EFFECTS OF β-GLUCAN AND NON-STARCH POLYSACCHARIDES ON ILEAL AND FAECAL ENERGY, NITROGEN, ILEAL APPARENT AND TRUE AMINO ACID DIGESTIBILITY IN THE GROWING PIG

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Abstract

It is generally accepted that soluble non-starch polysaccharides (NSP) in cereals such as barley have a negative influence on the digestibility of energy, nitrogen, and amino acids in broiler chickens. However, the evidence in growing pigs for a similar effect of barley NSP on nutrient digestibility is less convincing. A major reason for this is that detailed investigations into the effect of barley NSP, predominantly β-glucan, have not been conducted. Therefore, the overall aim of this study was to elucidate the effect of NSP from a cohort of Australian barleys on the ileal and faecal digestibility of energy, nitrogen, and amino acids in growing pigs.

This study was a collaborative project with the South Australian Research and Development Institute (SARDI). Eleven Australian barley varieties (including a control barley) were fed as the sole source of protein and energy to Landrace X Large White male pigs (35-55kg) fitted with a T-piece PVC cannula. Celite® was added as the indigestible marker. All diets were cold-press pelleted. Test diets were given to pigs based on a Latin Square design for five days prior to a two day collection period. An enzymically-hydrolysed casein (EHC) diet was fed to pigs for quantitative determination of endogenous amino acid flows at the terminal ileum. Ileal nitrogen, energy, apparent and true amino acid digestibilities were determined with reference to the marker. Faecal nitrogen and energy digestibilities were determined in six of the barley diets that were examined in New Zealand. An experiment with five barley varieties and one control barley was conducted in South Australia, and the same experimental protocol was followed at Massey University.

The range in nutrient composition of Australian barley varieties was 7.6-14.2% CP, 12-21% NDF, 3-6% ADF, 0.5-1.5% Lignin, 2-4% total β-glucan, 0.21-0.34% soluble β-glucan, and 6-12% total amino acids on a dry matter basis. The six barleys that were examined in New Zealand were analysed for NSP and contained 11-17% total NSP, 7-11% insoluble NSP, and 2-7% soluble NSP. The contents of total NSP, soluble NSP, and soluble β-glucan were significantly correlated to CP content in barley ($p<0.01$, $p<0.001$, $p<0.05$, $p<0.01$, respectively).

The ileal and faecal energy digestibilities of the barleys ranged from 53.6 to 71.0% and from 79.2 to 82.5%, respectively. Ileal and faecal nitrogen digestibilities ranged from 52.5 to 76.0% and from 64.3% to 75.6%, respectively. The mean apparent and

true amino acid digestibilities were 69.7% and 84.1%, respectively. The mean endogenous Lysine flow determined under conditions of EHC/Ultrafiltration was 472μg/g dry matter intake.

Correlation analysis between the chemical composition of the barleys and nutrient digestibility found significant positive relationships between ileal nitrogen digestibility and crude protein content ($p<0.05$), soluble β-glucan ($p<0.05$), soluble NSP ($p<0.05$), and faecal nitrogen digestibility ($p<0.05$). Ileal energy digestibility was negatively correlated to insoluble NSP ($p<0.05$). No correlation was found between the chemical composition of barley and faecal nitrogen digestibility, while faecal energy digestibility was negatively correlated with NDF, ADF, and hemicellulose ($p<0.05$). The apparent ileal digestibility of essential amino acids was positively ($p<0.05 - 0.01$) correlated to ileal nitrogen digestibility, whereas no relationship ($p>0.05$) was found between true digestibility of essential amino acids and chemical composition of barley.

Mathematical investigations found that the ileal ($r^2=0.66$) and faecal energy digestibility ($r^2=0.73$) could be predicted from the concentration of insoluble NSP and hemicellulose contents of barleys, respectively. Ileal nitrogen digestibility ($r^2=0.80$) could be predicted from the concentration of CP and faecal nitrogen digestibility. Also, apparent ileal digestible lysine content ($r^2=0.99$) could be predicted from faecal nitrogen digestibility along with the content of lysine in the barley.

The anti-nutritive effects of NSP of Australian barleys were not observed in apparent ileal and faecal digestibilities of energy, nitrogen, and amino acids. However, a consistent tendency of the negative influence ($p<0.05 - p>0.05$) of NSP to true amino acid digestibility was demonstrated.

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GENERAL INTRODUCTION

Swine require balanced diets that provide adequate levels of all nutrients, including energy and amino acids. Feeding costs account for 60–70% of the total costs of swine production. Therefore, accurate evaluation of nutrient in feedstuffs and optimal utilisation of the feed by animals are important factors in terms of animal production. Barley is one of the main cereal grains used in swine production, along with corn and wheat. In spite of the low digestible energy content of barley for swine compared to that of wheat or corn, economics favour the use of barley in swine feed in some countries and under some conditions.

