PREDICTION OF THE *IN VIVO* DIGESTIBLE ENERGY VALUE OF BARLEY FOR THE GROWING PIG ON THE BASIS OF PHYSICAL AND CHEMICAL CHARACTERISTICS AND *IN VITRO* DIGESTIBLE ENERGY

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JIAI CHEN

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ABSTRACT

The study aimed to develop statistical relationships to allow the prediction of apparent digetible energy in barley based on simple physical and chemical measures. A second aim was to evaluate a recently developed *in vitro* energy digestibility assay.

Seventeen barley samples representing nine varieties were obtained throughout New Zealand during the 1995 harvest. The samples were subjected to chemical analysis and several physical attributes were determined. Ten barley samples were selected on the basis of their crude protein and fibre contents to cover the range in gross chemical composition and digestible energy contents were determined after sampling faecal contents from 30 kg liveweight pigs, given barley as the sole source of energy. *In vitro* dry matter digestibility of the barley samples was determined using a multi-enzyme assay.

The physical characteristics of the barley samples were variable, especially the level of screenings (ranging from 1 to 11.6%) and to a lesser extent the moisture content (ranging from 12 to 16.2%) and 1000 seed weight. The chemical composition of the barley samples differed with the crude protein content ranging from 7.8 to 11.7%. The mean levels of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ANF) and lignin were 16.4 %, 4.2%, and 1.1%, respectively. Total β-glucan and GI extracted β-glucan contents were also determined with mean values of 4.5% and 1.4%, respectively. The *in vivo* apparent digestibility of energy (DE) ranged from 72.5% to 78.4% with a mean digestibility of 75.8%.

Among the physical and chemical characteristics, only the seeding rate was significantly correlated with *in vivo* energy digestibility ($r = 0.73$, $P < 0.05$). The gross energy (GE) content was significantly correlated with apparent digestible energy content ($r = 0.78$). When the gross energy value of a sample

is known, an approximation of the apparent digestible energy (ADE) content can be made using a simple prediction equation: ADE MJ/kg dry matter = -10.48 + 1.33 GE MJ/kg dry matter.

Repeatability of the *in vitro* digestibility of dry matter (DDM) was high (r =0.68) but the correlation coefficient between *in vivo* DE and *in vitro* DDM for the barley samples (r = 0.29) was not statistically significant. However, when combined with results for several wheat milling by-products, the *in vitro* DDM was significantly ($p<0.01$) correlated to the *in vivo* DE (r = 0.96) indicating that *in vitro* DDM is a good predictor for *in vivo* DE across feedstuffs but not within a feedstuff.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Amino acid
ADE	Apparent digestible energy
CP	Crude protein
Cr	Chromium
Da	Dalton
DDM	Dry matter digestibility
DE	Digestible energy
DEc	Energy Digestibility coefficient
DM	Dry matter
g	Gram
GE	Gross energy
ha	hectare
hl	hectolitre
IU	International unit
kg	kilogram
LW	Live weight
ME	Metabolizable energy
ml	millilitre
mm	millimetre
Mw	molecular weight
NSP	Non-starch polysaccharide

GENERAL INTRODUCTION

Archaeological findings have shown that barley has been cultivated by man since Neolithic times (Nash, 1978). It was the first cultivated cereal to be used for bread-making and as a feed for livestock. Bread made from ground barley was also the principal diet of the 15th Century English country peasants until the increasing availability of other grains displaced barley's popularity as a food grain at least in European societies (Kent, 1983).

Today, although barley is used in the production of alcoholic beverages, such as whisky and beer, the most important uses for barley throughout the world are as a grain feed for livestock and as a food for humankind (Wiebe, 1979). Barley is an important cereal in New Zealand and this dissertation addresses the nutritive value of New Zealand grown barley for the growing pig. The findings, however, are of more general significance.

Barley is a major cereal grain that makes up 46 % of the total area of planted cereals and peas in New Zealand (Table I). Most barley grown in New Zealand is used for the manufacture of stock feed or for malting. As barley is the major source of dietary energy in the New Zealand pig and poultry industries, there is a need to develop a standard system for evaluating the nutritive value of barley grain. From such a system, a rationale for payment based on quality could be developed.

The objective of the current study was to provide data on both the physical and nutritive variation between barley cultivars currently used in the New Zealand pig industry and secondly to provide a system for evaluating future varieties which may be selected specifically as feed grains.

Table I. Land areas sown and yields for cereals and peas (*Pisum sativum*) grown in New Zealand in 1993 (source: New Zealand Official Year-book 1995)

	Area sown			Yield		
Crop	North Island	South Island	Total	North Island	South Island	Total
	hectares	hectares	hectares	tonnes	tonnes	tonnes
Wheat	5686	35175	40861	27661	191753	219414
Oats	1416	12763	14179	3843	52950	56793
Barley	13825	65690	79785	65198	324325	389523
Peas	2041	17622	19663	6949	56319	63268
Maize	15540	385	15925	129394	3675	133069

Data on the physical attributes and gross chemical compositions of seventeen barley samples, representing nine varieties obtained from six cereal growing regions in New Zealand, were collected. Both North and South Island samples were obtained as well as early and late harvested grains. Digestible energy contents were determined on ten barley samples, which were selected to provide as wide a range of chemical parameters as possible.

Due to the fact that *in vivo* methods to measure the digestibility of energy are time-consuming and expensive, several rapid and much cheaper *in vitro* methods have been developed over the last decade. Boisen and Femandez (1991) described a three-step *in vitro* method which attempted to simulate gastric and intestinal digestion in pigs. In this method, samples of feed, ground to pass through a 1 mm screen, were incubated for 2 hours with a commercial pepsin preparation in acid solution followed by 4 hours of incubation with pancreatin solution. Then the undigested material was incubated for 18 hours with Viscozyme, which is a multi-enzyme complex containing a wide range of carbohydrases, including cellulase, hemicellulase, arabinase, xylanase and pectinase (Boisen and Eggum, 1991). Results indicate that the *in vitro* dry matter digestibility values closely correlate to *in vivo* faecal energy digestibility. The opportunity was taken in this study to further evaluate the *in vitro* method developed by Boisen and Femandez (1991). The evaluation of the *in vitro* method used was based on both the degree of relationship between *in vitro* and *in vivo* results from common samples and the repeatability of the *in vitro* analysis results. In addition, the results obtained on the ten barley samples were combined with data obtained previously, on other feed industry products, to further examine the reliability of the *in vitro* method.

CHAPTER 1

FEED EVALUATION AND THE NUTRITIVE VALUE OF BARLEY: A REVIEW

1. 1 Introduction

Barley is an excellent grain for pig feeding and produces pork of high quality, the fat being hard and firm (Morrison, 1957). It is an important source of energy and protein for the growing pig.Therefore, it is important to evaluate the nutritive value of barley.

There are many factors affecting the nutritive value of a feed, such as chemical composition and the digestibility of nutrients. Also the physiological status of the animal can influence the digestibility of nutrients in feeds. In this section the chemical composition of feeds and the evaluation of digestibility of nutrients in feeds will be discussed followed by an overview of digestion in the pig and the evaluation of energy and protein values in feeds. The general and nutritive characteristics of barley are also reviewed.

1. 2 Feed evaluation in the pig

A proper feed evaluation system must generate information about the requirement of the animal for the nutrient in question and the ability of the feed, or combination of dietary ingredients, to meet this requirement. In order to meet the animal's nutrient requirements and to maximise animal production or obtain a uniform quality product at least cost, it is important to estimate the nutritive value of feedstuffs to allow formulation of appropriate diets. The nutritive value of feedstuffs

depends on many factors, but two important ones are: (1) chemical composition and (2) nutrient digestibility or availability.

1. 2. 1 The chemical composition of feeds

Animals eat food to grow or to maintain their body mass. In addition to the adult animal maintaining its body mass, it may grow hair, produce eggs or sperm or secrete milk. The chemical compounds that make up the animal body and the energy used to fuel body processes are ultimately derived from the diet. It is pertinent, therefore, to examine the chemical composition of different feedstuffs.

In the study of nutrition, a nutrient is defined as any chemical element or compound in the diet that supports normal reproduction, growth, lactation, or maintenance of life processes (Pond *et al.,* 1995). The nutrients necessary for life are water, amino acids, carbohydrates, lipids, vitamins, and inorganic elements (minerals). Water is the only nutrient that consists of one uniform entity. Of the other nutrients, which make up the dry matter of the diet, none has a single homologous chemical composition. The composition of feeds can be determined by chemical analysis, which gives the amount or percentage of each substance. Also the energy content can be determined by combusting a sample in a bomb calorimeter.

The sources of energy in feeds are mainly carbohydrates, fats and proteins. In addition, protein, minerals and to some extent lipid are body structural materials. It is obvious that the quantity of carbohydrate, fat, and protein are helpful in measuring the usefulness of a feed. Unless a ration contains a certain minimum amount of each of these nutrients, the animal cannot be adequately fed.

To evaluate the nutritive value of a feedstuff, it is most desirable to have determined the common nutrients in animal feeds, such as gross energy, crude protein, crude fibre, crude ash, ether extract, calcium, phosphorus, etc. Also, it is helpful to have determined other nutrients such as various amino acids, fatty acids, vitamins and so on.

The simple approach used to determine the common nutrients in animal feeds is called proximate analysis or the Weende system. The different fractions that result from the proximate analysis include water, crude protein, ether extract, crude fibre, ash and nitrogen-free extract. Nitrogen-free extract is not determined directly but is obtained mathematically by deducting the sum of the other determined components of a product from 100%.

The analytical techniques used in proximate analysis are straightforward but not exact and do not provide the degree of detail which may be required. For this reason a number of other analytical procedures have been developed and are commonplace. These include the detailed analysis of the amino acid composition of proteins, the fatty acid composition of lipids, determination of the total energy content of feeds and the fractionation of the carbohydrate component of feeds. In the Weende system, carbohydrates are divided into crude fibre and nitrogen-free extract substances. Attempts have been made to replace these two terms with techniques that measure more nutritionally defined entities. Most effort has been directed towards more appropriate techniques (Robertson and Van Soest, 1981) for determining the fibre content, such as neutral-detergent fibre and acid-detergent fibre.

1. 2. 2 The digestibility of nutrients in feeds

Since the pig converts a variety of foods into meat for human consumption, the efficiency with which it carries out this conversion is of crucial importance to the

pig industry. For a diet to be useful to the pig, its components must be digestible and so the digestibility of nutrients in feedstuffs is of great economic importance. Because chemical analysis does not provide information on the availability of nutrients from feeds digestibility data are used extensively in animal nutrition to evaluate feedstuffs. The word digestibility, as used in animal nutrition, denotes the percentage of the feed or of any single nutrient in the feed which is released in the digestive tract so that it can be absorbed and thus put at the disposal of the body cells. Generally, digestibility measurements are reported in terms of 'apparent digestibility', since it is difficult to derive appropriate corrections for the amount of digestive secretions and other waste products (endogenous material) which are irretrievably mixed up with the undigested feed residues. According to Batterham (1992) there are considerable differences in what the term 'availability' means to nutritionists. For some, digestibility and availability are synonymous. This arises from the belief that if a nutrient is digested, it is available for use. Whilst this might apply in some areas of nutrition, it is inappropriate in the amino acid field, where it is possible for amino acids to be absorbed in forms that may be inefficiently utilised. A strict distinction is made between the terms 'digestibility' and 'availability'. Availability of an amino acid may be defined as the proportion of the total amino acid that is digested and absorbed in a form suitable for protein synthesis (Batterham, 1992).

Chemical analysis is the starting point for determining the nutritive value of feeds, but the value of a feedstuff does not depend entirely upon the amounts of the several nutrients it contains. The value of a feed also depends upon the amounts of these nutrients that the animal can digest, absorb and use. The chemical composition of any feedstuff is an imperfect standard to judge its nutritive value. The more important criterion is digestibility, since undigested nutrients do not enter the body at all. The composition alone does not determine the value of feed, but rather the value depends upon its composition, digestibility, and other factors. It is generally recognised that feedstuffs are not completely digested, that each contains some material which is not retained and utilised in the body. Only

that portion which is soluble or is rendered soluble by hydrolysis or some other chemical or physical change can be taken up into the circulation and assist in supplying the animal body with material for building and repair of tissue or to supply the energy necessary for body functions. The next step after chemical analysis is, therefore, to determine as accurately as possible what percentage of each nutrient is digested (Schneider and Flatt, 1975).

1. 2. 3 Digestion in the pig

Nutrients in the feed eaten by an animal must be absorbed and then used by the animal for its metabolism. However, large complex molecules in the feed cannot be absorbed through the gut wall and must be broken down into simple compounds. The process of splitting complex compounds into small compounds for absorption is called digestion (Pond *et al.*, 1995).

Digestion depends on (1) the enzymes present, their activities and the physiological environment in which they function; (2) the properties of the feeds which are being processed, including their susceptibility to enzymatic hydrolysis and the action of inhibitory substances which the feeds may contain; and (3) the total processing capacity of the animal's digestive tract. The process of digestion involves chemical and physical processes and involves microbial as well as the animal's own enzymes. The end products of digestion can (1) be absorbed into the body , (2) be volatilized as gases and released via the mouth or anus, (3) appear in the faeces. The overall function of the various digestive processes is to reduce food to a molecular size or solubility that allows absorption and cellular utilisation of the individual nutrients released during the process. Absorption consists of the processes that result in the passage of small molecules from the lumen of the gastrointestinal tract through the mucosal cells lining the surface of the lumen and into the blood or lymph systems.

1. 2. 3. 1 Morphology of the digestive tract

The digestive tract consists of the mouth, oesophagus, stomach, small intestine and large intestine. Connected to the tract are two major glands, the liver and pancreas.

The mouth region, which contains tongue, lips, cheeks and teeth, is adapted to procuring food, physically reducing the food size and secreting saliva via the salivary gland. The teeth include incisors for cutting feed and molars which grind feed into smaller particles. Saliva, which is secreted from the parotid, submaxillary, and sublingual glands under the control of the autonomic nervous system, serves to moisten the feed and lubricate the oesophagus to ensure easy swallowing of the food bolus. Saliva contains water, mucus and a digestive enzyme called salivary amylase. The consistency of mucus varies depending on the diet fed. Food remains in the mouth for a short time only, passing into the oesophagus. The oesophagus forms the first part of the tubular digestive system. The lumen surface consists of a stratified squamous epithelium, beneath which are numerous tubuloacinar mucus glands lubricating the food bolus on its passage to the stomach.

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

The small intestine can be considered in three parts: duodenum, jejunum and ileum. The central jejunum accounts for 80 - 90 % of the small intestinal length (Longland, 1991), the remainder being approximately equally divided between the cranial duodenum and the caudal ileum. 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 the digestion by host enzymes and the absorption of end products occurs 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. There is a microflora throughout the small intestine which becomes progressively more profuse towards the large intestine.

The large intestine, which begins at the junction of the ileum and colon, consists of a short, blind-ended caecum which continues into the colon at the point of ileal attachment. 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.

Undigested food and material from the animal's body (endogenous loss) pass into the large intestine from the ileum. The large intestine contains a dense population of micro-organisms (especially bacteria). The majority of microbial fermentation occurs in the large intestine, the end products of which are absorbed across the mucosa. As the material passes through the large intestine, water is absorbed and faeces are formed and eventually voided via the anus. Faeces consist largely of water, bacteria, some undigested food material and endogenous material.

1. 2. 3. 2 Digestive enzymes and nutrient digestion

The main function of the digestive system is the reduction of complex feeds by hydrolysis to component water-soluble units in preparation for absorption and subsequent metabolism. This is achieved by both enzymatic and non-enzymatic cleavage but it is the enzymatic processes which determine the ultimate extent of

hydrolysis of any constituent. Enzymes in the digestive tract are produced both by the host animal itself and by its resident microflora.

Digestion is concerned with the breakdown of a multiplicity of food constituents but this review will concentrate on just carbohydrate, protein and fat.

1. 2. 3. 2. 1 Digestion of carbohydrates

Plant carbohydrates are the predominant component for non-ruminant feeds, generally contributing over 70% of the dry matter (Graham, 1991). They are a complex group of compounds which differ considerably in chemical structure and physical activity. Carbohydrates in animal feeds can generally be classified as storage carbohydrates, e.g., starch (or glycogen) and certain disaccharides which can be hydrolysed by the host's enzymes to their constituent monomers, and the non-starch polysaccharides (NSP) of plant cell walls, (e.g. cellulose, hemicellulose and pectins). Starch, which is the main component of cereal grains and is usually the primary energy source for pigs and poultry, 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 NSP's are closely associated with lignin with which they form the dietary fibre complex. NSP's cannot be digested by the porcine enzymes and must be fermented by the gut microflora, which yields volatile fatty acids (VFA). In addition to these components, animal feeds may contain variable, but usually low amounts of free sugars and oligosacccharides, which may be absorbed directly or be fermented (Longland 1991).

The primary function of carbohydrates in animal nutrition is to serve as a source of energy for normal life processes. The digestion of carbohydrates begins in the mouth during chewing, through the action of α-amylase secreted by the

salivary glands. The α-amylase hydrolyses many of the α 1-4 glycosidic linkages of starch and glycogen to yield a mixture of dextrins, maltose and maltotriose.

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

The main site of starch digestion 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 products of the Brunner's glands. The digestion of starch, glycogen and other digestible polysaccharides to glucose continues in the small intestine under the action of pancreatic amylase. The action of 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 (especially maltose) compared 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. Maltose is finally hydrolysed by maltase to give two molecules of glucose. Disaccharides like lactose and sucrose are hydrolysed to monosaccharides by enzymes (such as lactase and sucrase) located in the outer border of the epithelial cells lining the small intestine, the brush border. Lactose is hydrolysed to glucose and galactose by lactase. Sucrose is hydrolysed to glucose and fructose by sucrase.

