasing the fibre in diets results in the level of feeding having a considerable effect on digestibility coefficients. In general the digestibility of rations containing less digestible nutrients are influenced by feeding levels to a larger extent (negative effect) than those with more digestible nutrients (Sugimoto, 1985). In the current work we were unable to confirm this relationship.

Roth and Kirchgessner (1985) have shown that increased rate of passage of digesta through the digestive tract as incurred by increasing levels of feeding may decrease digestibility coefficients. In our study, whereas lower digestibility coefficients were established for the wheat by-product over wheat and the commencement of daily faecal voidance occurred within 2 hours of feeding of wheat by-product as opposed to 5 hours in the case of wheat, there was no noticeable effects of feeding level on time of commencement of faecal voidance.

The response obtained in the current study suggests that the pigs irrespective of liveweight were able to digest the larger quantities of wheat based or wheat by-product based diets equally as well as the smaller quantities. Further the impact of endogenous excretions on the digestibility coefficients determined for the feeding levels were insufficiently large to cause significant effects.

Whereas food level had no significant effect on digestibility coefficients, larger pigs (90kg) achieved slightly improved coefficients ($P<0.05$) relative to smaller pigs (20kg). The response was consistent with those of Fernandez and Jorgensen (1986) and Noblet et al. (1994b). The increase in size of coefficients with increasing liveweight has been attributed to the influence of the hindgut to the digestive process. According to Cranwell (1968) the large intestine accounts for about 40% of the gastrointestinal volume and transit time through the large colon (35hrs) accounts for some 80% of the time feed particles spend in the gut. According to Nielsen (1962) the small intestine is almost fully developed at a pig liveweight of 20kg whereas the hindgut is still growing at 150kg pig liveweight. Noblet and Shi (1993) have ascribed the difference in digestibility coefficients between growing pigs and sows to a greater rate of degradation of fibre in the hindgut of the sows. This would appear to be the most widely accepted explanation. Notwithstanding the foregoing, endogenous excretions may be expected to be relatively smaller on a per kg liveweight basis in larger pigs than in smaller pigs and it is uncertain how effective feeding to a metabolic bodyweight basis is in eliminating the endogenous excretion effect of liveweight on digestibility coefficients (Dierick et al., 1990).

The effect of genotype on ADE and ADOM were investigated (chapter 4). The findings of this study showed that the ADE and ADOM was significantly higher for the Kune-Kune pigs than Large White X Landrace with the high fibre diet (wheat by-product based diet). However, the ADE and ADOM were slightly higher for the Large White X Landrace than the Kune-Kune pigs with the low fibre diet (wheat based diet).

Differences in digestibility due to differences in breed have been reported by Kemp et al. (1991) and have been reported for lines of pigs selected for leanness and fatness Wenk and Morel (1985) and Yen et al. (1983). Digestive morphological and physiological differences provide for the most tenable explanations. Longer digestion transit times in the small intestine per unit of intestinal length and/or greater small intestinal length and/or weight per unit of bodyweight may combine to improve digestibility of low fibre feeds, whereas genotypes of greater hindgut development may permit greater digestion of high fibre feeds (Pond et al., 1988).

Kune-Kune pigs are self sufficient through most of the year on pasture and Kune-Kune will fatten well on high fibre diets. The ability to utilize high fibre diets such as pasture and household scraps may lie in the structure of the digestive system and microflora of the Kune-Kune. In the future, studies should focus on the digestive tract especially the hindgut of the animal to discover why/how the Kune-Kune is capable of digesting high fibre diets so efficiently.

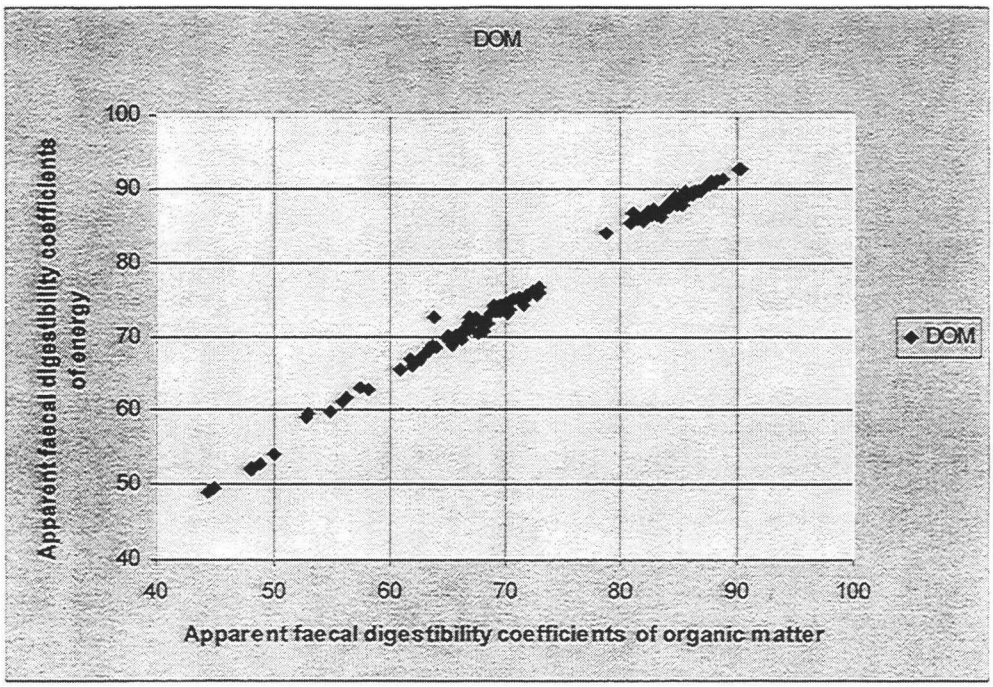


Figure 5.1 Relation between apparent faecal digestibility coefficients of energy and organic matter of experiments 1,2 and 3

The ADOM were significantly correlated to the ADE in each of the experiments 1,2 and 3. These results suggest that the apparent faecal digestibility coefficients of organic matter are a good predictor of apparent faecal digestibility coefficients of energy. The data pertaining to experiment 1 (total collection method and indicator method), experiment 2 (feeding level and liveweight) and experiment 3 (genotype) were combined and subjected to a correlation analysis.

Over all the ADOM was highly correlated to the ADE ($r= 0.99$, $P<0.001$), as shown in Figure 5.1. The apparent faecal digestibility coefficients of energy can be predicted using the following regression equation.

ADE= 7.24 + 0.96 ADOM(%)

$R^2= 0.99$ RSD= 0.07%

Apparent digestibility may be influenced by many factors. The present study investigated the effect of the collection period and method, feeding level and liveweight and genotype on the apparent faecal digestibility coefficients of energy and organic matter. Further studies should be focused on other effects such as sex, humidity and combinations of these factors in relation to the digestive physiology of pigs. Attention should be directed towards the modelling of factors influencing the digestion of energy to improve prediction of apparent energy digestibility.

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