The availability of nutrients in cereal grains is often limited by the presence of antinutritional factors that may act on the nutrients themselves or cause a deleterious, physiological effect on the animal. The carbohydrate and lignin fraction of most vegetable feedstuffs makes up 60-80% of dry matter. The nutritive value of feedstuffs is thus highly dependent on the digestibility and utilisation of carbohydrates, as they are by far the largest contributors to the energy of the animal.

In monogastric animals, which cannot utilise cell walls to the same extent as ruminants except through products of hindgut fermentation, it is the cell wall contents which possess anti-nutritive effect. The cell walls of cereals are comprised primarily of complex carbohydrates, which are termed non-starch polysaccharides (NSP). Originally NSP were considered to make minor contributions to the nutrition of monogastric animals through limited fermentation in the hindgut. However, NSP have recently become a focus of discussion in monogastric nutrition, because they are poorly utilised and because some soluble NSP have been shown to have anti-nutritive properties in monogastric animal diets. This has been particularly well characterised by studies with broiler chickens where the soluble NSP were shown to depress the digestion of macronutrients, which resulted in performance losses (Choct and Annison, 1990; 1992[a;b]).

Since barley contains considerable amounts of soluble NSP (β-glucan and arabinoxylan) that has anti-nutritive properties, the deleterious effect of barley-based diets in chicks and pigs has been observed (White et al., 1983; Graham et al., 1986; Li et al., 1996).

Due to the physiological and anatomical differences between pigs and chicks, however, literature values have shown that the pig is less responsible to the anti-nutritive effects of soluble NSP than chicks. Also, available data for pigs are seldom.

Therefore, the present study aimed to determine amino acid and energy digestibility coefficient value of barley in pigs and to find correlations between chemical composition and digestibility coefficients, with particular emphasis between soluble β-glucan contents and digestibility coefficients.

Since the soluble β-glucan depresses energy and amino acid digestibilities in the pig and chicken (White et al., 1981; Graham et al., 1989; Li et al., 1996; Leterme et al., 1998), decreased digestibilities of true ileal amino acid and energy are expected in barley cultivars with high soluble β-glucan contents. Also, as found in the study of rats (Meads et al., 1997), a strong correlation is expected between *in vitro* extracted β-glucan contents rather than total β-glucan contents in barley and *in vivo* nutrients digestibilities.

Chapter 1

Review of Literature

1.1 Introduction

Evaluation of feedstuffs is of importance since accurate diet formulation and the subsequent efficient use of available feeds requires an assessment of digestible energy and digestible amino acid profiles. The between and within variation in digestibility of feedstuffs is related to both chemical and physical factors, which may be related to the cultivars, variety and climatic condition.

This review first considers the physiology of digestion and absorption in the pig. Second, the energy evaluation system for feedstuffs and determination method in pigs will be discussed. Third, protein and amino acid evaluation method and the factors affecting the accuracy of ileal amino acid digestibility are reviewed. Finally, detailed consideration is given to the chemistry and anti-nutritive effect of cereal non-starch polysaccharides in monogastric animal nutrition, with special reference to the pig.

1.2 Digestion and absorption in the pig

1.2.1 Morphology of the digestive tract

The digestive tract can be considered as a tube extending from mouth to anus, lined with mucous membrane, whose function is the prehension, ingestion, comminution, digestion and absorption of food, and the elimination of solid waste material. The various parts are mouth, pharynx, oesophagus, stomach, small and large intestine (Vonk and Western, 1984; McDonald et al., 1995). The teeth in the mouth include incisors for cutting food and molars that grind food into smaller particles. Saliva that acts as a lubricant contains α-amylase and varies in consistency depending on the diet fed. Saliva is secreted by the three salivary glands, which are the parotids, the submaxillary glands and sublingual glands, under the control of the automatic nervous system (Longland, 1991; McDonald et al., 1995). The tubuloacinar

glands in the oesophagus secret mucus that lubricates the food on its passage to the stomach (Longland, 1991).

The stomach can be divided into the oesophageal, cardiac, gastric and pyloric regions, the cardiac and pylorus being sphincters controlling the passage of food through the stomach. Thus, it acts as a temporary storage organ. The oesophageal area has no glands. The cardiac area that comprises about a third of the surface secretes viscous mucus formed of a gel-forming glycoprotein, which protects the epithelium from acid attack. The gastric gland region that covers a further third of the surface also secretes glycoprotein and produce hydrochloric acid by oxyntic cells. This region also produces pepsinogen. The pyloric region secretes protective mucus (Longland, 1991; McDonald et al., 1995).

The small intestine has been classically divided into the sections duodenum, jejunum and ileum, with the jejunum accounting for about 85% of its length (Friedrich, 1989; Longland, 1991). The majority of digestion and absorption occurs in the small intestine. The function of the duodenum is mixing digesta and secretion with the jejunal area being the site of absorption. The Brunner's glands in the duodenum produce an alkaline secretion that acts as a lubricant and also protects the duodenal wall from the hydrochloric acid entering from the stomach. Bile and pancreatic juices are secreted into the duodenum (Longland, 1991; McDonald et al., 1995).