The animal's enzymes are unable to hydrolyse NSP. However, NSP may, together with any starch or sugars which escaped digestion by the enzymes, be fermented by the gut microflora. Significant numbers of micro-organisms are found at different locations throughout the pig's digestive tract, with a particularly high population in the large intestine, and are active in degrading and metabolising carbohydrates. It is known that the micro-organisms present degrade the carbohydrates by way of extra-cellular and wall-bound enzymes. Extracellular microbial enzymes break down the carbohydrates to simple sugars. These are subsequently absorbed by the bacteria and metabolised further. The end-products of this metabolism are short chain fatty acids (such as acetic acid, propionic acid, butyric acid, lactic acid), methane, carbon dioxide and hydrogen. Traditionally, fermentation of NSP has been considered to be a post-ileal activity of the indigenous microflora. There is increasing evidence, however, that some NSP is at least partly degraded anterior to the large intestine in the pig. NSP's make up only a small proportion of the potentially degradable material entering the hind-gut. According to Graham (1991) the energy value to the pig of microbial fermentation of carbohydrates is about 70% of that from glucose absorbed as such in the fore-gut. This is presumably the case irrespective of whether this fermentation occurs in the fore-gut or the hind-gut.

The upper or proximal section of the small intestine has the greatest capacity to absorb monosaccharides. The lower or distal small intestine (lower ileum) absorbs less, and the stomach and large intestine absorb little if any sugars. Most of the dietary carbohydrate is actively absorbed by the epithelial cells of the small intestine as monosaccharide (such as glucose, fructose and galactose), while traces of disaccharides, especially those not hydrolysed by the mucosa, are absorbed from the gut lumen. Different monosaccharides are absorbed from the lumen of the gut at different rates. At equal concentrations, galactose, glucose, fructose, mannose and xylose are absorbed in decreasing order of magnitude (Kidder and Manners, 1978).

1. 2. 3. 2. 2 Digestion of protein

After being ingested by the animal, dietary protein becomes progressively mixed with endogenous proteins and the total is subjected to digestive breakdown in the upper alimentary tract. The hydrolysis of protein is initiated in the stomach by the action of pepsin and hydrochloric acid. (Low, 1990). When protein enters the stomach, it stimulates the secretion of the hormone gastrin, which in tum stimulates the secretion of HCl by the parietal cells of the gastric glands, and pepsinogen by the chief cells. The gastric juice has a pH of between 1.5 and 2.5 and this causes globular protein to undergo denaturation or unfolding. The internal peptide bonds in the denatured protein are more accessible to the digestive enzymes.

Pepsin is secreted in inactive precursor forms (pepsinogens), which are converted to active pepsin by the enzymatic action of pepsin itself or HCl. The hydrolysis of pig pepsinogens to pepsins occurs in acid conditions, slowly at pH 4 and rapidly at pH 2 (Taylor, 1962), the pepsin produced catalysing the activation, so that the process is autocatalytic.

The pepsins each have two pH optima, one near 2, and the other about 3.5, and activity declines above pH 3.6 with no activity above pH 6 (Taylor, 1959). Pepsin only splits peptide bonds involving aromatic amino acids (tyrosine, phenylalanine, tryptophan). 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.

Proteins, peptides and possibly free amino acids passing from the stomach enter the duodenum whereby they are mixed with pancreatic and duodenal secretions, and bile. All these secretions are alkaline, with the result that the pH rises progressively, reaching nearly 7 by the end of the small intestine. The increased pH makes the pepsins inactive and the hydrolysis of protein and peptides is taken over by the proteolytic enzymes secreted by the pancreas and

intestinal brush border (Fruton, 1971). The proteolytic enzymes involved in intestinal digestion can be divided into three groups: the endopeptidases, the exopeptidases and the aminopeptidases.

The endopeptidases and exopeptidases are secreted by the pancreas, the former hydrolysing the protein at centrally located peptide bonds, the latter cleaving only the terminal bonds of proteins or peptides. The major endopeptidases of the pancreas are trypsin, the chymotrypsins and the elastases; the major exopeptidases are the carboxypeptidases A and B.

All pancreatic proteolytic enzymes are secreted into the duodenum as inactive precursors, or "zymogens". Enterokinase secreted by the duodenal mucosa activates trypsinogen to trypsin. The new formed trypsin then autocatalyses the production of more trypsin and also catalyzes the activation of all other pancreatic enzymes. Trypsin has the function of hydrolysing peptide bonds whose carboxyl groups are contributed by lysine and arginine residues. Chymotrypsinogen A, B, and C are converted by trypsin to active chymotrypsin A, B, and C. Chymotrypsin A is the most specific enzyme and it hydrolyses only phenylalanine, tryptophan and tyrosine bonds. Chymotrypsin B hydrolyses these bonds and also those formed with leucine. Chymotrypsin C is the least specific and splits all the bonds hydrolysed by chymotrypsins A and B and in addition bonds formed with glutamine and methionine. Elastase is secreted as pro-elastase and is activated to elastase by trypsin. Carboxypeptidases A and B catalyse the hydrolysis of peptide bonds adjacent to the terminal carboxyl group of proteins and peptides. The procarboxypeptidases are activated by trypsin.

Aminopeptidases are produced by the small intestinal mucosa and are located both in the brush-border membrane and within the cytoplasm of intestinal cells. The enzymes associated with the brush border can hydrolyse longer peptides, whereas cytoplasmic enzymes generally hydrolyse di- and tri-peptides.

Hydrolysis by the pancreatic proteases trypsin and chymotrypsin and the peptidases (carboxypeptidases A and B) reduces the size of the peptides to a chain comprising two or three amino acids. Free amino acids or small peptides, released by the digestive enzymes, are absorbed anterior to the end of the small intestine and enter the portal blood circulation. At the same time it is recognised that there are substantial endogenous inputs of amino acids, peptides and proteins into the digestive tract in the form of, for example, shed epithelial cells, enzymes, plasma protein and mucin. In addition it is now clear that the mixture of proteins found throughout the digestive tract includes a substantial component of bacteria, especially at the end of small intestine (Low, 1990).

The absorption rate of amino acids is different for the different parts of the small intestine. Although the ileum has considerable digestive and absorptive capacity, the absorption of dietary amino acids occurs mainly in the proximal jejunum (Grimble and Silk, 1989). The rates of absorption of various amino acids are different and depend on the concentration of amino acids in the intestine. Certain amino acids compete for the same absorption site.

At the terminal ileum there will be an amount of protein which has remained undigested and peptides and free amino acids which have not been absorbed. These along with other undigested dietary components will pass into the large intestine whereby they are subjected to a wide variety of bacterial metabolic processes. Some protein, peptides and free amino acids may escape breakdown in the hindgut and be excreted in the faeces. Although a considerable proportion of the nitrogenous material entering the hindgut will be metabolised by the microflora, no nutritionally significant absorption of peptides or amino acids occurs in this region. The bacterial flora hydrolyse the nitrogenous compounds and most of the nitrogen is absorbed as ammonia, amines or amides, which under normal circumstances are of no nutritional value to the host animal (Zebrowska, 1973; Low and Zebrowska, 1989).

1.2.3.2.3 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.

The hydrolysis of fats in the small intestine is catalysed 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 splits triacylglycerols into monoacylglycerols and fatty acids, (2) carboxylic ester hydrolase, which splits carboxylic esters, and (3) phospholipase A_2, which hydrolyses fatty acids in the 2-position of glycerophospholipid. The activity of lipase is enhanced by the presence of bile salts. Bile salts also promote the absorption of long-chain fatty acids and monoacylglycerols, due to their solubilizing effect, which increases the transport across the unstirred water layer between the gut lumen and brush border (Friedman and Nylund, 1980).

In the presence of bile salts, the end products of fat digestion (i.e. the monoglycerides, free fatty acids, lysolecithin, free sterols and sterol ester), pass into micellar solution which is absorbed by the mucosa to enter the lymphatic system. Fat absorption occurs mainly in the jejunum. The digestion and absorption of fats depend on their ability to form micelles, which are large molecular aggregates consisting of monocylglycerols, long-chain fatty acids, bile salts and phosphoacylglycerols, in the intestinal lumen. This in turn is affected by a number of factors such as chain length of the fatty acids, the degree of unsaturation, the positioning of fatty acids in the triglyceride molecules, the relative concentrations of free and esterified fatty acids, rate of passage of digesta (which is influenced by other factors such as level and type of dietary fibre), age of the animal and feeding method.

Generally, there is a decrease in the digestibility of fats as chain length increases. Unsaturated fatty acids are better utilised than saturated ones. However, the ability of long chain saturated fatty acids to form micelles with bile increases in the presence of unsaturated fatty acids. A synergistic effect is observed, and there are practical benefits from blending fats from different sources. The ratio of unsaturated to saturated fatty acids gives some indication of the likely digestibility of dietary fat. If the ratio is greater than 1.5, digestibility may be relatively high (85 - 95%). If on the other hand, the ratio is 1.0 to 1.3, overall digestibility will be substantially lower (35 - 75%) (Gurr *et al.*, 1989).

1. 2. 3. 3 Factors influencing digestibility *in vivo*

The digestibility of feedstuffs is affected by a number of factors such as the age and physiological status of the animal, and the food itself.

Digestive enzymatic activities develop with age. New-born animals are adapted to utilise highly-digestible milk. As they grow and develop and change their dietary habit, there are accompanying changes in digestive enzymes. The rate of change in respective enzyme activities occurs particularly rapidly when the young animal starts to ingest solid food. For example, the neonatal piglet has very low levels of pepsin, which increase slowly during the first two weeks of life, and then rapidly thereafter (Braude,*et al.*, 1958). Pancreatic α-amylase activity is very low at birth but increases rapidly with age. The activity of pancreatic α-amylase can increase 9-fold in the first week of life and 20-fold by eight weeks. Values for adult pigs are about 45 times the neonatal level (Longland, 1991).

The effect of diet on both the level of digestive enzyme activities and on the amounts of digestive tract secretions can be quite marked. For example, Zebrowska *et al.* (1983) found that the secretion of gastric juice by pigs on a barley-soya diet was approximately double that for pigs fed a starch-casein diet.

Generally, an increase in the amount of dietary protein induces an increased secretion of pancreatic proteolytic enzymes, while an increase in starch or lipid intake induces increased secretions of amylase and lipase respectively. Also, anti-nutritional factors (ANF) and dietary fibre affect enzyme secretion. Several specific responses of ANF on digestibility are known. For example, trypsin inhibitors increase the pancreatic secretion of trypsin and other enzymes, while tannins may induce a large increase in specific proline-rich proteins in the saliva. These proteins have a very high affinity for tannins and are assumed to play a role in reducing the adverse effect of tannins on protein digestibility. It is assumed that tannins form complexes not only with protein (feed and enzymes) but also with carbohydrates. Due to the formation of these complexes, the digestibilities of feed protein and carbohydrates are decreased and enzymes may be inactivated. Lectins have an affinity for binding to sugars present in glycoproteins in the glycocalix of the gutwall. As a result of lectin binding to the glycocalix of the gutwall, cells can be disrupted and the gutwall can be severely damaged. The damaged gutwall can limit the absorption of nutrients. The main effect of ANF on digestibility is probably an increased loss of endogenous protein rather than a negative effect on the digestibility of exogenous protein *per se*.

Dietary fibre can influence digestion in several ways, depending on the nature of the fibre. From a chemical point of view dietary fibre is defined as the sum of non-starch polysaccharides (NSP) and Klason lignin (Graham *et al.*, 1991). Not only is fibre not degraded by mammalian enzymes but it can also reduce the apparent digestibility of other dietary nutrients such as crude protein and ether extract (Noblet and Shi, 1993). Because fibre presents a barrier between protein- and starch- hydrolysis, enzymes arising from digestive secretions of monogastric animals and their substrates, so dietary fibre can reduce enzyme activity in the lumen. The digestibility of nutrients consistently decreases with increasing fibre content in the diet. It has been reported that for each 1% increase in the crude fibre content, the content of starch and sugars in the nitrogen free extract fraction decreased by 2.1 - 2.7%, resulting in a decreased ileal digestibility of gross energy

(Femandez and Jorgensen, 1986). It has also been reported that increasing levels of fibre (> 6 - 7% of the diet) reduced voluntary feed intake of pigs (Drochner, 1991). Furthermore, fibre will in general stimulate microbial activity in the digestive tract significantly and reduce transit time of the digesta (Boisen and Eggum, 1991).

NSPs in cereals consist predominantly of β-glucans and arabinoxylans with small amounts of cellulose and polysaccharides containing galactose, mannose and glucose (Fincher and Stone, 1986). In barley, a major cell wall polysaccharide is soluble β-glucan which is a linear polymer of glucose characterised by β-(1-3) and β-(1-4) glycosidic links (Annison, 1993). The anti-nutritive activity of barley β-glucan for chicks is associated with an inhibition of starch and nitrogen digestibility (Hesselman and Aman 1986), fat absorption (Classen *et al.* 1985) and energy digestibility (Rotter *et al.*, 1989a).

1. 2. 4 Evaluation of energy and protein values in feeds

Although a wide range of nutrients are present, feed evaluation for pigs is mainly based on an assessment of the energy and protein (with essential amino acids) components, which are the two major quantitative dietary components, while other nutrients, including minerals, trace elements and vitamins, are found in low amounts.

1. 2. 4. 1 Energy evaluation

It is generally assumed that the extent to which animals convert feed into usable products is primarily dependent upon the efficiency of dietary energy utilisation. It is important, therefore, to be able to precisely estimate the energy value of feedstuffs.

1. 2. 4. 1. 1 Energy and energy evaluation *in vivo*

Energy, defined as the ability to perform work, is an abstraction that can be measured only with reference to standard conditions. The animal derives energy by partial or complete oxidation of molecules ingested and absorbed from the diet or from the metabolism of energy stored in the form of fat, protein, or glycogen. Energy transfer from one chemical reaction to another occurs primarily by means of high energy bonds found in such compounds as ATP (adenosine triphosphate) and other related compounds. All animal functions and biochemical processes require a source of energy. This applies to all life processes and animal activities such as walking, chewing, digestion, maintenance of body temperature, maintenance of ion gradient, hepatic synthesis of glucose, absorption from the gastrointestinal tract, storage of glycogen or fat, or protein synthesis (Pond *et al.*, 1995).

Several factors are involved in defining the value of dietary energy to an animal, including diet composition, the animal's sex, genotype and physiological state, and the external environment. When evaluating the energy value of different feedstuffs it is important, therefore, to understand the biology behind energy utilisation in the animal. The different steps of energy utilisation are given in Figure 1. 1.

Gross energy (GE)

Gross energy is the amount of heat, measured in calories or joules, that is released when a substance is completely oxidised in a bomb calorimeter under 25 to 30 atmospheres of oxygen. GE is a poor guide as to the nutritional value of a feed as it tells nothing about the availability of the energy to the animal.

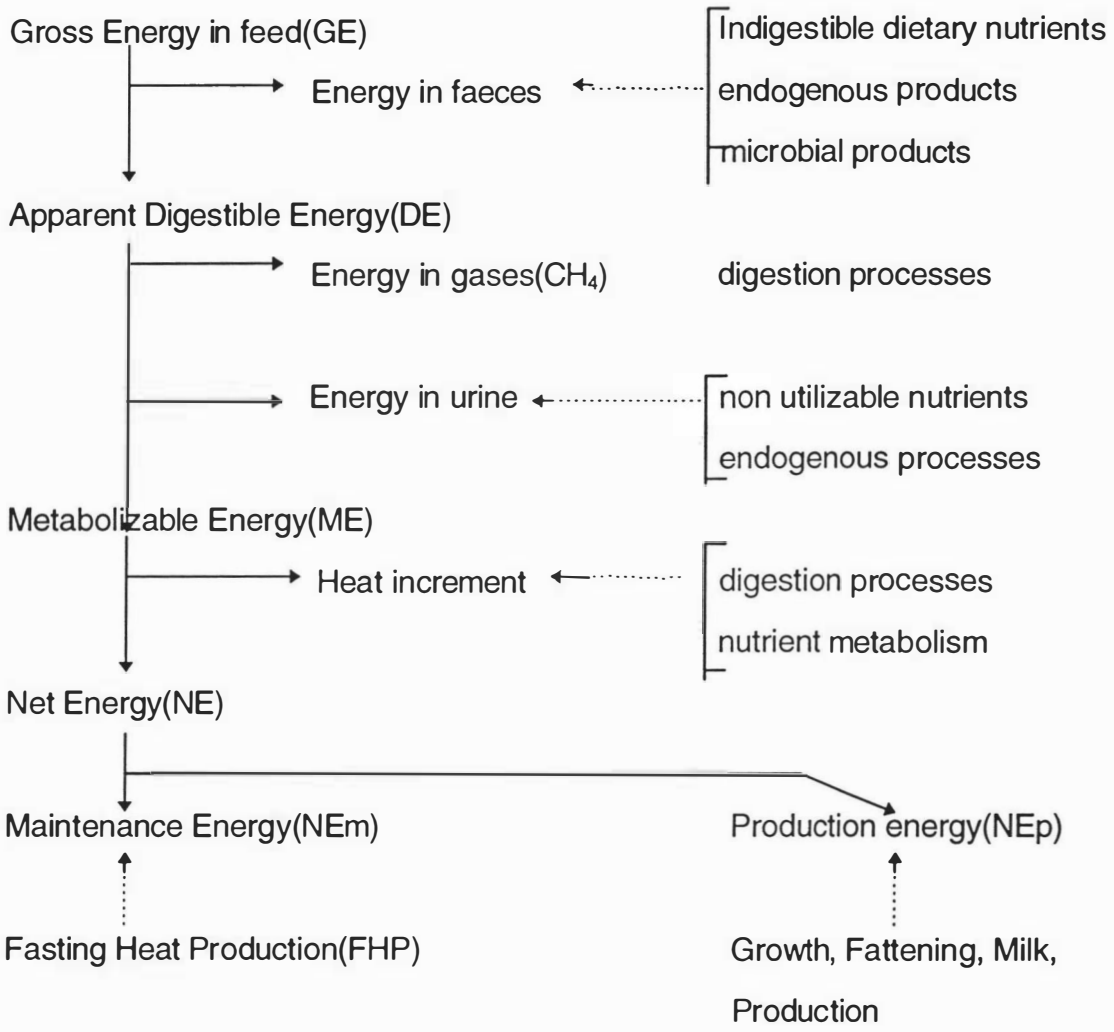

Figure 1.1 Energy utilisation in pigs.(Adapted from Noblet and Henry, 1991)

Digestible energy (DE)

Digestible energy (DE) is defined as the GE of the feed consumed minus the energy in the faeces. The DE value of a feed for pigs is simply measured in a digestibility trial by total collection of faeces or by using feed markers along with faecal sampling. In fact, DE is not a true measure of the feed energy absorbed from the digestive tract since faeces contain endogenous material (i.e., digestive secretions and intestinal cell debris). Furthermore, small amounts of various gases

and heat from fermentation processes are produced but not usually measured and then considered as digested energy. Accordingly, true digestible energy (which is seldom measured) is the intake of gross energy minus faecal energy of food origin (i.e. faecal energy minus endogenous and metabolic sources of faecal energy), heat of fermentation and digestive gaseous losses. The DE concept used in practice, however, is the apparent digestible energy (ADE) measure. Despite the shortcomings (time-consuming and expensive) of the traditional methods for determining DE, the DE system is still a commonly used measure for the energy value of pig feeds (ARC, 1981) and has the advantage of being independent of genotype and environment when similar feeding levels are used (Noblet *et al.*, 1985).

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 with DE values is that they are additive. Tabulated values are usually determined directly from digestibility trials. This method is feasible for routinely assessing limited numbers of mixed diets. However, on a large number of samples, the approach is time consuming and costly. Consequently, alternative indirect approaches have been proposed.

For raw materials, there have been two main indirect approaches adopted (Noblet and Henry, 1991). Theoretically, the DE value of a compound diet can be obtained by adding the DE value contributions of ingredients and assuming that DE is additive (i.e., energy contribution per unit of feed is constant and independent of the other components of the diets). However, in many circumstances, the ingredient composition is unknown and consequently methods for predicting DE are then required.

An alternative approach is to predict the energy content of diets based on their crude chemical compositions number of investigations have been conducted to define the relationship between chemical composition and energy content. As a

result, a considerable number of prediction equations have been developed (for example Morgan *et al.*, 1975; Wiseman and Cole, 1980; Just *et al.*, 1984; Morgan *et al.*, 1987). These relationships have shown that: (1) Crude protein, fat, and nitrogen-free extract (or starch and sugars) contribute positively to the DE and ME content of feeds. (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 equations: (1) those that account for the main chemical fractions that contribute towards the energy content of a diet; and (2) those that have a constant term and include one or more modifiers of this term. The former are 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, which is 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.

Metabolizable energy

The metabolizable energy (ME) content corresponds to the difference between DE content and energy losses in urine and gases. Most of the energy losses in gas are due to methane production. The measurement of methane production requires the animal to be housed in a respiration chamber. Usually, the losses of energy through methane are very low and are ignored. Accordingly, ME in the pig is simply determined by subtracting the energy loss in urine from the determined DE. 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) Therefore, for ME determination, it is necessary to standardise the level of nitrogen retention, either for optimum protein utilisation or for zero nitrogen balance. At a given physiological stage where the

amount of nitrogen retained in the body is stable, the urinary nitrogen will mainly depend on the amount of digestible protein and therefore on the crude protein content of the diet. Consequently, the ME/DE ratio is linearly related to dietary protein content. In most situations, the ME/DE ratio is considered relatively constant. With a balanced diet, ME represents a rather fixed proportion of DE of around 0.95 (Henry *et al.*, 1988). In single feedstuffs, however, the ME/DE ration is inversely related to protein level.

The indirect approach is to predict ME using regression equations. Tabular ME values for raw materials are usually calculated from DE values with the ME/DE ratio either constant or, preferably, related to the protein content of the diet (Noblet and Henry, 1991). ME may also be predicted from equations relating ME to digestible nutrient contents (Just, 1982).

Similar to DE, the ME value of compound diets can be predicted based on chemical composition (Noblet and Perez, 1993). In this case, the main difference between the corresponding equations for DE and ME concerns the coefficient obtained for CP, which is lower in the ME equations. The limitations concerning the DE equations also apply to ME equations.

Net energy

Net energy (NE) is defined as ME minus the heat increment (HI) associated with metabolic utilisation of ME and also the energy cost of ingestion and digestion of the feed. The NE is thus the energy available for maintenance (NE_m) or for production (NE_p).Theoretically, NE represents the best estimate of a feedstuff's "true" energy value to the animal as it is related to the value of different diets for the production in question.

However, determinations of net energy are both costly and complicated. In addition, the NE for any given diet cannot be accurately obtained using tabulated

values of the NE for individual feedstuffs making up that diet because the proportion of ME utilised as NE (i.e. NE/ME) depends on the interaction of two principal factors, these being the purpose for which compounds are used by the animal, and the nature of the chemical compounds (such as fat, carbohydrates and protein) which supply the ME. The ratio between NE for maintenance (NE_m) and ME for maintenance (ME_m) corresponds to the efficiency of utilisation of ME for maintenance (k_m). When the ME intake is higher than ME requirement for maintenance, a proportion of the additional energy supply (ME for production: ME_p) is retained in the body as protein or fat or exported as milk (NE_p); the ration NE_p:ME_p corresponds to the efficiency of utilisation of ME for growth (k_g) or milk production (k_l). During growth, energy gain includes protein and fat energy; the efficiency of utilisation of ME for energy gain as protein or as fat are defined as k_p and k_f, respectively. The numerical values of these efficiency values differ. It is clear, therefore, that the same feedstuff will have a different NE value according to its final utilisation. Moreover, the k value for a particular process will differ depending on what form the energy is supplied in. Efficiency of fat deposition (k_f) has been shown to range from 70 to 85% and 98% when ME is provided by protein, carbohydrate (as glucose) and fat, respectively (Armstrong, 1969). Finally, the efficiency of utilisation of ME will be affected by the climatic environment since the HI of feeding is partly used for thermoregulatory purposes. Therefore, the net energy value of feeds is usually calculated from prediction equations. There have been two types of prediction equations developed for assessing the NE content of diets. They are based on the prediction of NE for fattening or for growth of pigs.

Several comprehensive reviews of the various prediction equations used by different countries to predict the DE, ME or NE content of pig diets were undertaken by Morgan and Whitemore (1982), Henry *et al.* (1988), and Noblet and Henry (1991).

1. 2. 4. 1 .2 Prediction of Energy value by *in vitro* methods

Due to the fact that traditional feed evaluation is time-consuming and costly, efforts have been made to develop rapid, feasible and accurate *in vitro* methods for feed evaluation as alternatives to *in vivo* trials.

In vitro techniques with rumen fluid or semi-purified enzyme preparations, or both, have been routinely used for the evaluation of ruminant feeds (Osboum and Terry, 1977). In recent years, such methods have been proposed for the nutritive evaluation of compound feeds and ingredients for monogastric animals. Furuya *et al.* (1979) developed a two-stage method using pepsin and pig intestinal fluid to estimate the digestibility of dry matter (DDM) and crude protein (DCP) of pig diets. The system attempts to simulate gastric and intestinal digestion in swine and other monogastric animals. Samples of diets, ground to pass through a 1 mm screen, were incubated for 4 hr with a commercial pepsin preparation in acid solution followed by 4 hr of incubation with prepared 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.

Since this method only assesses stomach and small intestinal digestion, a further modification might be required for fibrous foods. Several authors (Clunies *et al.*, 1984; Lowgren *et al.*, 1989) have conducted further studies to examine the method and its possible application to predict digestion in the small intestine and large intestine of pigs, respectively. The results indicated that the correlations between *in vitro* and *in vivo* were high (r=0.99, 0.93 for dry matter and crude protein digestibility, respectively). However, this method requires a surgically-prepared animal. Dierick *et al.* (1985) found that pancreatin fluid can replace the jejunal fluid, thus providing an animal-independent method. Babinszky *et al.* (1990) used commercially available enzymes for prediction of the *in vivo* digestible crude protein content and dry matter. The results showed that the correlation coefficients 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 can be used to predict the energy digestibility for individual feedstuffs based on the *in vitro* dry matter or organic matter digestibility. 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).

1. 2. 4. 2 Evaluation of protein

The evaluation of feed proteins for pigs requires two successive steps to be considered: (1) the gross chemical composition in terms of nitrogen and amino acids of feed protein by reference to the requirements, (2) the digestibility of protein and more generally the availability of the amino acids. The digestibility of amino acids is highly variable and accurate data on the digestibility of amino acids in feeds is needed to allow the animal's daily requirement for individual dietary amino acids to be met more precisely and economically. There are many different methods, including *in vivo* and *in vitro* approaches, that have been used to measure the digestibility of amino acids in feedstuffs.

1. 2. 4. 2. 1 Faecal versus ileal

For many years faecal apparent amino acid digestibility measurement was widely accepted as a valid means for estimating amino acid digestibility in pigs. However, the microbial flora hydrolyse the nitrogenous compounds which are absorbed from the hindgut mainly as ammonia, amines or amides. Under normal circumstances these end products are of no nutritional value to the host. In fact, 5-35% of the total ingested amino acids is lost from the large intestine (Zebrowska,

1973, 1975). Just, *et al*. (1981) confirmed this by demonstrating that amino acids infused into the caecum of pigs did not improve overall nitrogen balance.

The amino acid loss in the hindgut is not constant, therefore the faecal values cannot be corrected by any constant to provide estimates. The degree of fermentation is a function of the feedstuff, as well as residence time in the tract. Large amounts of fibre appear to decrease amino acid digestibility.

With the understanding that 80% of faecal nitrogen is bacterial in origin (Low and Zebrowska, 1989), and that amino acids entering the large intestine are not metabolised by the pig, along with the development of gut cannulation technology, it becomes clear why digestion at the terminal ileum is the prefered measurement chosen by scientists today.

There is a small amount of fermentation in the upper digestive tract. Ideally, this should be accounted for, but to date it is not. Ileal digestibilties of amino acids are now universally accepted and are almost always lower than the corresponding faecal values. The amount of amino acids lost in the large intestine increases with decreasing overall protein digestibility. This is to be expected, as lower digestibility means that more protein will reach the hindgut. This means that it is even more important to use ileal digestibility with low quality proteins.

1. 2. 4. 2. 2 Apparent versus true digestibility

The absolute value for amino acid absorption as measured at the terminal ileum, is termed "apparent" digestibility. It is the amount of amino acid ingested, less that remaining at the terminal ileum. The amino acids at the terminal ileum are not, however, all dietary and this problem needs to be addressed. There are considerable proteinaceous endogenous losses from the intestine, in the form of

materials such as mucin and epithelial cells. When correction is made for these the true value is found.

There are two schools of thought as to whether the endogenous loss should be counted as a cost against the need of the animals or as a penalty against the food. Low (1990) acknowledged that true digestibility is a function of the feed alone. Moughan (1991) also argued that true digestibility, after endogenous losses are accounted for, is a function of the feed only. Traditionally, the endogenous loss has been considered to be constant when related to the food dry matter intake. Several recent studies, however, have demonstrated that it may vary considerably, dependent on the composition of the ingested feed (Boisen and Moughan, 1996). The most important dietary factors leading to an increased endogenous protein loss seem to be protein, dietary fibre and anti-nutritional factors (ANF's). It is now generally agreed that true digestibility has an advantage over apparent digestibility in that it is a fundamental property of the feedstuff, being independent of dietary conditions. For a given amino acid, the apparent digestibility increases disproportionatelly with the ingested quantity because endogenous excretion, as a percent of total excretion, decreases proportionally (Moughan and Donkoh, 1991). By contrast, true amino acid digestibility is not affected by the ingested quantity. Moughan (1991) observed that the true value is less affected by the digestibility assay method. Furthermore, the advent of computer modelling has arrived in the feed industry. True digestibility measures are more useful in current computer models (Moughan, 1991).

In trying to determine the level of endogenous loss, traditional methods involving the use of protein free diets have been challenged (Low, 1980a; Moughan and Donkoh, 1991), as these may lead to a decreased rate of whole body protein synthesis (Millward *et al.*, 1976; Muramatsu, 1990). The protein free method underestimates the endogenous losses. Several new approaches, which allow more definitive study of the effect of peptides and protein on endogenous amino acid losses, have been proposed.

One approach which involves feeding animals guanidinated proteins (lysine has been transformed to homoarginine by treatment with o-methylisourea) allows determination of the endogenous loss of lysine (Hagemeister and Erbersdobleer, 1985) when dietary protein is present in the gut. In a study with growing rats, Moughan and Rutherfurd (1990) demonstrated that when dietary protein is present in the gut, endogenous lysine loss from the terminal ileum is considerably enhanced above that found with protein-free alimentation.

A second new method proposed by Moughan *et al.* (1990) involves feeding an animal a semi-synthetic diet containing enzymically hydrolysed casein (peptides < 5000 Da) as the sole source of nitrogen. Ileal digesta are collected and the protein (>10000 Da) is immediately separated physically by ultra-filtration. Any unabsorbed peptides or amino acids along with endogenous amino acids and small peptides which are found in the ultra-filitrate are discarded and the retentate fraction is analysed to give a measure of endogenous loss. This technique has been applied by Butts *et al.*, (1991) with growing rats and the results suggested that dietary peptides have a stimulatory effect on endogenous amino acid secretion and loss from small intestine. A smilar effect has been found with growing pigs (Butts *et al.*, 1993; Moughan and Schuttert, 1991).

Another method for determining specific feed-induced endogenous protein loss is the tracer technique, by which endogenous protein can be distinquished from feed protein after labelling either the food of body protein using radioactive or stable isotopes. Among several isotopes used, ^{15}N is the most common (Boisen and Moughan, 1996). The endogenous losses determined with this technique are usually higher than those found using the traditional methods.

In addition, endogenous ileal protein and amino acids losses can be estimated from the difference between *in vivo* apparent ileal digestibility values and

in vitro digestibility values (Boisen and Eggum, 1991; Boisen and Fernandez, 1995).

1. 2. 4. 2. 3 Cannulation methods

There are several methods of cannulation in use today to allow the total collection of digesta or sampling of digesta from the terminal ileum of pigs. The different approaches have been recently reviewed (Sauer and Ozimek, 1986; Low, 1990; Moughan, 1993). It was concluded that more work is required before drawing firm conclusions as to which is the superior procedure. However, some general conclusions can be made. Ileo-ileo and ileo-caecal re-entrant cannulation involve total transection of the ileum and, this is considered to be undesirable as it interferes with gut motility. The ileo-colic (post-valve) re-entrant cannulation, post-valve T-caecum cannulation and simple T-ileum cannulation all have the distinct advantage that the function of the ileo-caecal valve is preserved and the ileum is not transected.

An important question concerns whether or not the procedure of cannulation disturbs the processes of digestion and absorption. Most reports show no major physical disturbances. Moughan and Smith (1987) demonstrated that cannulation (simple T-piece cannula) of the terminal ileum of the growing pig had little effect on amino acid digestion and absorption.

However it is difficult and costly to obtain samples of ileal digesta from cannulated pigs on a routine basis. An alternative to collecting digesta via intestinal cannula, is to sample digesta from the terminal ileum of animals while under anaesthesia (Moughan *et al.* 1989). The so-called slaughter technique, has the distinct advantage of involving minimal disruption of normal digestive function in the animal and allows samples of digesta to be taken from several parts of the digestive tract. Digestibility data derived using the slaughter technique in pigs were

no more variable than those found with cannulation techniques. Some drawbacks, however, are the relative cost of the slaughter technique as the meat cannot be sold with anaesthetic in it, and the animals are only used once. This is in contrast to cannulated pigs which survive for up to two years. The ease of collecting ileal contents from rats after slaughter suggests this species could be a useful model for the determination of ileal digestibility of dietary protein in the growing pig. There was agreement between the rat and pig for apparent ileal digestibility of protein and amino acids in several feed ingredients (Moughan *et al.*, 1984; 1987).

1. 2 .4. 2. 4 *In vivo* versus *in vitro* methods

There is much interest in using *in vitro* techniques to determine the digestibility of amino acids for pigs. Various *in vitro* methods have been developed, using either a single enzyme (usually pepsin), or a mixture of enzymes. Such determination of amino acid digestibility is simple, inexpensive and rapid.

The *in vitro* technique seeks to simulate the natural digestion process. In some cases close *in vivo* / *in vitro* relationships have been reported (Furuya *et al.*, 1979; Boisen, 1991; Boisen and Fernandez, 1991). Boisen and Eggum (1991) reviewed several major techniques and made the observation that a very important determinant of the accuracy of the *in vitro* techniques was the specificity of the enzymes employed. Most naturally occurring enzymes are not commercially available, hence, some workers use digesta preparations. Pancreatin, however, seems to simulate the pancreatic enzymes sufficiently.

Boisen and Fernandez (1995) reported a high correlation (r = 0.92) between the *in vitro* and *in vivo* ileal digestibility of protein and AAs in barley. The *in vitro* digestibilities of essential AAs were all higher than corresponding values of apparent ileal digestibility. It was concluded that the difference was due to

endogenous losses. In theory, the *in vitro* techniques measure true digestibility. Also, the *in vitro* assays measure digestibility not availability.