The mucosa is that layer of the intestinal wall in which all uptake and transport processes take place. The extensively increased surface area provided by the villi on intestinal mucosa facilitates nutrient absorption. Low and Zebrowska (1989) indicated that the presence of villi increases the mucosa surface of pigs five times at the age of 4-5 months. The height and density of villi gradually decreases from the duodenum to the ileum and this results in a decrease in the total absorptive surface of the mucus membrane in the distal direction (Mouwen, 1970). The functional unit of intestinal absorption is the enterocyte. The whole of the luminal region of the enterocyte formed by the microvilli is called the brush-border region. The function of this region is hydrolysis of carbohydrates and proteins, and it has specific transport systems (Friedrich, 1989). Microvilli increase the apical surface of the absorptive cells by approximately 14 to 40 times. Goblet cells are mucus-secreting cells that are present throughout the epithelium and with increasing relative frequency from the proximal jejunum to the distal ileum. The mucus acts as a barrier that helps to protect the epithelium from potentially noxious intraluminal substances (Low and Zebrowska, 1989).

The large intestine consists of blind-ended caecum which continues in the colon at the point of ileal attachment (Longland, 1991). 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 mucosal membrane of both the caecum and colon is flat and does not have villi as in the small intestine, but there are small projections which increase the surface area (McDonald et al., 1995). The columnar cells are interspersed by large numbers of goblet cells that secrete a protective sulphated carbohydrate-protein complex that also acts as a lubricant (Low and Zebrowska, 1989). There is microflora throughout the small intestine that becomes progressively greater towards the large intestine. The majority of microbial fermentation occurs in the large intestine and the end products are absorbed across the mucosa mainly as short chain fatty acids (Longland, 1991; McDonald et al., 1995).

The muscular rectum has a simple structure of columnar epithelial cells interspersed with a few goblet cells and the anus is purely muscular with no mucosal cells (Low and Zebrowska, 1989).

1.2.2 Digestion in the pig

1.2.2.1 Carbohydrate digestion

The carbohydrate fraction in monogastric animal feeds is heterogeneous, but can generally be classified as storage carbohydrates (starch) and the non-starch polysaccharides (NSP) of plant cell walls (cellulose, hemicellulose and pectins). While the storage carbohydrates can be hydrolysed by the animal's enzymes, the NSP-fraction cannot be digested by the animal's enzymes but must be fermented by the gut microflora, yielding volatile fatty acids (VFA) and gases (Longland, 1991; Drochner, 1991). Low amounts of free sugars and oligosacchrides which are also found in animal feeds may be absorbed directly or fermented (Drochner, 1991).

Digestion in the mouth of the pig is negligible. Intake of dry feed induces secretion of saliva, containing water, mucus and α-amylase. However, the activity of salivary α-amylase is low. Salivary α-amylase acts on starch, glycogen and other oligo- and poly-saccharides that contain three or more α-(1$\rightarrow$4)-linked D-glucose units (McDonald et al., 1995). The internal linkages of amylose are hydrolysed with equal ease but the breakdown of terminal linkages is considerably low (Walker and Whelan, 1960). Therefore, the breakdown products of pig salivary α-amylase are maltose, maltotriose and some dextrins (Longland, 1991; Drochner, 1991). On the

other hand, amylopectin that contains a number of branched α-(1→6)-glucosidic bonds is not hydrolysed by α-amylase (Roberts and Whelan, 1960). The optimum pH for α-amylase is 7.0 and the lower pH limit is 3.5.

In the stomach, small amounts of sugar, starches, hemicelluloses and pectic substances breakdown due to the fermentative activity of the gastric microflora, and the main end product is lactic acid (Friend et al., 1963). The gastric mucosa is able to absorb limited amounts of VFA, but its efficiency is negligible (Argenzio and Southworth, 1974).

Storage carbohydrates are digested extensively in the small intestine. Digesta of low pH entering the small intestine from the stomach stimulates secretion of alkaline pancreatic juice, bile and the products of Brunner's glands that provide a suitable level of pH for carbohydrase activity. Pancreatic α-amylase break down α-(1→4) bonds in starch and produce large amounts of reducing sugars (especially maltose). Pancreatic α-amylase is produced 5-10 times more than the amount of substrate eaten by pigs (Longland, 1991; McDonald et al., 1995). The brush border in the small intestine mucosa produces maltases, lactase and trehalase. Among the maltases, isomaltase break down isomaltose and limited dextrins, sucrase hydrolyses sucrose into fructose and glucose, and glucoamylase I splits maltodextrose, starch, isomaltose, limited dextrins, tyranose and maltosucrose. Glucoamylase II has the same properties to those of glucoamylase I but this is quite a heat-resistant maltase (Drochner, 1991; Longland, 1991). Therefore, these maltases hydrolyse α-(1→6) linkages and the resulting end-products degraded to glucose either by α-amylase or maltases. The brush border lactase hydrolyses not only different types of β-galactosides but also β-glucosides. However, the concentration of lactase decreases with age. Trehaloses are hydrolysed by trehalase to two molecules of α-glucose (Longland, 1991; Drochner, 1991).

The structural carbohydrates such as cellulose, hemicellulose, and lignin are not hydrolysed by any other enzymes present in the digestive secretions of the pig. Also certain starches, such as raw potato starch, are not broken down by amylase, although more than 95% of commercial feed starches are hydrolysed at the end of the small intestine by gastrointestinal carbohydrate degrading enzymes (Bach Knudsen, 1991). In addition, carbohydrates trapped by lignified tissues are resistant to the action of digestive enzymes.

During the first 40 days of life, α-amylase secretion in the pancreatic tissue is almost absent, therefore, the pig has a limited capacity to digest starch at this stage.

Consequently, high starch intake at this time may induces digestive disturbances and proliferation of hindgut microflora (Mason, 1980).

Extensive microbial activity in the large intestine, especially in the caecum, is responsible for degrading those undigested carbohydrates by bacterial enzymes producing mainly VFA and gases (Longland, 1991; McDonald et al., 1995).

1.2.2.2 Protein digestion

Proteolysis starts in the stomach with the action of pepsin which hydrolyses the peptide bonds, mainly those adjacent to aromatic or dicarboxylic L-amino acids. Pepsins secreted in the inactive precursors, pepsinogens, and activated by hydrolytic removal of a peptide from the N terminal end of the molecule. Four pepsins have been found in the pig which have two pH optima, at 2 and 3.5, and activity declines above pH 3.6. Pepsins A and D are secreted by the gastric fundic mucosa and B and C are secreted by the pyloric mucosa. The end-products of gastric proteolysis are mainly polypeptides of variable chain length, and a few amino acids (Zebrowska, 1980; Low and Zebrowska, 1989; Longland, 1991; McDonald et al., 1995).

As the low pH gastric digesta moves into the small intestine, the hormone secretin and cholecystokinin are liberated from the mucosa of the small intestine into the blood. Secretin liberation is affected by low pH of digesta and stimulates secretion of bicarbonate ions in the pancreatic cells, while cholecystokinin, which is liberated by peptides and other digestive products in digesta, stimulates secretion of proteolytic enzymes (McDonald et al., 1995)

In the pancreatic juices two groups of proteases exist, the endo-peptidases and the exopeptidases (Low and Zebrowska, 1989; Longland, 1991). Trypsin, chymotrypsin and elastase are classified as endopeptidases which hydrolyse any susceptible peptide links in the chain. The major exopeptidases are the carboxypetidases A and B, which cleave only the terminal bond from the carboxyl end of the peptide chains. The end products of this enzymatic hydrolysis are di-, tri-, and oligo-peptides, with some free AAs. Before absorption in the small intestine, aminopeptidases which are produced in the small intestinal mucosa and are located both on the brush border and in the cytoplasm, complete protein digestion by removing a single AA residue from the amino end of a peptide chain (Low and Zebrowska, 1989; Longland, 1991).

In the same way as pepsin, all pancreatic proteolytic enzymes are secreted as inactive zymogens. Trypsinogen (zymogen of the trypsin) is activated by the action of

enterokinase, an enzyme liberated from the duodenal mucosa, and activates trypsinogen by removing a peptide from the N-terminal end of trypsinogen. Formation of trypsin is then autocatalytic, and once formed, trypsin catalyses the activation of the other pancreatic proteases (Longland, 1991; McDonald et al., 1995).

Microorganisms in the large intestine attack undigested exo- and endogenous AAs. There, considerable bacterial deamination, decarboxylation and transamination as well as synthesis of bacterial protein occur (Fauconneau and Michel, 1970; Mason, 1980; Wrong et al., 1981).

Due to minimal secretion of proteases in the caecum and colon mucosa and the rapid inactivation of proteolytic enzymes entering hindgut from the terminal ileum by hindgut microorganisms, endogenous proteolytic enzymes play a limited role in the digestion of nitrogenous compounds within the large intestine. The major end products of microbial protein breakdown in the large intestine are ammonia and VFA (Marson, 1980).

1.2.3 Absorption in the pig

1.2.3.1 Carbohydrate absorption

The digestion and absorption of carbohydrates have been reviewed by Wiseman (1964), Reiser (1976), Low (1980), Rérat (1981), and Linder (1991).