1. 2. 4. 2. 5 Availability of amino acids

An assumption of ileal digestibility assays has been that an amino acid not recovered at the terminal ileum has been absorbed in a form suitable for protein synthesis. This is often so for the high quality proteins, but not for low quality proteins, or for those that have been heat treated (Moughan, 1991). There is a strict distinction between the terms 'digestibility' and 'availability'. Generally, digestibility is defined as the difference between the amount of an amino acid ingested and that in the ileal digesta or faeces, divided by the amount in the diet. Availability of an amino acid is defined as the proportion of the total amino acid that is digested and absorbed in a form suitable for protein synthesis (Batterham, 1992). In heat-treated feedstuffs, protein amino acids may have undergone reactions with other chemical compounds like reducing sugars present in the feed, to form new chemical compounds resistant to the digestive enzymes. This will change the availability of the amino acids to the animal, rendering amino acids such as lysine, for example, unutilisable. It is well known that the most important reaction making an amino acid 'unavailable' is the so-called Maillard reaction. The term Maillard reaction or nonenzymatic browning is related to reactions between amines and carbonyl compounds, especially reducing sugars. It has also been said to include the aldehyde or the ketone produced by oxidation of fatty acids. Such substances can indeed react like sugars with amino acids. In general the primary ε-amino groups of the lysine side chains preferentially react with sugars. The Maillard reaction may be divided into three stages as described by Mauron (1981): early, advanced, and final. In the advanced stages the amino acid will be completely destroyed and will not be recoverable following acid hydrolysis during amino acid analysis. However, the deoxyketosyl compound formed in the early stages of the Maillard reaction is of no nutritional value to the pig, but it can be

hydrolysed back to lysine in the presence of strong acids. Thus for feeds which have undergone the early Maillard reaction during processing, conventional acid hydrolysis will lead to an over-estimation of the lysine content (Hurrell and Carpenter, 1981). Consequently, numerous techniques have been developed as a means of estimating amino acid availability, such as the slope-ratio assay, chemical analyses and microbiological techniques. The features of the methods which have been used to measure the availability of amino acids in feedstuffs have been summarised by McNab (1979).

Growth assay

The most common technique for determining amino acid availability is a growth assay (slope-ratio), where the response to increasing increments of the test amino acid in a protein is compared to the response to the standard free amino acid. The method involves the formulation of a control diet deficient only in the amino acid under study and the relative growth of pigs fed this diet is compared with those fed diets to which graded levels of the limiting amino acid have been added in the crystalline form. It is assumed that the availability of the added amino acid is 100%. The response between growth of pigs fed the diet to which a known amount of test protein has been added allows the calculation of the amount of amino acid in the protein source. Procedures using rats, chicks and pigs have been developed for lysine, methionine, isoleucine and tryptophan (Batterham, 1992).

The results of a series of assays conducted by Batterham (1992) indicated that lysine availability (proportion of total) varied from 0.27 in cottonseed meals to 1.13 for blood meal. That the latter coefficient is greater than unity points to the inherent inaccuracy of this assay. The assay has also been applied to determining the effects of processing on meat and bone meal quality, where processing conditions were shown to alter the availability of lysine from 0.97 to 0.38 (Batterham *et al.*, 1986).

The slope-ratio assay has the advantage of being a biological assay, which measures availability by definition. However, it also has some disadvantages, such as being time-consuming and expensive. Only one amino acid can be assessed at a time; dietary formulations are complex. Moreover, standard errors around the estimate are high, and results may have a low repeatability.

Chemical assays for availability

Chemical techniques have the advantage of being rapid and comparatively inexpensive. Most of the chemical techniques used to estimate amino acid availability are concerned with lysine, because heat treatment and/or storage may render it nutritionally unavailable more easily than other amino acids by promoting its irreversible reaction with carbonyl compounds (i.e. Mailard reaction) to form indigestible colourless browning intermediates.

The majority of chemical techniques used to estimate lysine availability are based on binding of the free ε-amino group of lysine with a specific receptor or dye. This technique is based on the assumption that if the ε-amino group of lysine was free, then that lysine molecule would be nutritionally available.

A number of chemical compounds have been used, the principal one being 1-fluoro-2,4-dinintrobenzene (FDNB) (Carpenter, 1960; Carpenter and Ellinger, 1955). Several other chemical methods have been developed, such as 2,4,6-trinitrobenzene sulphonic acid (TNBS) (Kakade and Liener, 1969) and o-methylisourea (Mauron and Bujard, 1964, cited by Carpenter, 1973), but none has attracted as much interest as the FDNB method.

Microbiological Assays

The use of microbiological assays to estimate amino acid availability and protein quality has been applied for many years. In general, certain micro-

organisms, such as *Streptococcus zymogenes* and *Tetrahymena pyriformis* (McNab, 1979), have a specific requirement for an amino acid. By determining this requirement with standard or free amino acids, the organism can be used to estimate how much of an amino acid is available within a test protein. The procedure involves the measurement of the response of the organism to graded supplements of the material under test and a comparison with graded doses of the one amino acid; the basal medium contains all other nutrients necessary for optimal growth of the organism. 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 the digestion and metabolism.

Microbiological assays can estimate the bioavailability of certain amino acids in some feedstuffs. Such assays are reasonably fast and can be made in close proximity to feedstuffs preparation and use, where more complex animal assays would be impractical. The problem of microbiological assays is the inability to differentiate between peptides that can or cannot be used as sources of amino acids by domestic animals.

1. 3 Nutritive value of barley

1. 3. 1 General characteristics of barley

Barley is a grass the seeds of which are useful to man. Taxonomically it belongs to the family *Gramineae*; subfamily *Festucoideae*; tribe *Hordeae* and genus *Hordeum*, which includes cultivated barleys and various wild barleys and barley grasses (Reid and Wiebe 1979). Varieties differ greatly in their morphological and other characteristics (Briggs, 1978).

Barley is grown in many parts of the world, but it is more popular in temperate countries where the cereal is grown mainly as a spring crop. Barley is a crop with tremendous adaptability. It can be found beneath date palms in the Sahara Desert. Barley has adapted to the high plateaus of Bolivia, South Africa and Tibet; to the hills of western China; and to the north of the Arctic Circle. Barley is man's most dependable cereal crop where alkali soils, summer frost, or drought are encountered (Lee, 1973).

The Barleys have either a winter or a spring habit of growth. Their classification into these two types is based principally on whether they are normally autumn- or spring-sown in farm practice, and on the habit of growth of the plant.

The rate of growth depends on the weather, the climate, the water supply, soil fertility, the degree of competition with weeds and other plants, the depredations of pests and diseases, and whether the crop is autumn or spring-sown. Barley grows particularly well where the ripening season is long and cool, where the rainfall is moderate rather than excessive, and where the soil is well drained but not sandy. Barley can stand high temperatures if the humidity is low, but it does not thrive where both are high. As a winter crop, it is more hardy than oats but less hardy than wheat or rye. It is suggested, that for vegetative growth an air temperature of about 15°C is best, while at heading the optimum is around 17-18°C. Soil temperatures of about 15°C are optimal for barley growth. Certain strains of naked spring barley from Tibet are characterised by remarkable resistance to cold as well as being early ripening. Consequently, spring barley is grown farther north and at higher altitudes than any other cereals (Briggs, 1978).

The duration of the growth period varies, being about 105 days in Britain, and 45-60 days on the Canadian Prairies (Briggs, 1978). Some varieties of spring barley mature in 60-70 days.

There are two types of barley cultivated, the hulled and the hull-less varieties, with the cultivation of hulled varieties far exceeding that of the hull-less varieties.

1. 3. 2 Chemical composition and nutritive value of barley

Gross chemical analysis indicates that starch is the major constituent of barley grain, followed by dietary fibre, protein, fat, low molecular weight sugars and ash. For example, average values in the study by Knudsen *et al.* (1987) were: starch 58.4%, dietary fibre 22.3%, protein 12.0%, fat 3.4%, low molecular weight sugars 2.2% and ash 2.2%. In addition to starch, barley contains both water-soluble and insoluble polysaccharides (ß-glucan), which constitute between 2 and 16% of the dry matter (Aman and Graham, 1987; Bhatty *et al.*, 1991).

However, many investigations have shown considerable variation in the chemical composition of barley, particularly with regard to starch and dietary fibre. For example, Aman *et al.* (1985) reported that the starch content (% of dry matter) varied from 52.9 to 66.6% with a mean value of 62.2% in a 2-row barley and from 52.9 to 64.1% with a mean value of 58.9% in a 6-row barley. The starch content was inversely correlated with fibre. Most of the fibre is in the husk, pericarp and testa.

1. 3. 2. 1 Factors influencing the chemical composition of barley

The nutrient content of barley grain varies with different varieties. For example, in the study of Knudsen *et al.* (1987) the starch content of the spring malting varieties was 1.7% higher and the fibre content 2.1% lower than that of spring feeding varieties. The lower dietary fibre was accounted for by both a lower soluble dietary fibre and insoluble dietary fibre. Total and soluble ß-glucans were 4.1% and 2.0% in spring malting varieties compared to 4.6 and 2.6% in spring

feeding varieties. The relative distribution between the two forms of ß-glucans (soluble : insoluble) was 50 : 50 in spring malting varieties compared to 57 : 43 in spring feeding varieties. The protein content was higher in malting than in feeding barley varieties. Bhatty *et al.* (1974) reported that the ranges in protein, starch, amylose, and gross energy varied from 5 to 15% in 29 cultivars of 2-row and 6-row barley, whereas, ether extracts, fibre, ash and ß-glucan contents varied from 1 to 3%. The 2-row barley starch contained about 3% more amylose than 6-row barley starch. The average content of ß-glucan ranged from 1.2 to 2.7%. The 2-row barley had higher glucan content than the 6-row barley.

The chemical composition of barley also varies with cultivar, within varieties. For example, the crude protein content of individual cultivars within a 2-row barley variety ranged from 12.7 to 16.8% with a mean of 14.5%, and ranges from 12.7 to 17.2% for a 6-row barley variety with a mean of 14.1% (Bhatty *et al.*, 1975). Generally, hulless barley contains more starch than hulled barley, while the hulled barley contains more crude fibre and ash than hulless barley. In addition, there is more protein and nitrogen-free extract content in hulless barley than in hulled barley. It has been reported that the crude fibre content in hulled barley is 4% more than that in hulless barley (6% versus 2%). The protein content in hulled barley is somewhat lower than that in hulless barley (17.56% versus 18.53%) (Mitchall *et al.*, 1976).

There are no major differences in the essential amino acid composition of the hulled and hulless types (Bhatty *et al.*, 1979).

1. 3. 2. 2 Effect of Locality, climate and soil fertility on the chemical composition of barley

The starch, dietary fibre and protein are affected by locality. For instance, barley grown on clay soils contains less protein (11.3% DM), more starch (59.6%

DM) and less total dietary fibre (21.9% DM) than barley grown on sandy soils (12.5% protein, 57.2% starch and 22.7% total dietary fibre). The variation in chemical composition of barley grown on clay soils is significantly lower than that found for those grown on sandy soils (Knudsen *et al.*, 1987).

Apparently, hot and dry weather conditions favour the formation of barley (1-3, 1-4)-ß-glucan (Hesselman and Thomake, 1982) and it is well known that nitrogen fertiliser can increase the crude protein content of barley (Buchmann, 1979).

1. 3. 3 The digestibility and utilisation of nutrients in barley

The nutritive value of feedstuffs is highly dependent on the digestibility and utilisation of nutrients by the animal. The primary function of cereals in pig and poultry rations is to provide digestible energy (DE) or metabolizable energy (ME) for growth and production. Thus, DE or ME content is a major nutritional criterion for the evaluation of feed barley. However, DE or ME content is related to many physical and chemical characteristics of barley. For example, the results of the study conducted by Bhatty *et al.* (1974) using mice, who examined the relationships between a number of physical and chemical characters and DE in 29 cultivars of barley, indicated that DE content was significantly correlated with protein content and GE content. The digestion coefficient was positively correlated with bulk weight, plumpness, ether extract and was negatively correlated with fibre.

Hull content is a major factor affecting the DE content of barley. For example, Bell *et al.*, (1983) reported that energy digestibility was highly correlated with percent hulls and percent crude fibre (r = -0.9 for both factors). Digestible energy prediction values were developed, whereby barley with 0 % hull was 84 % digestible and had 15.73 MJ DE/kg dry matter whereas barley with 30% hulls was 64% digestible and contained 12.01 MJ DE/kg dry matter.

The hull content of barley varied with variety, thus the digestibility of energy and digestible energy content varied among varieties. For example, the mean digestibility of energy and digestible energy value for six hulless cultivars were 85.7% and 16.39 MJ/kg, respectively, compared with means of 79.2% and 15.18 MJ/kg for the 10 hulled cultivars (Bhatty *et al*, 1975).

As mentioned above, the chemical composition was affected by variety, so that the digestibility of energy and digestible energy contents were different with different varieties. For example, the DE values quoted by Peers *et al.* (1977) ranged from 13.76 MJ/kg dry matter to 15.10 MJ/kg dry matter for different varieties. The digestible energy (MJ/kg dry matter) value for a 2-row barley for pigs was 12.69 and that of a 6-row barley was 12.09 (Wiseman, 1984).

The DE or ME value of barley is highly dependent on the digestibility and utilisation of carbohydrates as they are by far the largest contributors to the energy of the animal. By the Weende method, carbohydrates are divided into crude fibre and nitrogen-free extract substances. From a nutritional point of view, the carbohydrates can be divided into one fraction that is digested by the enzymes present in the gut lumen and another fraction that is resistant to mammalian enzymes in the small intestine. The former fraction includes starch and sugars, while the latter includes non-starch polysaccharides (NSP), primarily cellulose, hemicellulose, pectin, ß-glucans and lignin and resistant starch (Graham *et al.,* 1986).

It is generally accepted that starch, which is quantitatively the most important energy source for animals, and most sugars are broken down by a combination of pancreatic and mucosal enzymes in the small intestine. Studies with monogastric animals have shown that less than 5% of dietary starch escapes digestion in the small intestine (Graham *et al.*, 1986). Studies with pigs have also shown that most cereal fibre (i.e. non-starch polysaccharides, NSP, and lignin) escapes digestion in

the small intestine (Aman *et al.*, 1985; Knudsen *et al.*, 1987). Although a significant amount of NSP is broken down by microbial enzymes in the hindgut, the net energy of carbohydrates fermented and absorbed as short chain acids from the hindgut is appreciably lower than that of carbohydrates hydrolysed and absorbed as monosaccharides from the small intestine. The extent of microbial break down of NSP is influenced by the composition of the NSP-fraction and the degree of lignification. Therefore, a higher dietary fibre content at the expense of starch and sugars has two negative implications for the net energy value. Firstly, DE and ME are reduced in proportion to the ratio of dietary fibre to starch and sugars, and secondly, the utilisation of ME is lower as the DE derives from short chain acids.

Studies have shown that the DE is strongly correlated with the composition of the carbohydrate fraction, in particular the ratio of starch to fibre (Knudsen *et al.*, 1987). Many studies have also found considerable variation in carbohydrate composition of barley varieties (Bhatty *et al.*, 1974; 1975).

The actual nutritive value also varies with the animal to which the barley is being fed. For example, barley is less digestible and yields less energy when fed to poultry than when fed to pigs or ruminants (Fincher and Stone, 1986). The ME (MJ/kg) values of 2-row barley and 6-row barley for pigs are 12.28 and 11.76, respectively. The ME values of the same varieties for broiler are 10.04 and 9.62, and for the adult bird are 11.67 and 11.28 respectively (Wiseman, 1984).

In addition to energy, knowledge of protein and amino acid (AA) digestibility for individual feedstuffs is very important in formulating animal diets. Similar to the energy component, the digestibility of crude protein varies with different varieties of barley and different species of animal. For instance, the apparent faecal digestibility of protein in 2-row and 6-row barleys for pigs were 78 and 76% respectively, which were higher than those values for poultry (70% for the two varieties) (Wiseman, 1984).

The digestibility of crude protein is dependent on the fibre and hull content. Bell *et al.* (1983) reported that apparent faecal digestibility of protein decreased from 75.0 to 72.0% as hulls increased from 0 to 30% and from 82.2 to 62.5% as dietary CP decreased from 21.9 to 8.0% (dry matter basis) in barley. Metabolic faecal protein increased from 1.49 to 2.79g/100 g dietary dry matter as barley hulls increased from 0 to 30%. The apparent faecal digestibility of hull protein was 44% and the corresponding value for non-hull protein was about 75%. Buchmann (1979) observed that naked barley had a higher average digestibility of protein than normal barley.

The nutritive value of a protein is not only determined by its AA composition but also by the digestibility of the individual AAs in the protein. Since the AAs that are hydrolysed in the large intestine are not used by the pig for protein synthesis, the ileal measure of AA digestibility is generally recognised as a more acceptable approach than the traditional faecal method (Tanksley and Knabe, 1984; Low, 1980b). Studies have shown that ileal nitrogen and most AA digestibilities are lower than faecal values. For example, a review by Tanksley and Knabe (1984) indicated that the average ileal digestibility of essential AA was 4.5% lower than corresponding faecal values for barley. Bacterial degradation of AAs to ammonia in the large intestine is probably partly responsible for this discrepancy. In addition, ileal AA digestibilities in barley were generally lower than those in wheat, maize and sorghum. This was probably attributable primarily to a higher fibre content. Further, Moughan and Smith (1984) showed that the coefficient of faecal digestibility of crude protein in barley was greater than the corresponding ileal coefficient and Moughan and Smith (1985) demonstrated that the ileal digestibility coefficients of AAs for barley were reasonably accurate in describing the extent of uptake of AAs from the gut.

Because it is expensive to obtain samples of ileal digesta from the pig on a routine basis, a method using small animals such as the laboratory rat as an alternative approach for the routine measurement of ileal AAs digestibility has been

developed. Moughan *et al.* (1987) demonstrated that there were no significant differences between the rat and pig for the apparent ileal digestibility of most essential AAs in barley except for methionine, phenylalanine, and several of the non-essential AAs. It was concluded that the apparent ileal digestibility of all dietary essential AAs in ground barley except for methionine determined in the rat can be used to predict digestibility in the pig.