Only monosacchrides are absorbed from the intestinal lumen by passage through the mucosal epithelial cells into the blood stream (Orten and Neuhaus, 1982; Linder, 1991). The digestion of carbohydrates by pig gastrointestinal enzymes results in the production of monosaccharides. The formation of these simple sugars from disaccharides takes place on the surface of the microvillus membrane. Although passage of monosaccharides through the absorptive cell membrane may occur to some extent by simple diffusion (e.g. pentoses such as ribose, xylose), this is not the primary mechanism for the absorption of the hexoses, especially glucose and galactose (Wiseman, 1964; Reiser, 1976; Orten and Neuhaus, 1982; Linder, 1991; McDonald et al., 1995).

Glucose and galactose are transported by an energy dependent carrier, where the sugar molecules move into the absorptive cell along with sodium ions. Thus, glucose and galactose are transported against concentration gradient. Fructose is absorbed by facilitated diffusion on a membrane carrier. This is not an energy

requiring process, but the fructose is absorbed more quickly than by simple diffusion (Reiser, 1976; Orten and Neuhaus, 1982; Linder, 1991; McDonald et al., 1995).

The results of microbial fibre digestion in the large intestine are not sugars but mainly the VFAs. The VFAs are absorbed by simple diffusion through the membrane of the large intestine and contribute to the energy supply of the pig (Mason, 1980; McDonald et al., 1995).

1.2.3.2 Protein absorption

The digestion and absorption of protein have been reviewed by Gilter (1964), Cuthberston and Tilsone (1972), Erbersdobler (1973), Snook (1973), Rérat et al. (1976), Zebrowska (1980), Rérat (1981), Low and Zebrowska (1989), Friedrich (1989), and Rérat and Corring (1991).

Feed proteins are almost completely digested to AAs and peptides in the gastrointestinal tract and are absorbed in the small intestine. There is no evidence so far for a possible absorption of AAs or peptides from the stomach. The ability to absorb both free AAs and peptides is less in the duodenum than in the jejunum and the ileum (Zebrowska, 1980). It is well known that AAs and peptides are transported across the absorptive cells by different systems. The absorption of AAs involves an active transport mechanism requiring energy and specific transport proteins in the intestinal mucosal cells. The exact numbers and specificities of AA carriers which exist is still open to some debate but four main groups are generally accepted: neutral AAs, basic AAs, acidic AAs, and glycine and immino acids transporter. However, these mechanisms are not completely rigid and some AAs can be transferred by more than one system.

The absorption of peptides across the intestinal mucosa has been reviewed by Holdworth (1972), Silk (1974), Adibi (1975, 1985), Matthews (1975[a;b]), Adibi and Kim (1981), Smith (1983), Gardner (1984), Ganapathy and Leibach (1985), Silk et al. (1985), Steinhardt (1987), Friedrich (1989), Grimble and Silk (1989), Webb (1990), Rérat and Corring (1991), and Webb et al. (1992).

Only di- and tri-peptides with free AAs can be absorbed across the intestinal mucosa by specific peptide transporters, which operates against the concentration gradient. The absorbed tripeptides are split by tripeptidases that are distributed equally between the membrane and the cytoplasm. Enzymes that cleave dipeptides into two AAs are predominantly in the interior of the cell. The rate of peptide uptake from the intestinal lumen is generally higher than the rates of free AAs uptakes

(Matthews, 1975[a;b]; Adibi and Kim, 1981; Webb, 1990). The transport of peptides has several advantages to organisms. The competition among AAs for places in the four carriers of individual molecules is avoided when intact peptides are taken up across the lipid membrane. Moreover, the presence of peptide carriers minimises the energy expended to counter concentration gradients; dipeptides and tripeptides are quickly cleaved inside the cell, so that the concentration of the peptide chains does not rise to a level higher than in the lumen (Webb, 1990).

Since the AAs that are not hydrolysed in the small intestine are broken down into ammonia and VFAs in the large intestine by microorganisms, ammonia is the main nitrogenous compound absorbed from the large intestine. However, the active transport of methionine in the proximal colon of piglet within the 10 days of life (James and Smith, 1976) and simple diffusion of few AAs in the colon mucosa of the pig (Binder, 1970) have been reported. Since the digested and absorbed nitrogen in the large intestine is excreted in the urine, however, protein absorption in the large intestine has little significance in pig nutrition (Zebrowska, 1973; 1975; Just et al., 1981; Schmitz et al., 1991).

1.3 Energy evaluation in feedstuffs for the pig

A proper evaluation of the energy value of the monogastric animals is of importance because all animal feeding standards are based on energy needs; daily gain, feed conversion and carcass composition are influenced by the feeding standard. Therefore, a precise estimate of the energy value is important in order to meet the energy requirements of monogastric animals and maximise animal production or obtain a uniform quality product.

Energy value for monogastric animals is expressed in terms of digestible energy (DE), metabolisable energy (ME), and net energy (NE), along with various ways of calculating the energy value within each system. It is measured directly from calorimetric determinations (kJ or kcal) with correction factors for differences in chemical composition in the case of DE or ME, or based on regression equation derived from digestible nutrients or simply from crude nutrients. However, a given diet or ingredients is given different energy values according to the system of energy utilisation by animals and to the prediction methods used for each system.