1. 3. 4 Anti-nutritional factors in barley

The use of barley in poultry diets has been limited because of its inconsistent nutritional value, contribution to wet and sticky faeces and depressed animal performance (Ghol and Ghol, 1977; Rotter *et al.*, 1989a).

Fibre is the major anti-nutritional factor in barley. Because its fibre content is relatively high (about 5-6%) and poorly digested by birds, barley is generally classed as a low energy cereal (Herstad, 1987). Studies have shown that the growth rate and the efficiency of feed conversion of pigs fed barley rations are often inferior to those of pigs fed diets based on lower fibre grain such as corn or wheat (Mitchall *et al.*, 1976). Fibre mostly exists in the hull fraction and it has been shown that hulls have a negative effect on energy digestibility (Bell and Keith, 1994; Bell *et al.*, 1983). Larsen and Oldfield (1961) reported that the addition of barley hulls to either corn or pearled barley reduced feed conversion efficiency and rate of gain in growing pigs more than a comparable addition of fibre from wood cellulose. Other workers have also demonstrated that hull material depresses rate and efficiency of growth in swine (Dinusson *et al.*, 1956, 1960). Consequently, hulless cultivars of barley which are low in fibre and high in energy have been developed. Bhatty *et al* (1974, 1975) reported that the hulless cultivars of barley had a higher (8.2%) digestibility of gross energy and a higher digestible energy content (16.39 MJ/kg vs 15.18 MJ/kg) than hulled barley. The hulless variety had a higher crude protein content (15.0 versus 13.1%) and starch content (62.5 versus

58.2%) and a lower fibre level (NDF; 7.5 versus 12.9%). However, several reports indicate that when these cultivars are given to chicks (Anderson *et al.*, 1961) or swine (Newman *et al.*, 1968, Mitchall *et al.*, 1976), their feeding value is at best only slightly better than that for hulled barley. The rate of gain of chickens fed a ration based on yellow corn averaged 17% more than that of chicks fed a ration based on hulless barley. Gain/feed ratio averaged 12% more with the corn ration. Rations based on hulless barley were not superior to rations based on regular barley (Anderson *et al.*, 1961). The daily gain and efficiencies of feed conversion for pigs were not significantly different between rations based on regular and hulless barley (Gill, *et al.*, 1963; Mitchall *et al.*, 1976). Furthermore, the digestibility of protein in hulless barley was lower than in regular barley (Mitchall *et al.*, 1976).

There is considerable evidence that the overriding feature which makes barley unpopular as a constituent of poultry diets and the efficiency of feed conversion for pigs often inferior to corn or wheat is the presence in the grain of a polysaccharide known as mixed-linked (1-3), (1-4)- ß - D -glucan, often abbreviated to ß-glucan (McNab and Smithard, 1992; Hesselman *et al.*, 1981; Hesselman and Aman, 1986; White *et al.*, 1981; Henry, 1987; Miller *et al.*, 1994). The ß-glucan is the principal endosperm and aleurone cell wall component in cereals (White *et al.*, 1981). In barley ß-glucan constitutes about 70% of the starchy endosperm walls and about 25% of isolated aleurone cell walls (Aman and Graham, 1987).

It is well known that ß-glucan decreases digestibility and absorption of all nutrients, produces sticky droppings and consequently depresses chick growth (Campbell *et al*, 1989). Barley is unique among cereals, in that it contains a relatively high concentration of the ß-glucans. Hulled barley may typically contain 3-7% ß-glucan (Aman and Graham, 1987) and hulless barley as much as 16% (Newman *et al.*, 1989). Both soluble and insoluble ß -glucans are present in cereals with factors such as particle size, ß -glucanase activities of the flour, and temperature, pH, and ionic strength of the extraction media affecting solubility (Aman and Graham, 1987). Soluble ß -glucan, on average, formed 32.7% and 54%

of the total ß-glucan content in hulless barley cultivars (Knudsen *et al.,* 1987; Bhatty, 1987) and in covered barley cultivars (Aman and Graham, 1987), respectively. Insoluble ß-glucans in grain cell walls encapsulate easily available nutrients such as starch, intracellular protein, and fat and act as a physical hindrance to nutrient hydrolysis and utilisation, while soluble or solubilized ß - glucans give rise to viscous solutions, which also decrease digestibility and absorption of all nutrients, produce sticky droppings and consequently depress chick growth (Hesselman and Aman, 1986; Campbell *et al.*, 1989). The poorer nutritive value of hulless barley for chicks was related to its high content of ß-glucan (Bhatty *et al.*, 1991) and high viscosity (Rotter *et al*, 1989b, 1990) compared with hulled barley.

In addition, barley also contains tannins (Jansman, 1993), which are defined as naturally occurring water-soluble polyphenolic compounds with a molecular weight between 500 and 3000, capable of precipitating alkaloids as well as gelatin and other proteins from aqueous solutions. Tannins are usually classified as either being hydrolysable or condensed tannins, on the basis of their structure. The condensed tannins are considerably more reactive than the hydrolysable tannins. Numerous studies have been conducted on the effects of tannins in feedstuffs on nitrogen, amino acid and energy digestibility of rats, poultry and pigs (Yu *et al.*, 1995; Herstad, 1979; Jansman, 1993). The results indicated that tannins reduce the apparent digestibility of nitrogen (protein), amino acids, and to a lesser extent, energy. It is assumed that tannins form complexes with protein (feed protein and enzymes) and also with carbohydrates (Longsttaff and McNab, 1991; Griffiths, 1979, 1981). Due to the formation of these complexes, the digestibility of feed protein and carbohydrates are decreased and enzymes may be inactivated. Terrill *et al.* (1992) reported that the condensed tannin concentration in barley meal was 0.1% on a dry matter basis.

1. 3. 5 Treatments to improve the nutritive value of barley for pigs and poultry

There is considerable evidence that ß-glucans of barley have an anti-nutritive effect in poultry. The anti-nutritive effect is manifested by poor growth of the chickens and sticky droppings accompanied by a depressed utilization of nutrients. The viscosity effects may be less relevant in pigs than in poultry, however, the viscosity may still have a significant effect on digestibility and animal performance. Therefore, much effort has been committed to developing methods which can decrease the ß- glucan content of barley and improve the nutritive value of barley.

Studies have indicated that the anti-nutritional properties of barley can be effectively overcome by gamma irradiation, water treatment and subsequent drying, or by enzyme addition (Herstad and McNab, 1975; Classen *et al.*, 1985; Campbell *et al.*, 1986; Rotter *et al.*, 1989a).

Gamma irradiation is the most recent and least studied of the measures which have been used in attempts to ameliorate the adverse effects of barley on poultry nutrition. Although gamma irradiation has received most attention as a means of decontaminating or sterilizing feedstuffs, it has also been reported to improve the feeding value of barley and its hulless counterpart for chicks (Classen *et al.*, 1985; Campbell *et al.*, 1986). These effects have been interpreted in terms of depolymerization of ß-glucan.

Studies have indicated that soaking barley in water improves the nutritive value of barley for poultry (Leong *et al.*, 1962; Potter *et al.*, 1965;). Leong *et al.* (1962) reported that water-treatment of ground barley (pearled and regular), which consisted of adding one part tap water (40°C-60°C) to one part of the ground barley, mixing, drying in a forced-draft oven at 70°C and regrinding before using, markedly increased the ME of the barley. It is assumed that water treatment may disrupt the protective barrier of the ß-glucan and release the contents, mainly

starch and protein, to the digestive processes (Hesselman and Aman, 1985). Lawrence (1976) suggested that soaking improves the nutritive value of barley to the pig by converting starch into the more digestible maltodextrins. However, the demonstration by Potter *et al.* (1965) that the increase in metabolizable energy of water-treated barley could be accounted for by significant increases in the amounts of digestible crude protein and crude fat, and that there were only minor increases in the digestibility of nitrogen-free extract casts some doubt on the suggestion that changes in starch is a significant factor in the improvement induced in barley by water treatment. In this study coefficients of digestibility of the crude protein in barley were increased from 45 to 75 and 84 % by the presence of enzymes and by water treatment of barley. The coefficient of digestibility of the crude fat in barley was increased from 0 percent to 76 and 84 %, and the coefficient of digestibility of the nitrogen-free extract was increased from 75 to 81 and 78 percent by enzyme and water treatment, respectively.

The benefit of enzymes in diets containing barley for poultry has been known for many years. Early studies used crude amylase and protease preparations (Jensen *et al.*, 1957; Burnett, 1962) which were later shown to contain β-glucanase activity (Rickes *et al.*, 1962). β-glucanase is an enzyme present in certain crude enzyme preparations, particularly from bacterial and fungal sources (Bumett, 1966). The result from the experiment conducted by Mannion (1981), who studied the influence of bacterial and fungal enzyme supplementation at 0.15 and 0.3% of barley based diets on the growth and nutrient utilization by 4 week old female broiler chickens, indicated that body weight gain was improved by 12 to 25% and food consumption increased by 3 to 21 % with feed enzyme treatment. The metabolizable energy content of the diets was improved 1.53 MJ/kg dry matter. Another study with chickens indicated that the improvements in body weight gain and feed to gain ratio following enzyme supplementation were 16% and 6% for a barley diet (Marquart *et al*, 1994).

It is well known that the anti-nutritional properties of barley can be largely overcome by the addition of enzyme preparations which contain β-glucanase (Gohl *et al.*, 1978; Hesselman and Aman, 1986; Rotter *et al.*, 1989a, 1990). β-glucanase can increase the nutritional value of barley, resulting in improved animal growth and feed efficiency (Burnett, 1966; Gohl *et al.*, 1978; White *et al.*, 1981; Hesselman and Aman, 1986). β-glucanase hydrolyses the β-glucans into smaller polymers in the alimentary tract (White *et al.*, 1981; Hesselman and Aman, 1986) and reduces the viscosity of the intestinal contents (Burnett, 1966; White *et al.*, 1981, 1983). Rotter *et al.* (1989a) reported that enzyme supplementation of a diet containing hulless barley improved weight gain (11.2%) and feed efficiency (8.5%) in Leghorn and broiler chicks over an entire 6-week feeding period. Rotter *et al,* (1990) have also shown that β-glucanase supplementation significantly increased available energy for young broiler chicks as the barley component of the diet increased. In this study the addition of an enzyme preparation having high ß-glucanase activity to a diet containing Scout barley, which is a hulless barley containing a high content of the highly viscous water-soluble ß-glucans, increased its apparent metabolizable energy value by 25% and greatly reduced its viscosity *in vitro*.

In pigs the major responses to enzyme supplementation of barley-based feeds are likely to be related to improvements in nutrient digestibility brought about by the break-down of arabinoxylans and β-glucans. Considerable effort has been directed towards establishing applications for enzyme additives in swine diets. Some improvement in growth and feed conversion (as well as digestible energy and protein) has been reported in pigs fed barley diets (Bedford *et al.*, 1992) in response to enzyme supplementation. Recent work has shown that enzyme supplementation of barley-based feeds for early weaned pigs improved ileal starch and non-starch polysaccharide (NSP) digestibility (Inborr *et al.*, 1993). Another study (Inborr *et al.*, 1995) indicated that supplementation of the diet based on barley with β-glucanase changed the conditions in the digestive tract by reducing digesta viscosity and the concentration of short chain fatty acid. The liveweight

gain and feed conversion ratio tended to be improved in the presence of the enzyme although not significantly so.

Other studies have found, however, that pigs were not affected by β-glucan (Campbell and Bedford, 1992). Dietary β-glucanase resulted in only a small improvement in ileal starch and β-glucan digestibility (Graham *et al.*, 1989b). A high viscosity hulless barley that had consistently given substantial growth depression in young chicks gave comparatively good results when fed to pigs (Bhatty *et al.*, 1979). This is due to the fact that significant quantities of β-glucan are degraded by endogenous enzymes in the pig (Bass and Thacker, 1996). Graham *et al.* (1989b) reported that over 95% of mixed linked β-glucans were degraded prior to the terminal ileum in pigs fed barley-based diets. Weltzien and Aherne (1987) reported digestibilities of 76-82% for β-glucans at the terminal ileum of growing pigs. Thus the potential to improve pig performance by using β-glucanases is dramatically less than with poultry.

1. 4 Conclusion

Barley is an excellent grain for pig diets and produces pork of high quality, the fat being hard and firm. Making up 46% of the total area of planted cereals and peas in New Zealand, barley is widely used as an important source of energy and protein for the growing pig.

The nutritive value of barley may be affected by nutrient digestibility and by its chemical composition, which in turn is influenced by many factors, such as variety and culitivar within variety, locality, climate, and soil fertility. Several anti-nutritional factors, (for example, fibre, β-glucans and tannin) can decrease the digestibility of nutrients in barley. Evidence has shown that β-glucan can affect the digestibility of protein, and to a lesser extent, energy.

As a major energy source for the growing pig, it is important to have information on the digestible energy value of barley. Traditionally, such information has been obtained through *in vivo* digestibility experiments. *In vivo* methods, however, are expensive and time consuming. Accordingly, efforts have been directed towards predicting digestible energy content based on chemical composition, physical characteristics or *in vitro* digestbility methods. At present, there is a lack of information on how to best predict the digestible energy content of barleys for the growing pig.

CHAPTER 2

THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF NEW ZEALAND BARLEYS

2. 1 INTRODUCTION

It is necessary to be able to accurately evaluate the nutritive value of feeds for farm livestock. A first step in the evaluation process is to characterise the physical characteristics of the feed and its chemical composition.

Barley serves as an important dietary source of energy for pigs in New Zealand, and to a lesser extent, poultry. Barley is also used without restriction in rations for growing and finishing cattle and for lactating dairy cows. The digestible or metabolizable energy content of barley may be affected by its chemical composition, which is known to differ between varieties and between cultivars within varieties (Bhatty *et al.*, 1974; Coates *et al.*, 1977). Information on differences in chemical composition, between and within varieties, is important in the feeding of monogastric animals (Fuller *et al.*, 1989).

The objective of this study was to determine the variation in the chemical composition and in certain physical characteristics, among barley cultivars grown in New Zealand. The information on the chemical composition and physical characteristics of the barley samples was used, subsequently, to predict their *in vivo* digestibility of energy.

2. 2 MATERIALS AND METHODS

2. 2. 1 Preparation of Barley Samples

Representative (100 kg) samples of seventeen barley grains representing nine varieties (Fleet- 6 samples from both early and late harvest times, 5 North Island and 1 South Island sample; Magnum- 2 South Island samples; Valetta and Nugget- both North and South Island samples; and 1 sample each of Triumph, Liberty, Opiki, Regatta and Corniche) were obtained from six major growing regions throughout New Zealand during the February to June period of 1995. For each sample complete information on variety, yield, sowing and harvest dates and other production parameters was collected. The grain bulk density (kg/hl), moisture content, percent screenings and 1000 seed weight were determined. The samples were stored in sealed dark containers at air temperature (storage < one month) until submission for chemical and physical analysis.

2. 2. 2 Chemical analysis

Prior to laboratory analysis, all barley samples were ground to pass through a 1 mm mesh diameter sieve (Wiley *mill*, USA).

Dry matter was determined on duplicate samples of barley. Samples of approximately 1 g were placed in pre-weighed beakers and weighed to 0.1 mg. The samples were oven-dried at 105°C for 16 h. After cooling in a desiccator, the beakers and contents were again weighed. Dry matter content was expressed as a proportion of the weight of the original sample.

Gross energy content was determined in duplicate by the conventional method (AOAC, 1984). The analysis was performed using an adiabatic bomb calorimeter. The ground samples were pelleted and weighed before combustion. Gross energy values were expressed in megajoules (MJ) per unit weight of sample.

Total nitrogen (N) content was determined on duplicate samples of all of the 17 barley samples using the macro Kjeldahl procedure (Kjeltec Auto 1030 analyser, Tecator, Sweden). The material was digested in hot concentrated sulphuric acid in the presence of selenium as a catalyst. The digested solution was cooled, diluted with distilled water and the nitrogen content was determined using a Kjeltec Auto 1030 analyser by the distillation of ammonia into boric acid and subsequent titration against standardised 0.1N hydrochloric acid. Crude protein was calculated as total N $\times$ 5.83 (Jones, 1931; Kerese, 1984).

The neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin contents were determined in duplicate using the method described by Robertson and Van Soest (1981).

Determination of NDF: samples of approximately 1 g were placed in pre-dried crucibles, which were dried in a furnace at 500°C for 1h and cooled in a desiccator before being weighed, and were then weighed to 0.1 mg. The crucibles with the samples were placed in a Hot Extraction Unit and 100 ml neutral detergent solution, 2ml amylase solution and 2-4 drops 1-octanol antifoaming reagent were added. The reagents were boiled for approximately 60 minutes and filtered. Following a rinse with distilled H_2O, the crucibles with NDF residues were oven-dried at 105°C for 16 hours. After cooling in the desiccator, the crucibles with the NDF residues were weighed

Determination of ADF: The weighed crucibles with NDF residues from the above step were placed in a Hot Extraction Unit and 100 ml Acid Detergent Solution were added to each crucible. The reagents were boiled for 60 minutes and filtered Following a rinse with distilled H_2O, the crucibles with ADF residues were oven dried at 105°C for 16 hours. After being cooled in a desiccator, the crucibles with the ADF residues were weighed.

Determination of lignin: The weighed crucibles with ADF residues from the above step were placed in a Cold Extraction Unit. Twenty five ml of 72% H_2SO_4 were added to each crucible and stirred with a glass rod. The crucibles were left to stand for 3 hours and stirred with a glass rod hourly. Then the crucibles were rinsed with distilled H_2O until they were acid free and dried at 105°C for 16 hours. After cooling in a desiccator, the crucibles with the lignin were weighed.

The cellulose content was calculated as ADF minus lignin, and hemi-cellulose as NDF minus ADF.

The total ß-glucan contents were determined in duplicate using the conventional total ß-glucan procedure (Jφrgensen and Aastrup, 1988). Fifty mg of ground sample (0.5 mm) was suspended in 10 ml H_2O and incubated in a boiling waterbath for 1 hour. Ten ml of 0.075 M H_2SO_4 were added and the sample was further heated for 10 minutes. The samples were centrifuged and the supernatants collected. The extract was chilled until analysis of ß-glucans using Flow Injection Analysis (Jφrgensen, 1988).

ß-glucan contents were also determined using an extraction procedure (Crop and Food CRI, Christchurch, New Zealand), which simulates natural digestion in the gastro-intestinal (GI) tract. In this procedure, 4 g of diet were suspended in 20 mls of HCL buffer, pH 1.5, and incubated for 2 hours at 37°C. Then 0.8 ml of 10% NaOH were added to neutralise the suspension, and it was

incubated a further 3.5 hours at 37°C, before analysing the supernatant using Flow Injection Analysis (Jφrgensen, 1988).

2. 2. 3 Physical analysis

The grain bulk density was determined in duplicate using the International Standard Routine Method (ISO, 1995). A one littre measuring cylinder was filled with air-dry sample and the contents weighed to the nearest 1 g.

One thousand seed weight was determined in duplicate by the International Standard Method (ISO, 1977). Five hundred whole grains from the sample were randomly selected and weighed in total to the nearest 0.01 g.

Moisture was determined in duplicate using the International Standard Method (ISO, 1985). Approximately 5g of sample was placed in a pre-dried and weighed dish. The dish was weighed to the nearest 1 mg and dried in the oven at 130°C for 2 hours. After cooling in the desiccator, the dish with contents was weighed to the nearest 1 mg.

2. 2. 4 Data analysis

The data were analysed using a generalised linear model (SAS). Correlations between physical characteristics and chemical composition were calculated and tested for statistical significance.

2. 3 RESULTS

The seventeen barley samples were representative of nine varieties (Table 2. 1).

Table 2. 1. Variety, location grown and harvest date for the New Zealand barley samples

Sample Number	Variety	Location	Harvest Date
1	Fleet	Manawatu	7 February 1995
2	Fleet	Hawke's Bay	18 December 1994
3	Nugget	Hawke's Bay	7 March 1995
4	Corniche	Rangitikei	7 March 1995
5	Valetta	Rangitikei	10 March 1995
6	Magnum	Mid-Canterbury	2 February 1995
7	Regatta	Mid-Canterbury	10 February 1995
8	Nugget	Mid-Canterbury	10 February 1995
9	Fleet	Rangitikei	16 February 1995
10	Fleet	Rangitikei	22 March 1995
11	Opiki	Manawatu	13 March 1995
12	Fleet	South-Canterbury	20 January 19995
13	Valetta	South-Canterbury	10 January 1995
14	Magnum	South-Canterbury	15 January 1995
15	Liberty	South-Canterbury	20 January 1995
16	Triumph	South-Canterbury	20 January 1995
17	Fleet	Wairarapa	22 January 1995

For the one variety (Fleet) different harvest times were represented, as were different locations of cultivation. Varieties grown both directly for animal feed and those grown primarily for the brewing industry were sampled. Samples came from six major cereal growing regions including Manawatu, Rangitikei, Hawke's Bay, Wairarapa and South Canterbury. Nine samples were from the North island and eight from the South Island. The yields, seeding rates and intervals from sowing to harvest are given in Table 2. 2 and certain physical characteristics in Table 2. 3.

As expected, the measured physical characteristics of the samples were variable (Table 2. 3), especially the level of screenings (ranging from 1 to 11.6%) and to a lesser extent the moisture content and 1000 seed weight.

The determined chemical compositions of the barley samples are shown in Table 2. 4.

Table 2. 2. Production data for the New Zealand barley samples

Sample Number	Yield (t/ha)	Interval Sowing-harvest (days)	Seeding Rate (kg/ha)
1	1.60	112	140
2	-	74	135
3	3.75	135	160
4	3.93	100	143
5	3.85	103	150
6	6.00	146	100
7	7.50	130	125
8	7.50	129	125
9	4.90	97	150
10	5.90	112	150
11	4.40	113	165
12	5.00	122	115
13	5.60	112	115
14	5.50	148	105
15	6.00	132	110
16	4.50	122	105
17	5.20	127	111
Mean	5.07	118.47	129.65
S.D	1.46	18.71	20.96

Table 2. 3 Physical characteristics of the New Zealand barley samples

Sample Number	Grain bulk density (kg/hectolitre)	Screening (%)	Moisture (%)	1000 seed wt (g)
1	66.10	7.60	13.40	39.00
2	64.70	11.60	12.00	37.90
3	65.50	2.20	12.80	42.80
4	62.40	1.20	12.80	49.80
5	65.00	1.00	13.70	47.20
6	68.30	3.00	14.10	49.60
7	66.90	3.40	12.90	47.80
8	69.00	1.60	13.70	49.20
9	66.30	5.80	14.50	44.00
10	62.90	4.60	16.20	47.60
11	65.90	5.00	14.50	35.60
12	65.00	8.00	12.30	39.20
13	63.50	8.00	12.70	40.20
14	65.50	7.80	12.50	42.60
15	67.20	5.40	13.40	40.80
16	64.80	5.80	12.00	42.80
17	69.60	1.60	13.70	47.40
Mean	65.82	4.92	13.36	43.74
S. D	2.03	3.02	1.07	4.51

Table 2. 4. Chemical composition of the New Zealand barley samples

Sample Number	[1]GE (MJ/kg)	[2]GE (MJ/kg)	[1]CP (%)	[1]NDF (%)	[1]Lignin (%)	[1]ADF (%)	[1]Hemi-cellulose (%)	[1]Cellulose (%)	[1]Total ß-glucan (%) [3]	[1]GI ß-glucan (%) [3]
1	16.16	18.38	9.77	19.09	1.13	4.42	14.67	3.29	-	-
2	15.69	18.05	8.67	20.40	1.48	1.09	15.31	3.61	4.72	1.24
3	15.94	18.16	9.53	18.34	1.53	4.44	13.90	2.91	-	-
4	15.85	18.51	10.02	18.06	1.55	4.05	14.01	2.50	4.55	0.55
5	15.63	18.23	9.14	17.09	1.73	3.33	13.76	1.60	4.39	1.02
6	15.96	18.21	9.44	15.54	0.84	3.88	11.66	3.04	-	-
7	15.56	17.83	7.48	15.53	0.94	4.49	11.04	3.55	4.02	1.73
8	15.32	17.90	8.21	16.05	1.24	4.69	11.36	3.45	4.01	1.23
9	15.63	17.79	7.8	16.43	1.14	4.68	11.75	3.54	-	-
10	15.23	18.13	9.76	15.78	1.11	4.58	11.20	3.47	4.33	1.78
11	15.58	18.25	9.42	14.86	1.01	4.49	10.37	3.48	5.56	1.25
12	16.03	18.13	9.64	15.75	1.08	4.44	11.31	3.36	-	-
13	16.15	18.37	11.68	14.69	0.85	4.11	10.58	3.26	-	-
14	15.76	18.04	10.33	15.19	0.89	3.65	11.54	2.76	3.49	1.26
15	16.61	18.77	11.14	15.07	1.12	4.43	10.64	3.31	4.96	2.66
16	16.12	18.24	11.39	14.30	0.88	3.60	10.70	2.72	-	-
17	15.55	18.01	10.59	17.49	1.18	3.66	13.83	2.48	5.47	1.86
Mean	15.81	18.18	9.65	16.45	1.16	4.24	12.21	3.08	4.55	1.46
S.D	0.34	0.25	1.18	1.71	0.27	0.48	1.63	0.53	0.65	0.58

[1] as fed basis

[2] dry matter basis

[3] only barleys subjected to an *in vivo* trial (refer 3. 2. 1)

The crude protein content (as fed basis) averaged 9.7 % (ranging from 7.8 to 11.7 %), with 4 samples being below 9 %. These came from Manawatu, Hawke's Bay and Canterbury and were from different varieties. The mean fibre levels in terms of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Lignin were 16.45%, 4.24%, 1.16%, respectively. The gross energy (GE) content for the 17 samples varied from 15.2 to 16.6 MJ /kg on as fed basis with an average of 15.8 MJ /kg. The mean content of GI extracted β-glucans was quite lower than that for the total β-glucan's.

There appeared to be no close statistical associations between any of the individual physical parameters measured, except for a high negative correlation ($r = -0.76$, $P<0.001$) between screenings content and 1000 seed weight (Table 2. 5).

A significant negative correlation between crude protein content and NDF was observed ($r = -0.49$, $P < 0.05$). The correlation between crude protein and GE was positive ($r = 0.60$, $P < 0.05$). Due to the fact that cellulose content was calculated as ADF minus lignin and the hemi-cellulose as NDF minus ADF, there was a high correlation between lignin and NDF ($r=0.71$, $P< 0.01$); hemi-cellulose and NDF ($r =0.96$, $P < 0.001$); hemi-cellulose and lignin ($r=0.73$, $P < 0.001$); and cellulose and ADF ($r = 0.86$, $P < 0.001$) (Table 2. 5).

Table 2. 5 Correlation coefficients between chemical composition and physical characteristics of the barley Samples (as fed)

Items	GE	CP	NDF	LIG	ADF	HEM	CEL	TG	GIG	GBD	SCR	MOI	SW	YIL	SR	IV	RO
GE																	
ADE	0.78 **																
CP	0.60 *																
NDF	-0.11	-0.30															
LIG	-0.19	-0.27	0.71 **														
ADF	-0.16	-0.49 *	0.30	0.06													
HEM	-0.07	-0.17	0.96 ***	0.73 ***	0.03												
CEL	-0.04	-0.31	-0.09	-0.45 #	0.86 ***	-0.34											
TG	0.20	0.35	0.15	0.11	0.08	0.13	0.02										
GIG	0.43	0.33	-0.41	-0.50	0.18	-0.46	0.36	0.24									
BUS	-0.08	-0.24	-0.06	-0.20	-0.04	-0.05	0.06	0.21	0.46								
SCR	0.31	0.15	0.09	-0.33	0.39	-0.02	0.52 *	-0.07	0.14	-0.31							
MOI	-0.49 *	-0.22	-0.17	-0.04	0.14	-0.22	0.15	0.21	0.24	0.09	-0.35						
SW	-0.38	-0.24	-0.05	0.16	-0.33	0.05	-0.38	-0.42	-0.18	0.21	-0.76 ***	0.30					
YIL	-0.32	-0.25	-0.62 *	-0.39	0.21	-0.66 **	0.37	-0.42	0.46	0.34	-0.15	0.12	0.42				
SR	-0.40	-0.43 #	0.40	0.58 *	0.38	0.31	0.04	0.30	-0.48	-0.35	-0.18	0.41	-0.15	-0.42			
IV	0.19	0.22	-0.34	-0.50	-0.38	-0.45	-0.08	-0.32	0.43	0.48	-0.33	-0.004	0.26	0.43	-0.52		
RO	-0.02	0.02	0.43	0.60 *	-0.17	0.48	-0.38	0.14	-0.80 **	-0.52	-0.28	-0.22	0.17	-0.65	0.48	-0.36	

= p<0.1, * = p<0.05, ** = p<0.01 *** = p<0.001. GE = Gross Energy, CP = Crude protein, NDF = Neutral detergent fibre, LIG = lignin, ADF = Acid detergent fibre, HEM = Hemicellulose, CEL Cellulose, TG = Total ß-glucan, GIG = GI Extracted ß-glucan, GBD =grain bulk density, SCR=screenings, MOI = Moisture, SW = 1000 seed weight, YIL Yield, SR = Seed rate, IV=interval sowing-harvesting, RO = ratio of GI Extracted ß-glucan and total ß-glucan,

2. 4 DISCUSSION

The grain bulk density for the barley samples used in the present study was lower than that observed by Bhatty *et al.* (1974) but for all the barley samples it exceeded 60 kg/hl which, according to industry norms, indicates that the barley used in the present study was of "good quality". The large variation in screenings, and to a lesser extent, 1000 seed weight is in support of Bhatty *et al.*, (1974). The high negative correlation ($r=-0.76$, $P<0.001$) between screenings and 1000 seed weight indicates that 1000 seed weight is a useful indicator for farmers to select barley at market.

The determined chemical compositions of the barley were comparable to those recorded in other studies on barley quality (Batterham *et al.*, 1980; Bhatty *et al.*, 1974, 1975). The contents of NDF, ADF and crude protein found in the present study were similar to those obtained by Batterham *et al.* (1980). In the study of Batterham *et al.*(1980) the reported mean values (on an as fed basis) were 17.2, 17.2 and 9.2 for NDF, ADF and crude protein, respectively. However, the recorded mean crude protein content was lower than that found for hulless barleys reported by Mitchall *et al* (1976) and Bhatty *et al.* (1979). It is well known that hulless barley contains more crude protein and less fibre.

The mean gross energy content (18.2 MJ/kg dry matter) for the samples studied here was slightly higher than the value obtained by Bhatty *et al.* (1974). In the study of Bhatty *et al.* (1974) the mean value for gross energy in 29 Canadian cultivars of barley was 17.9 MJ/kg (dry matter). However, the gross energy content (dry matter basis) in regular and hulless Canadian barley observed by Mitchall *et al.* (1976) was slightly higher (18.8 and 18.7 MJ/kg dry matter for regular and hulless barley, respectively) than that found in this study. The differences may be due to the different varieties or cultivars and different climatic and soil conditions, which have all been shown to affect protein, fibre

and starch contents which in turn affect energy content (Knudsen *et al.,* 1987; Buchman, 1979; Hesselman and Thomake, 1982).

The mean content of total β-glucan in this study was consistent with that measured by Bhatty *et al.* (1991) but slightly lower than that rreported by Miller *et al.* (1994). The range of total β-glucan values was low (4.01-5.56%) for the barley samples measured in the present study. A higher range of total β-glucan values was reported for Australian (Henry, 1986), Scandinavian (Lehtonen and Aikasalo, 1987), and U. S. barleys (Aman and Graham, 1987). The total content of β-glucans varies with both genetic and environmental factors (Henry, 1986; Bhatty *et al.*, 1991; Campbell *et al.*, 1993).

The GI extracted β-glucan contents were lower than the total β-glucan contents. This is due to the different methodology used, with a different extraction reagent and different temperatures which would have extracted different amounts of β-glucan solubilized in the solution for analysis (McNab and Smithard, 1992). From a physiological point of view extraction at body temperature, 37°C, would seem more appropriate. It is interesting to note that for the ten barleys for which β-glucans were measured in the present study, the order of ranking across the barleys differed with the two methods. If the physiological GI extraction procedure is a reasonable simulator of *in vivo* digestion then this method may give rise to preferred data.

In the present study several physical characteristics and chemical components of New Zealand barley samples have been determined. The study provides previously unknown data on New Zealand barleys. However the effects of different varieties, locations or harvest times on the physical characteristics and chemical components have not been analysed due to the limited data and these should be investigated.

The ranges in gross energy and crude protein contents, although not large, were of economic significance, which means that it would be useful to be able to predict these entities. Although, numerous associations between various chemical and physical characters were studied here, few useful predictors were found.

CHAPTER 3

PREDICTION OF THE DIGESTIBLE ENERGY CONTENT OF NEW ZEALAND BARLEYS

3. 1 INTRODUCTION

The cost of feed is 60-70% of the total cost of pig production (Noblet *et al.*, 1993), with energy and protein representing the major quantitative components of the diet. Thus a proper evaluation of the energy content of feedstuffs and diets is of critical importance. It is important to be able to estimate the energy value of feedstuffs with precision and accuracy. It is not surprising, therefore, that considerable attention has been directed towards the development of systems for expressing both the energy requirements of animals and the energy value of feedstuffs. In the latter respect there has also been considerable emphasis placed on developing methods to rapidly predict the energy value of feeds. Several rapid and relatively inexpensive *in vitro* methods have been developed to allow the prediction of dietary energy digestibility in simple-stomached animals. These use either duodenal-jejunal fluid or a mixture of commercially available microbial enzymes (Furuya *et al.*, 1979; Graham *et el.*,1989a; Boisen and Eggum, 1991; Babinszky *et al.*, 1990; Boisen and Fernandez, 1995). The limited number of studies conducted to date indicate a high degree of correlation between *in vitro* predictions and *in vivo* values for the apparent faecal digestibility of dry matter and energy, especially when studies have included a wide range of ingredients. In spite of such methods showing promise, there have been few controlled studies evaluating their application within single ingredients. Boisen and Fernandez (1991) have developed an *in vitro* method to predict *in vivo* digestibility of energy and amino acids in single ingredients for the pig. Results have shown that *in vitro* dry matter digestibilities were highly correlated to *in vivo* energy digestibility within several feed ingredients.

The aims of this study were to determine the digestibility of energy (*in vivo*) in a range of New Zealand barley samples and to correlate the *in vivo* digestibility with chemical components and various physical characteristics. Secondly, the barley samples were subjected to a recently developed *in vitro* digestibility assay (Boisen and Fernandez, 1991) and the results were correlated with observations *in vivo.*

3. 2 MATERIALS AND METHODS

3. 2. 1 Determination of Apparent Energy Digestibility *in vivo*

3. 2. 1. 1 Preparation of barley diets

Ten barley samples were selected (from a total of 17 samples collected, refer 2. 2. 1) on the basis of their crude protein and fibre contents, to cover the range in gross chemical composition. Each sample was ground through a hammer mill using the normal screen size (4mm sieve) and diets were prepared using individual barley samples as the sole source of energy. Each diet was supplemented with a commercial (Danmix, Nutritech Ltd. Auckland, New Zealand) grower vitamin and mineral supplement (0.25% of the diet). Chromic oxide was added to each diet (0.4% as fed) as an indigestible marker for the determination of faecal digestibility values.