1.3.1 Concepts of energy value

Alternative energy evaluation systems for pigs have been extensively reviewed by Just (1981; 1982), Morgan and Whittemore (1982), Morgan et al. (1987), Henry et al. (1988), Batterham (1990), Noblet and Henry (1991; 1993), Noble and Perez (1993), Noblet (1996), and Moughan and Smith (1996).

1.3.1.1 Gross energy (GE)

Gross energy (GE) 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. Since some energy is lost in faeces, in urine, as gaseous products of hind gut fermentation (methane, hydrogen) and as heat, GE is a poor guide to the nutritional value of a feed (Noblet, 1996).

1.3.1.2 Digestible energy (DE)

Digestible energy (DE) is defined as the feed GE consumed minus the energy losses in faeces. Total collection of faeces or use of feed markers along with faecal sampling are involved in digestibility trial to measure DE value of feeds for pigs. In fact, DE is not a true measure of the feed energy absorbed from digestive tract since endogenous losses (i.e. digestive secretion, and intestinal cell debris) are included in faeces. Furthermore, small amounts of various gases and heat from fermentation processes are produced but not usually measured and then considered as digested energy. Therefore, the DE concept corresponds to the apparent DE in feed (Henry et al., 1988; Noblet and Henry, 1993; Noblet, 1996).

The most significant disadvantage of the DE system is the fibre content of compounds, because high fibre contents causes of high digestive energy loss. Again, DE does not provide any indication of the real energy value of absorbed nutrients because it over estimates protein-rich foods and fibrous feeds to some extent, while the value of fat is underestimated (Noblet and Henry, 1993; Noblet, 1996)

1.3.1.3 Metabolisable energy (ME)

The Metabolisable energy (ME) content corresponds to the difference between DE content and energy losses in urine and gasses. Most of energy losses in gas are due to methane production. The measurement of methane production requires the animal to be housed in a respiration chamber. In fact, the losses of energy through methane are very low and are usually ignored. Henry et al. (1988) indicated that the energy losses in methane represent less than 0.5% of GE. Accordingly, ME in pig is simply determined by excluding the energy losses in urine from DE.

Since the urinary energy losses are closely dependent on the level and quality of dietary protein, the level of nitrogen retention for optimum protein utilisation or for zero nitrogen balance must be corrected to determine precise ME values (Henry et al., 1988; Moughan and Smith, 1996). The ME value takes accounts the metabolic process for energy evaluation and it allows a better evaluation of protein-rich feedstuff but it shares other shortcomings with the DE value.

1.3.1.4 Net energy (NE)

The net energy (NE) content of a food corresponds to its ME content minus the heat increment (HI), which is the amount of heat released due to the energy cost of the ingestion and digestion processes and nutrient metabolism. The NE is, thus, the energy available for maintenance (NEm) and production (NEp). The NE value can be measured by a feeding a particular diet and determining the energy lost in the heat increment, either by calorimetry or by comparative slaughter technique (Just, 1982). Net energy determinations require sophisticated equipment (respiration chambers or calorimeters) or complex methods (comparative slaughter techniques). Furthermore, this system is largely depend on assumptions which are based on more variable or complex measurement of heat production, especially for fasting heat production, and should be accomplished with balanced diets at constant levels. Additionally, the value of a given feed is related to its final utilisation such as maintenance, growth or milk production and their combinations for the pig (Noblet and Henry, 1991; Noblet and Perez, 1993; Noblet, 1996).

Net energy is the closest estimate of the "true" energy value as it is proportional to the value of different diets for production in question. However, determinations of NE are both costly and complicated. Therefore, the NE value of feeds usually

calculated from prediction equations, either for fattening or for growth. A problem of growing importance with the use of diversified feedstuffs and complex diets is that of non-additivity in digestive and metabolic utilisation. Thus, another problem of the NE system is that the compound feeds are not additive with this system. For practical application, it has to be proved that NE gives a significant advantage over ME for predicting the energy value (Henry et al., 1988; Noblet and Henry, 1991; 1993; Noblet, 1996).

1.3.2 In vivo determination of energy values in feedstuffs

1.3.2.1 Digestible energy (DE)

The DE value for manogastric animals can be obtained directly for animals kept in metabolism crates from determination of the amounts of dietary and faecal energy. Tabulated DE value are usually determined from digestibility trials. This method is feasible for routinely assessing limited numbers of mixed diets. However, on a large number of samples it is not realistic, because the digestibility trial is inadequate in terms of both time and cost (Noblet and Henry, 1993; Henry et al, 1988). An advantage of DE value is that it is additive. Theoretically, the DE value of compound diets can be obtained by adding the DE value contributions of ingredients and assuming that DE is additive (i.e., energy contributions for unit of feed is constant and independents of the other components of the diet). However, in many circumstances, the ingredient composition is unknown and consequently methods of predicting DE is then required. The alternative approach is to predict energy content of raw materials based on their crude chemical composition and estimation of digestibility coefficients of nutrients. The DE content is then predicted from regression equations. However, this method assumes that digestibility coefficients are constant irrespective of the nutrient level in the feed and the presence of other nutrients (Noble and Henry, 1993).