3. 2. 1. 2 Animals and Feeding

The digestibility trials were conducted at the Pig Research Unit, Massey University and involved a total of 60 Large White × (Landrace × Large White)

entire male pigs of 29.5 ± 3.75 kg (mean ± S. D) liveweight. The pigs were randomly selected and housed individually in single pens in a temperature-controlled room (20 ± 2°C) for 15 days. Six pigs were randomly assigned to each barley diet. The level of feeding was constant and was restricted to 10 per cent of metabolic body weight ($W^{0.75}$) and the food was given in two equal portions (09:00 and 16:00 h) daily. The diets were fed wet (2:1 ratio w/v), with fresh water being available between meals.

The pigs were fed the barley-based diet without chromic oxide for 5 days, after which time they were fed the test diet with chromium for a further 10 days (test period). Every morning the floors were washed as clean as possible. During the final 5 days of the test period fresh faecal samples were collected from each pig immediately upon being voided. These were frozen and later thawed, and bulked on a pig basis, sub-sampled, and then freeze-dried. Sub-samples of the test diets were built up by sampling the feed each day.

3. 2. 1. 3 Chemical Analysis

Prior to analysis, both diets and freeze-dried faecal samples were ground in a laboratory mill (1 mm mesh diameter sieve, Wiley *mill.* USA) Dry matter determination was performed in duplicate, as described earlier (refer 2. 2. 2). Duplicate determinations were also performed on diets and faeces for gross energy (AOAC 1984) using an adiabatic bomb calorimeter (refer 2. 2. 2).

The chromium contents of duplicate diet and faecal samples were determined using the method of Costigan and Ellis (1987). Samples of approximately 20 mg were placed in dry pre-weighed beakers and were weighed to the nearest 0.1 mg. The samples were oven dried at 105° C for 16 hours and cooled. After weighing, the samples were transferred to a furnace at 500°C overnight. Three ml $MnSO_2$/phosphoric acid solution were added to each

sample. The sample was covered with a glass plate and placed onto a pre-heated block (140°C) for 20 minutes. The beaker was removed from the block and 4 ml of 4.5% w/v KBO_3 added to each beaker. After covering the beakers with a glass plate, they were put onto a heated block (220°C) for a further 45 minutes. After removal from the heat block, 15 ml of 60°C distilled water were added to each beaker. The sample solution was made up to a volume of 50 ml in a flask and decanted to a P35 container. The Cr 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).

3. 2. 2 Determination of the *in vitro* Digestibility of Dry Matter

Seventeen barley samples (refer 2. 2 1), of which ten were used in the above described pig trials, were ground to pass through a screen with a pore size of 1.0 mm. Dry matter digestibility was determined using a three-step *in vitro* digestibility assay developed and described in detail by Boisen and Fernadez (1991).

3. 2. 2. 1 *In vitro* procedure

Step 1

0.5 g of each of the 17 barley samples was weighed (± 0.1 mg) into a 100 ml conical flask. A blank determination (without added sample) was also made. A small magnetic stirrer and 25 ml phosphate buffer A (0.2 mol/l, pH 6.0) were added to each flask, then 10 ml 0.2 mol/l HCL was added and the pH was adjusted to pH 2 with 1 mol/l HCL or 1 mol/l NaOH solution.Then 1 ml freshly prepared pepsin (2000FIP U/g, Merk art. no.7190) solution was added. The flask was closed with a rubber stopper and the samples were incubated at 40°C for 75 minutes under constant stirring.

Step 2

5 ml of a 0.6 mol/l NaOH solution and phosphate buffer B (0.2 mol/l, pH 6.8) were added to the mixture. The pH was adjusted to 6.8 with 1 mol/l NaOH or 1 mol/l HCL and then 1 ml of freshly prepared pancreatin (grade VI Sigma no. p-1750) solution was added. The flask was closed with a rubber stopper and the samples were further incubated at 40^{0}C for 3.5 hours.

Step 3

10 ml 0.2 mol/l EDTA solution was added to each sample and the pH was adjusted to 4.8 with 30% acetic acid. After adding 0.5 ml Viscozyme (Novo 120L, Denmark), the samples were incubated overnight (18 hours) at 40^{0}C with constant magnetic stirring.

The undigested residues were then collected in a filtration unit by using dried and pre-weighed glass filter crucibles (dia 3, pore size 40-90μm) and were dried overnight at 80^{0}C.

The *in vitro* digestibility of the dry matter was calculated using the sample dry matter (DM) and the undigested residue DM after correction for DM in the blank. The procedure was repeated three times for each barley sample, using duplicate sub-samples each time.

3. 2. 3 Data Analysis

In vivo apparent digestibility coefficients for gross energy (GE) were calculated using the following equation (Maynard *et al.*, 1979):

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

The energy digestibility data were subjected to a simple ANOVA (SAS Institute Inc, 1985). Liveweight was also included as a covariate in the model to test for any effects of liveweight on energy digestibility.

A linear model with the sample as a fixed effect was fitted to the *in vivo* energy digestibility data and to the *in vitro* dry matter digestibility data to calculate repeatability. Repeatability was calculated using the following equation:

$$\text{Repeatability (R)} = \delta_w^2 / (\delta_w^2 + \delta_e^2)$$

Where δ_w^2 represents mean square of differences among individual samples ($MS_w = \delta_e^2 + k_1\delta_w^2$, $\delta_w^2 = (MS_w - \delta_e^2)/k_1$), δ_e^2 represents mean square of the differences among measurements within the individual samples ($\delta_e^2 = Ms_e$), k_1 = number of measurements per individual.

Statistical correlations between physical measures, gross chemical characteristics (refer 2.2. 2), *in vitro* dry matter digestibility and *in vivo* energy digestibility were calculated. A stepwise regression analysis model (SAS) was used to derive equations to allow prediction of the apparent energy digestibility coefficient and the apparent digestible energy content of barley.

3. 3 RESULTS

3. 3. 1 The *in vivo* digestibility of energy in the New Zealand barley samples

The *in vivo* apparent digestibility of energy (DEc) in the ten barley samples was calculated (Appendix I) and mean (± SE) values are presented in Table 3. 1. The DEc ranged from 72.5 to 78.4%, with a mean of 75.8%.

Energy digestibility was significantly ($P<0.001$) different between the barley samples (Appendix IV). There was no effect ($P>0.05$) of liveweight of pig on the determined energy digestibility. The significance of differences between samples is presented in Appendix II. There was also a significant ($P<0.001$) effect of barley sample on the apparent digestible energy content (Appendix V)

Table 3. 1 The mean (± SE) apparent digestibility of energy and mean (± SE) digestible energy contents for the ten barley samples

Sample *	DEc (%)	ADE (MJ/kg) (as fed basis)	ADE (MJ/kg) (DM basis)
2	76.3(±0.46)	12.0(±0.07)	13.8(±0.08)
4	77.9(±0.71)	12.3(±0.11)	14.4(±0.13)
5	76.6(±0.80)	12.0(±0.12)	14.0(±0.15)
7	76.1(±0.86)	11.8(±0.13)	13.6(±0.15)
8	75.3(±0.46)	11.5(±0.07)	13.5(±0.08)
10	75.3(±1.08)	11.5(±0.16)	13.6(±0.19)
11	78.4(±0.39)	12.2(±0.06)	14.3(±0.07)
14	72.5(±1.03)	11.4(±0.16)	13.1(±0.19)
15	76.5(±0.50)	12.7(±0.08)	14.4(±0.09)
17	73.6(±1.40)	11.5(±0.22)	13.3(±0.25)
Overall Mean	75.8	11.9	13.8
S. D.	1.78	0.44	0.47

* for code see chapter 2 (Table 2.1)

DEc = Digestibility of Energy (%),

ADE = Apparent Digestible Energy (MJ/kg)

3. 3. 2 The *in vitro* digestibility of dry matter in the barley

The repeated measurements for the digestibility of dry matter (DDM) in the barley samples are presented in Table 3. 2. The *in vitro* DDM for the barley samples ranged from 84.5 to 87.6% with an overall mean value of 86.1 %. The DDM was significantly ($P<0.001$) affected by sample (Appendix VI).

The repeatability of *in vitro* DDM in seventeen barley samples was calculated based on a linear model with the sample as a fixed effect (Appendix VI). Repeatability of *in vitro* DDM was 0.68.

3. 3. 3 Prediction of energy digestibility and digestible energy contents

Simple correlation coefficients were calculated between various physical and chemical characteristics, and *in vitro* dry matter digestibility (DDM) and apparent energy digestibility (DEc) and the apparent digestible energy contents (ADE) (Table 3. 3).

The *in vitro* DDM was not significantly correlated to the *in vivo* energy digestibility or other physical/chemical characteristics, except for the GI extracted ß-glucan, which was significantly and negatively ($r = -0.62$, $P<0,05$) correlated to the *in vitro* DDM (Table 3. 3).

Table 3. 2 Repeated measurements (means of duplicates) for *in vitro* digestibility of dry matter (DDM %)

Sample Number	Assay Number			Mean ± S. E
	1	2	3	
1	85.6	85.5	85.9	85.6±0.09
2	84.7	84.8	84.5	84.7±0.13
3	86.4	86.7	86.5	86.5±0.13
4	86.9	87.1	87.0	87.1±0.21
5	87.5	87.6	87.7	87.6±0.07
6	86.9	86.8	86.9	86.9±0.13
7	86.3	86.7	86.7	86.6±0.30
8	87.2	87.2	87.6	87.3±0.15
9	85.1	85.8	86.8	85.9±0.32
10	85.2	86.3	85.5	85.6±0.31
11	84.6	85.4	86.3	85.4±0.30
12	86.1	86.6	87.4	86.7±0.27
13	87.2	87.3	87.5	87.4±0.08
14	85.1	85.0	86.0	85.4±0.41
15	83.6	84.5	85.5	84.52±0.46
16	87.0	87.2	85.6	86.0±0.33
17	83.7	85.7	83.9	84.5±0.40
Overall mean	85.8	86.4	86.3	86.1
S. D	1.21	0.98	1.07	0.99

Table 3. 3 Statistical correlations between physical characteristics, chemical components, *in vivo* energy digestibility and *in vitro* digestibility of dry matter.

	DEc (%)	Sig.	ADE (MJ/kg)	Sig.	DDM (%)	Sig.
GE (MJ/kg)	0.19	NS	0.78	**	-0.08	NS
Crude protein (%)	-0.20	NS	0.24	NS	-0.13	NS
NDF (%)	0.10	NS	0.00	NS	-0.23	NS
Lignin (%)	0.38	NS	0.26	NS	0.11	NS
ADF (%)	0.34	NS	0.16	NS	-0.28	NS
Hemicellulose (%)	-0.01	NS	-0.05	NS	-0.16	NS
Cellulose (%)	0.12	NS	0.03	NS	-0.30	NS
Total β -glucan (%)	0.44	NS	0.41	NS	-0.47	NS
GI β -glucan (%)	-0.24	NS	0.13	NS	-0.62	*
Grain bulk density (kg/hectolitre)	-0.38	NS	0.23	NS	-0.26	NS
Screenings (%)	-0.13	NS	0.04	NS	-0.40	NS
Moisture (%)	0.08	NS	-0.22	NS	-0.11	NS
1000 Seed wt (g)	-0.24	NS	-0.38	NS	0.42	#
Yield (t/ha)	-0.34	NS	-0.33	NS	0.08	NS
Seeding rate (%)	0.73	*	0.21	NS	0.08	NS
Interval Sowing-harvesting (days)	-0.56	#	-0.27	NS	0.04	NS
Ratio (GI/TG)	0.54	#	0.33	NS	-0.23	NS
ADE (MJ/kg)	0.77	**			-0.05	NS
DDM (%)	0.29	NS				

# P < 0.1, * P < 0.05, **P < 0.01. Ge=gross energy, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADE=apparent digestible eneergy, DDM=dry matter digestibility DEc=digestibility of energy.

Significant correlations with apparent energy digestibility were observed for seeding rate (r=0.73, P<0.05) and interval between sowing and harvesting (r = -0.56, P<0.1). None of the other physical measures were significantly correlated with the energy digestibility, though the correlation for the ratio of GI extracted β-glucan and total β-glucan was significant (r=0.54, P<0.1).

When physical data alone are available, stepwise regression analysis shows that the apparent digestibility coefficient of energy (DEc) can be predicted from seeding rate (SR):

DEc (%) =67.32 + 0.064×SR

Coefficient of determination (R^2) = 0.53
Residual standard deviation (RSD) =1.39 (%)

Correlations between seeding rate and other physical and chemical characteristics were not significant, except in the case of lignin (r=0.58, P<0.05). The reason for seeding rate being so highly correlated with apparent digestibility remains unclear.

Correlations between apparent energy digestibility (DEc) and gross energy content, crude protein, fibre content, total ß - glucan and GI extracted ß - glucan, were low and non-significant.

Since mathematically, digestible energy is the product of GE and a digestibility measure, there was a high correlation between ADE and DEc (r=0.77, P<0.01). There were no significant correlations between ADE and the chemical characteristics measured, except for the gross energy content which was highly significantly (r = 0.78, P<0.01) correlated with the ADE content of the barley samples.

The results of the stepwise regression analysis showed that the apparent digestible energy content (ADE) can be predicted from the gross energy content (GE) when only the gross chemical data are known.

ADE (MJ/kg fed basis) = -2.32 + 0.91 ×GE (MJ/kg as fed basis)

$R^2 = 0.61$ RSD = 0.31 MJ/kg

ADE (MJ/kg dry matter basis)= -10.48 + 1.33 × GE (MJ/kg dry matter basis)

$R^2 = 0.65$ RSD = 0.32 MJ/kg

When both physical and chemical data are available, stepwise regression analysis gave the following equations to predict apparent energy digestibility (DEc) and apparent digestible energy content (ADE):

DEc (%) = 12.94 - 0.62 × CP + 3.7 × GE + 0.08 × SR

$R^2 = 0.90$ RSD = 0.77%

Seeding rate alone explained 53% of the total variation, GE a further 27% and CP 9%.

ADE (MJ/kg as fed basis) = - 9.9 - 0.096 × CP + 1.34 × GE + 0.013 × SR

$R^2 = 0.96$ RSD = 0.12 MJ/kg

Gross energy alone explained 60% of the total variation, SR a further 32% and CP 4%.

3. 4. DISCUSSION

The overall mean value and the rather low degree of variation in *in vivo* energy digestibility (75.8 ± 0.56, Mean ± SE) between samples in this study agrees with the data of Batterham *et al.* (1980). Similar results were also obtained by Mitchall *et al.* (1976) and Bhatty *et al.* (1979). However, a slightly higher difference in energy digestibility (79 versus 86 %) has been observed by Bhatty *et al.* (1975), who used mice to evaluate sixteen Canadian barley varieties including hull and hulless cultivars. It is assumed that the difference in fibre content would be considerable between the hulled and hulless varieties. It is well known that fibre mainly exists in the hull fraction, and not only is fibre not digestible itself but also affects the digestiblity of other nutrients in the feed (Bell and Keith, 1994). The present values for the apparent digestible energy content of barley are similar to those reported in the literature (Batterham *et al.*, 1980; Just *et al.*, 1978, Bhatty *et al.*, 1974).

Although the variation in the digestible energy content of the barley samples studied was not very large, the maximum difference of 10.8% is economically significant, underlying the need for rapid methods for predicting energy digestibility.

Except for seeding rate, correlations between various physical characteristics (such as 1000 seed weight and grain bulk density) and energy digestibility or digestible energy content were not significant. This is in support of Bhatty *et al.* (1974) who used mice to determine the digestible energy content of 29 cultivars of Canadian barley and reported that physical measures of grain bulk density, plumpness, 1000 seed weight, and chemical components of the samples, with the exception of fibre content, were of little value in predicting digestible energy content. However, grain bulk density, 1000 seed weight, and other physical characteristics add market value to barley grain feed

and could be selected for without affecting energy digestibility (Bhatty *et al.*, 1974).

Total β-glucans have been found to be negatively correlated with the digestible energy content of barley for pigs (Miller *et al.*, 1994), but in the present study no significant correlation was found. There were also no significant correlations between apparent digestible energy content and other chemical characteristics determined, except for the gross energy content. Batterham *et al.* (1980) also reported that most of the variation in digestible energy content of barley was due to its gross energy content and a similar conclusion was made by Bhatty *et al.*(1974). Accordingly, the apparent digestible energy content of an individual barley sample can be predicted by knowing the gross energy content and multiplying by the digestibility factor of 0.76. This assumes that the sample was grown in New Zealand and has physical characteristics (such as seeding rate, 1000 seed weight,and grain bulk density) similar to those recorded in this study.

The *in vitro* DDM was not significantly correlated to *in vivo* energy digestibility and other chemical and physical characteristics, except for the GI extracted β-glucan, which was significantly and negatively correlated to the *in vitro* DDM. The high repeatability of measurements between batches and between duplicates, however, suggests that the *in vitro* method is precise.

The *in vitro* procedure described here (Boisen and Fernandez, 1991) has also been used (Jiai Chen, unpublished) at Massey University to determine the *in vitro* DDM for pollards and brans (wheat milling by-products). The wheat by-products were representative samples from experimental diets used in previous pig trials (Pearson, 1995). The samples were stored in a freezer until the *in vitro* assays were performed.

The *in vivo* digestibility was based on a total faecal collection method. For each by-product six 35 kg live-weight entire male pigs (Large White × Landrace) were included in a 6 × 6 randomised Latin Square Design. A basal diet (80% barley, 10% Chilean fish meal, 10% casein, plus a vitamin and mineral supplement) and the ten samples each combined with the basal diet (70%/30%) were fed (8% $LW^{0.75}$) to each pig for a 10 day period. *In vivo* digestibility was determined by correcting the determined digestibility of the experimental diet for the contribution of the basal diet.

The *in vivo* digestibility of energy (DEc) for the wheat by-products ranged from 52.7 to 84% and the *in vitro* dry matter digestibility (DDM) ranged from 53.7 to 86.8% (Appendix III). Correlation analysis indicated that the *in vitro* DDM was significantly related to the *in vivo* DEc ($r = 0.985$, $P < 0.001$). The prediction equation generated from regression analysis was:

In vivo DEc (%) = 1.42 + 0.93 *in vitro* DDM (%)

$R^2 = 0.97$, RSD = 2.05.

The data pertaining to barley reported here and those relating to the latter wheat milling by-products were combined and subjected to correlation analysis.

The effects of different feeds and their *in vitro* DDM on the *in vivo* energy digestibility of barley and those relating to the latter wheat milling by-products were analysed using a General Linear Model. The model included DDM as a covariate and feed (barley or wheat by-products) as a fixed effect. The interaction between DDM and feed was also included in the model. The results indicated that DEc was significantly affected by DDM ($p<0.001$) and different feed ingredients ($p<0.001$), but no difference between the slopes was observed (Appendix VII).

The *in vitro* DDM was highly correlated to the *in vivo* DEc (r = 0.96, P < 0.001) (Figure 3. 1). Therefore, DEc for barley and wheat milling by-products combined can be predicted using the following regression equation:

DEc (%) = 11.291 + 0.7664 DDM (%)

R^2 = 0.9248, RSD = 2.648 %.

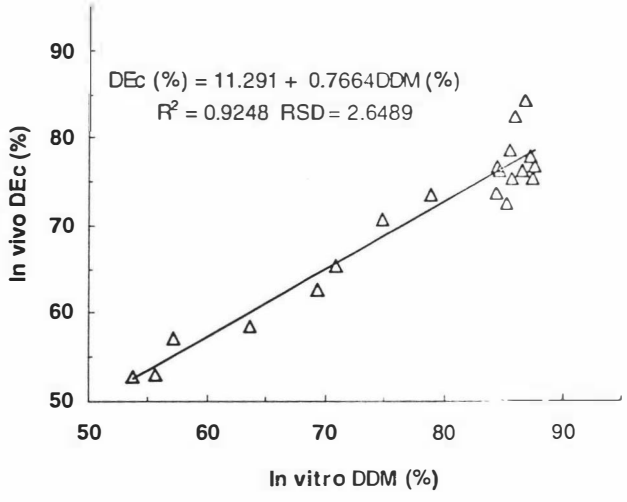

Figure 3 1 Relationship between *in vivo* DEc (%) and *in vitro* DDM (%) for barley and wheat by-products

The correlation between the predicted ADE (calculated by multiplying gross energy content by DEc predicted using the above equation,) and *in vivo* ADE was also calculated. As shown in Figure 3. 2, the predicted ADE was highly correlated to the *in vivo* ADE (r = 0.96, P < 0.001). The relationship between the *in vivo* ADE and the predicted ADE was expressed by the following equation:

In vivo ADE (MJ/kg fed basis) = -0.70550+ 1.0624 predicted ADE (MJ/kg fed basis).

$R^2 = 0.9157$, RSD = 0.4231.

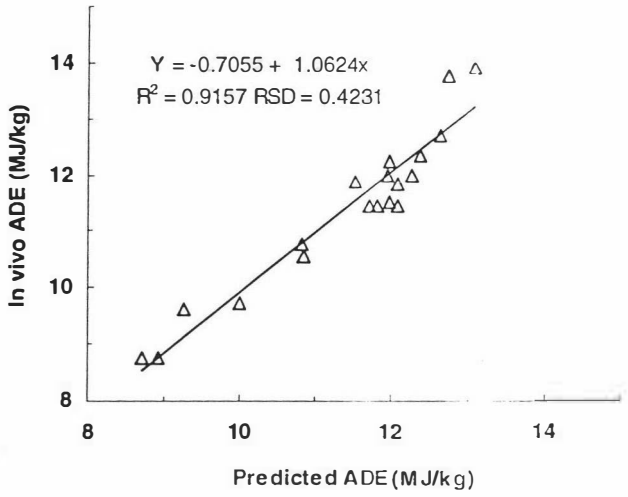

Figure 3. 2 Correlation between *in vivo* ADE and predicted ADE for barley and wheat by-products

A high correlation was obtained between the *in vitro* DDM and *in vivo* energy digestibility for the results of barley combined with that of pollards and brans. In some studies very high correlations between *in vitro* DDM and *in vivo* DEc have also been obtained (Boisen 1995, Pearson 1995) when a wide range of feedstuffs has been studied. The small range in *in vivo* energy digestibility values for the barley samples alone in this study could be the reason for the lack of correlation between the two methods. Overall, these results indicate that the *in vitro* dry matter digestibility assay is an accurate predictor for *in vivo* energy digestibility across feedstuffs but not necessarily accurate within feedstuffs.

The observed higher mean *in vitro* DDM than *in vivo* DEc is in support of the observations of Boisen (1991) and Eggum and Boisen (1991). This is likely to be because the *in vivo* measurements include variable amounts of endogenous and bacterial matter and are therefore 'apparent' measures of digestibility, while *in vitro* measurements correspond to 'true' values of

digestibility. Therefore, the *in vitro* values are expected to be higher than the *in vivo* values. However, the differences between the two methods for barley were higher than that observed for the wheat milling by-products. This may indicate that some anti-nutritional factors (such as β-glucans) in barley cause more *in vivo* endogenous matter than in wheat milling by-products.

In summary, the energy digestibility values provide important data. The small variation in energy digestibility between samples suggests that if the gross energy content of a sample of barley is known the apparent digestible energy content can be predicted from the gross energy. The high correlation between seeding rate and digestibility of energy may be useful from an agronomic respect, however this needs further investigation. The *in vitro* method used here is precise and may be able to be used to predict the apparent digestible energy content for alternative or new varieties of barley. Further work is required, however, before the method can be routinely applied within a feedstuff to predict relatively small but economically significant differences in digestible energy content.

CHAPTER 4

GENERAL DISCUSSION

The nutritive value of feedstuffs depends on both their nutrient content and nutrient digestibility. Therefore, in order to evaluate the nutritive value of feedstuffs, it is necessary to determine the contents of various nutrients and nutrient digestibility. The primary function of cereals in pig rations is to provide digestible energy for growth and production. Digestible energy is thus a key attribute of feed grains and should be a major parameter investigated in an evaluation of feedstuffs and should be a major factor for developing new cultivars. Determination of the digestible energy content, however, through the conduct of *in vivo* digestibility trials is expensive. Accordingly, much effort has been devoted to developing indirect methods to predict the digestible energy content of feedstuffs.

In this study no significant correlations between physical characteristics of barley and *in vivo* energy digestion coefficients determined in the pig were obtained, except in the case of seeding rate. Lack of correlation between some physical characteristics and digestible energy has been reported previously by Bhatty *et al.* (1974). The present results thus confirm that physical characteristics of barley are of little or no value in predicting the digestible energy content of barley. However, seeding rate seems to be a good predictor for the digestible energy content of barley. The reason for seeding rate being a good predictor for the digestibility of energy in barley is not clear. The finding has not been reported elsewhere and the observation needs to be confirmed by further investigations. In spite of a highly statistically significant correlation having been found, however, it is unlikely that seeding rate would ever be useful in feed evaluation practice. The finding, if it can be confirmed, is of particular agronomic interest.

No statistically significant correlations between chemical characteristics and digestible energy content or digestibility coefficient of energy were found, except for the gross energy content, which was highly correlated with apparent digestible energy content ($r = 0.78$, $P < 0.01$). Large significant correlations between digestible energy and gross energy have been obtained by Bhatty *et al.* (1974). A positive correlation between gross energy and digestible energy was also reported by Batterham *et al.* (1980). The present work further confirmed that gross energy is a useful indicator of the digestible energy content of barley.

No significant correlation between *in vitro* (Boisen 1991) dry matter digestibility and *in vivo* energy digestibility for barley samples was found. However, the high repeatability of measurements between batches and between duplicates suggests that the *in vitro* method is precise. Furthermore, when combined with results for pollards and brans (Pearson, 1995) correlation between the two methods was highly significant ($r = 0.96$, $p < 0.0001$). The lack of correlation between *in vitro* dry matter digestibility and *in vivo* energy digestibility for the barley samples alone may have resulted from the small variation in *in vivo* energy digestibility observed in the barley samples. This suggests that *in vitro* dry matter digestibility obtained with the assay described here is a good predictor for *in vivo* energy digestibility across feedstuffs but not necessarily within feedstuffs. Similar conclusions have been discussed by Boisen and Eggum (1991). More validation work, within feedstuffs needs to be conducted. If such assays are to have practical application in diet formulation, they need to differentiate among batches within a feed.

In conclusion, this study has identified characteristics in barley which are associated with digestible energy or the digestibility of energy. The mean digestibility values for energy and the range in values for a wide range of well defined barley samples, provides important data. The observed low variation in

energy digestibility between samples suggests that if the gross energy value is known a good approximation to the apparent digestible energy content can be made using the prediction equation (ADE = - 2.32 + 0.907 GE). According to this study, New Zealand-grown barleys often contain less energy than previously assumed based on tabulated values. The average value for digestible energy content for the seventeen barley samples tested here was 11.9 MJ/kg while the value used historically in New Zealand feed formulation has been 12.75 MJ/kg (Barugh, 1996). In this case, when formulating for a given lysine to DE ratio, pork producers may add unnecessary and expensive protein to diets resulting in an increased feed cost. Use of the presently reported digestible energy values for barley sown and harvested in 1995 can reduce feed cost by up to $10 per tonne for growers (Barugh, 1996).

The low statistical correlation between *in vitro* DDM and *in vivo* DEc for barley samples but a significantly high correlation for barley and wheat milling by-products combined indicates that the *in vitro* DDM can be a good predictor for feedingstuffs having a relatively high variation in digestibility. In addition, the high repeatability of the *in vitro* dry matter digestibility indicates that alternative or new varieties of barley can be screened for their apparent digestible energy content using this procedure. For the purposes of feed formulation, it is possible to quickly, easily and inexpensively identify a 'poor' barley from a 'good' one using the *in vitro* (Boisen and Fernandez, 1991) method. The method is promising and worthly of further refinement and testing.

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Appendices

Appendix I Liveweights of the pigs and chromium and energy contents of the barley diets and faeces

Sample	LW (kg)	GE (f)	Cr % (f)	GE (D)	Cr % (d)	DEc (%)
2	35.2	19.26	1.452	18.057	0.31	77.2278
2	33.8	19.88	1.366	18.057	0.31	75.0149
2	27.6	20.02	1.391	18.057	0.31	75.2911
2	28.2	19.59	1.524	18.057	0.31	77.9319
2	29.6	19.58	1.394	18.057	0.31	75.8862
2	35.8	19.3	1.399	18.057	0.31	76.316
4	35.0	19.75	1.598	18.512	0.321	78.569
4	30.2	20.12	1.553	18.512	0.321	77.5349
4	31.6	19.99	1.513	18.512	0.321	77.09
4	30.0	19.84	1.739	18.512	0.321	80.2169
4	29.2	20.15	1.405	18.512	0.321	75.1315
4	44.4	19.94	1.624	18.512	0.321	78.7093
5	31.6	20.16	1.52	18.232	0.337	75.4844
5	27.0	19.92	1.693	18.232	0.337	78.2516
5	32.6	20.11	1.767	18.232	0.337	78.9636
5	24.4	22.27	1.666	18.232	0.337	75.2918
5	28.6	20.14	1.645	18.232	0.337	77.3698
5	22.4	20.23	1.434	18.232	0.337	73.9239
7	30.0	19.59	1.808	17.831	0.338	79.4611
7	27.2	19.41	1.613	17.831	0.338	77.1896
7	30.4	19.72	1.397	17.831	0.338	73.2421
7	32.8	19.8	1.556	17.831	0.338	75.8789
7	26.6	19.52	1.508	17.831	0.338	75.4631
7	21.8	19.89	1.519	17.831	0.338	75.1791
8	24.4	19.61	1.456	17.9	0.337	74.6433
8	31.4	19.68	1.603	17.9	0.337	76.8864
8	26.0	19.62	1.479	17.9	0.337	75.0249
8	24.6	19.98	1.435	17.9	0.337	73.7868
8	29.2	19.83	1.497	17.9	0.337	75.0611
8	30.6	19.81	1.571	17.9	0.337	76.2598

To be continued

Sample	LW (kg)	GE (f)	Cr % (f)	GE (d)	Cr % (d)	DEc (%)
10	32.0	20.24	1.246	18.13	0.332	70.2537
10	30.6	20.22	1.572	18.13	0.332	76.4458
10	25.8	19.98	1.642	18.13	0.332	77.7176
10	26.8	19.99	1.546	18.13	0.332	76.3221
10	27.2	19.83	1.432	18.13	0.332	74.6417
10	33.2	20.29	1.557	18.13	0.332	76.1365
11	25.4	19.95	1.394	18.249	0.292	77.1006
11	30.8	19.73	1.511	18.249	0.292	79.1067
11	23.4	19.67	1.526	18.249	0.292	79.375
11	27.0	19.98	1.493	18.249	0.292	78.5869
11	28.6	20	1.517	18.249	0.292	78.9046
11	27.0	20.28	1.434	18.249	0.292	77.3711
14	34.6	19.65	1.431	18.042	0.321	75.5689
14	27.4	19.65	1.241	18.042	0.321	71.8284
14	26.4	20.28	1.145	18.042	0.321	68.4875
14	33.4	19.79	1.309	18.042	0.321	73.1016
14	28.8	20	1.251	18.042	0.321	71.5558
14	28.8	19.93	1.397	18.042	0.321	74.6177
15	25.2	20.16	1.548	18.771	0.327	77.3129
15	28.2	20.12	1.546	18.771	0.327	77.3286
15	30.8	20.08	1.519	18.771	0.327	76.9715
15	31.4	20.52	1.39	18.771	0.327	74.2828
15	31.6	20.33	1.479	18.771	0.327	76.0542
15	31.8	20.33	1.56	18.771	0.327	77.2975
17	26.2	20.35	1.509	18.008	0.337	74.7629
17	29.4	20.2	1.534	18.008	0.337	75.3572
17	29.6	21.1	1.185	18.008	0.337	66.6782
17	31.4	20.58	1.5	18.008	0.337	74.3245
17	30.8	20.23	1.514	18.008	0.337	74.9946
17	31.4	20.42	1.559	18.008	0.337	75.4883

LW = live weight of pigs, GE (f) = Gross energy content in faeces (MJ/kg), Cr % (f) = Chromium content in faeces, GE (d) = Gross energy content in barley diets, Cr % (d) = chromium content in barley diets, DEc = coefficient of energy digestibility, calculated by the equation in Chapter 2 (refer 2. 2. 3).

Appendix II Mean of energy digestibility and apparent digestible energy content for the barley samples

Sample	[2]Mean (DEc%)	[1]Level of significance	[3]ADE(MJ/kg) (fed basis)	[1] Level of significance	[3]ADE(MJ/kg) (dry matter)	[1]level of significance
2	76.28	A B	11.97	B C	13.77	B C
4	77.88	A B	12.34	B	14.42	A
5	76.55	A B	11.97	B C	13.96	A B
7	76.07	A B C	11.83	C D	13.56	B C D
8	75.28	B C	11.53	D	13.48	C D E
10	75.25	B C	11.46	D	13.64	B C D
11	78.41	A	12.22	B C	14.31	A
14	72.53	D	11.43	D	13.09	E
15	76.54	A B	12.71	A	14.37	A
17	73.60	D C	11.45	D	13.25	D E
Mean	75.84		11.89		13.78	
S. D.	1.78		0.44		0.47	

[1] Means with the same letter are not significantly different

[2] DEc = Digestibility Coefficient of Energy (%)

[3] ADE = Apparent Digestible Energy (MJ/kg)

Appendix III Energy content (as fed basis), digestibility of energy, and dry matter digestibility in pollard and bran samples

Items	B1	B2	B3	B4	B5	P1	P2	P3	P4	P5
GE	16.79	16.53	16.59	16.48	16.65	16.79	16.84	16.93	16.67	16.37
DEc	57.22	52.93	52.67	65.31	58.43	70.71	62.71	82.26	73.36	84.14
ADE	9.61	8.75	8.74	10.76	9.73	11.87	10.56	13.93	12.23	13.77
DDM	57.21	55.62	53.68	70.93	63.57	74.80	69.33	86.08	78.92	86.82

B = brans, P = pollards, GE = gross energy (MJ/kg), DEc = digestibility of energy (%),
ADE = apparent digestible energy content (MJ/kg), DDM = *in vitro* digestibility of dry matter.

Appendix iv Variance Analysis for energy digestibility in the barley samples

Source	DF	Sum of Square	Mean Square	F Value	Pr > F
Between Samples	9	0.01717443	0.00190827	4.65	0.0002
Within Samples	50	0.02052016	0.00041040		
Total	59	0.03769459			

Appendix v Variance analysis of apparent digestible energy content in different barley samples

Source	DF	Sum of Square	Mean Square	F Value	Pr > F
Between Samples	9	10.34461388	1.14940154	11.51	0.0001
Within Samples	50	4.991955	0.09983910		
Total	59	15.33656888			

Appendix vi Variance analysis of in vitro dry matter digestibility in seventeen barley samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Between Samples	16	99.57700915	6.22356307	13.98	0.0001
Within Samples	85	37.82717540	0.44502559		
Total	101	137.40418455			

Appendix vii. The effect of DDM and different feed on the digestibility coefficient of energy

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DDM	1	1552.934224	1552.934224	416.25	0.0001
Feed	1	63.487993	63.487993	17.02	0.0008
DDM*Feed	1	3.119913	3.119913	0.84	0.3740