The DE content of a diet can be obtained using prediction equations based on chemical composition of diets when the actual composition of feed is unknown. Numerous investigations have shown that (1) Crude protein (Wiseman and Cole, 1980; Batterham et al., 1980[b]; Morgan et al., 1987), fat (Wiseman and Cole, 1980; Morgan et al., 1987), and nitrogen free extract (or sugar and starch) contributes positively to the DE value of diets; (2) ash tends to act as an energy diluent and thus has a negative influence (King and Taverner, 1975; Just et al., 1984; Morgan et al.,

1987); (3) Fibre contributes in a negative manner (King and Taverner, 1975; Batterham et al., 1980[a]; Morgan et al, 1987). The limitation of these equations is their inability to consider the nature of fibre and the composition of fat. Therefore, when the DE value is predicted from these equations, low digestible fibre will be overestimated while high digestible fibre is underestimated.

1.3.2.2 Metabolisable energy (ME)

In order to measure ME precisely, energy losses in urine as well as gases should be taken into account, and thus a respiration chamber is required. However, losses of gas energy in pigs are small (<1%) and usually ignored. In contrast, energy lost in urine is not constant and highly related to the amount of nitrogen in the urine. Therefore, it is closely dependent on the raw material or dietary protein level and especially the AA balance. Consequently, ME values are not additive. Therefore, the ME values are normally corrected to a given level of nitrogen retention (normally 30% or 50%) or to a zero nitrogen balance in order to overcome this shortcoming (Batterham, 1990; Noblet and Henry, 1993; Noblet, 1996).

1.3.2.3 Net energy (NE)

Net energy value are determined by feeding a particular diet and measuring heat increment (HI) either by calorimetry or by comparative slaughter technique (Batterham, 1990). When the animal is fasted, body reserves are used for maintenance (HI_m). Therefore, the efficiency of utilisation of ME(K_m) is as follow;

$$K_m = (\Delta ME - HI_m)/ \Delta ME$$

Where, ΔME is the difference between maintenance energy requirements and metabolisable energy intake, HI_m is maintenance heat increment.

However, only total energy retention or total heat production can be measured directly and then HI is calculated as the slope of the linear regression of heat production on ME.

In order to compare HI or efficiencies of utilisation or ME in different feeds, feeding similar energy levels and keeping a constant composition of the retained energy (weight gain) are required due to HI not being constant over a large range of ME intakes for a given feed. In practice, only one feeding level is usually applied for each diet and the HI slopes are calculated by taking an estimate of fasting heat production (FHP), because HI measurement which involves feeding different energy levels for each diet are too complex and time-consuming. Again, the FHP value is not

measured but estimated by extrapolating heat production as measured at different energy levels to zero ME intake under the assumption that the efficiencies of ME for maintenance and for energy gain for a given diet are the same. Therefore, incorrect estimation of FHP will affect absolute NE value. Consequently, in order to overcome this problem, use of same genotype, sex, body weight, keeping controlled environment and same feeding level are usually recommended in the measurement of NE values (Batterham, 1990; Noblet and Henry, 1991; 1993; Noblet, 1996).

1.4 Protein and amino acid evaluation in feedstuffs for the pig

The nutritive value of protein is determined not only by its amino acid (AA) composition but also by its ability to supply biologically available AAs for protein synthesis. A very important factor in the practical formulation of diets for pig is a knowledge of AA digestibility. The better this information is, the more effectively the aim of increasing the efficiency of feedstuff conversion to meat can be realised. This is the reason that during last three decades much work has been directed towards determining AA digestibilities. As a result, many methods have been proposed for determining AA digestibilities.

1.4.1 Concepts of protein and AA digestibility values

Due to different methods of determining AA digestibilities the terms of digestibilities are different. Generally speaking, apparent digestibility is defined as the difference between the amount of the AA in the diet and in the ileal digesta or faeces, divided by the amount in the diet. True digestibility is defined in the same way except that the amounts of endogenous AAs in faeces or ileal digesta are subtracted from the total amount of AAs in the faeces or ileal digesta. Detailed concepts of digestibility values will be discussed below.

1.4.1.1 Faecal versus ileal digestibility

The most commonly used procedure for determining AA digestibility is the faecal digestibility method, which measures the difference between the amount of each of the AAs consumed in the feed and excreted in the corresponding faeces (Kuiken and Lyman, 1948). Faecal AAs are a mixture of those from undigested diet residues, endogenous secretions and bacteria (Just, 1980); those of undigested dietary origin

appear to account for less than 10% of the total (Low, 1982[b]). Therefore the digestibility value determined by this method is the apparent digestibility. Apparent AA digestibilities corrected for endogenous AA losses, determined via direct or regression method, are referred to as true AA digestibilities.

Until the 1970s it was generally thought that faecal apparent or true AA digestibility measurements were a reliable indicator of protein quality in monogastric animals. Therefore, the digestibilities of AA in a wide range of feedstuffs have been determined using this procedure (Tanksley and Knabe, 1984). However, experiments in which protein and free AAs were infused into the pig's large intestine (Zebrowska, 1973; 1975; Hodgdon et al., 1977; Gargallo and Zimmerman, 1981; Just et al., 1981) have shown that most of the nitrogen disappeared from the large intestine is not retained, indicating that the compounds absorbed are not used for protein synthesis by the pig (Buraczewaski, 1980; Wrong et al., 1981; Low and Zebrowska, 1989). The hindgut bacterial flora hydrolyse the nitrogenous compound and most of the nitrogen is absorbed as ammonia, amines or amides, and is largely excreted in the urine (Michel, 1966). Therefore, protein or AAs entering the large intestine make little or no contribution to the protein status of the pig. Furthermore, the predominance of bacterial nitrogen (62-76%) in the faeces provides evidence for hindgut fermentation of endogenous and exogenous nitrogen (Stephen and Cummings, 1980; Mason, 1984; Low and Zebrowska, 1989). Consequently, the breakdown of AAs in the hindgut to non-utilisable products of absorption results in the faecal method overestimating AA digestibility (Low, 1982[b]; Sauer et al, 1982[a]). Therefore, it is generally agreed that the ileal measurement is preferred to the faecal method as a means of determining AA digestibility in pigs (Zebrowska, 1973; Rérat, 1981; Tanksley and Knbe, 1984; Sauer and Ozimek, 1986; Low, 1990; Lenis, 1992).

1.4.1.2 Ileal digesta collection method

Numerous studies have been done on ileal collection methods in pigs and the comparison of the different methodologies for the measurement of digestion have been discussed in recent reviews (Sauer and Ozimek, 1986; Sauer et al., 1989[a]; Low, 1990; Fuller, 1991; Köhler, 1992; Batterham, 1994; Nyachoti et al., 1997).

- **Slaughter technique**

The most simple method to determine ileal digestibility is the slaughter technique which involves feeding experimental diet for 5-7 days and removal of the last 20cm of

the terminal ileum under anaesthesia (Payne et al., 1968; Kies et al., 1986; Moughan and Smith, 1987; George et al., 1988; van Barneveld et al., 1991). The AA digestibility is then measured with reference to an indigestible marker given with the experimental diet. It is relatively quick to conduct and there is no disruption of normal digestive function. Moreover, samples of digesta can be obtained from several parts of the digestive tract.

However, it is more difficult to collect representative samples of digesta, and replicated observations in the same animal are not possible which means animal variation cannot be taken into account in the statistical analysis. Furthermore, it can be expected that the shedding of mucosal cells into the intestinal lumen at death (Badawy et al., 1957; Fell, 1961) may have an influence on digestibility measurement of the nitrogenous compounds. Therefore, anaesthesia or euthanasia with a barbiturate such as sodium pentobarbitone should used to avoid shedding of mucosal cells (Badawy, 1964). In addition, this technique is expensive when applied to large animals since a large number of animals are needed to take into account the animal variation in this technique (Fuller, 1991) and is suited to small animal digestibility assay in which large numbers of animals can be used for treatment replication. The comparative study has demonstrated that the digestibility coefficients between slaughter technique and T-piece cannulation were similar (Moughan and Smith, 1987; Donkoh et al., 1995).

- **Cannulation technique**

T-cannulation

The simple T-cannulation, which involves surgical implantation of a T-piece cannula 5-15cm anterior to the ileo-caecal valve with spot sampling of ileal digesta, also requires the use of an indigestible marker. Unlike the re-entrant cannula, this technique avoids the transection of the small intestine and thus a more normal physiological state is maintained (Sauer et al., 1989[a]). In fact, similar voluntary feed intake and slightly lower growth rate (Livingston and McWilliam, 1985), similar growth rate and greater digestibility in dry matter, nitrogen and lysine (Jorgensen et al., 1985) have been reported when compared simple T-cannulation technique to unmodified animal or slaughter technique, respectively. Also, Moughan and Smith (1987) and Donkoh et al. (1994) reported no statistically significant differences in apparent ileal amino acid digestibility between T-cannulated and intact pigs. Furthermore, no significant differences in the digestibilities of dry matter, nitrogen or

AAs when a comparison of simple and re-entrant cannulas was made by Taverner et al. (1983). However, a difficulty in obtaining representative samples in T-cannulated pigs has been claimed in several studies (Zebrowska, 1978; Sauer and Ozimek, 1986; Leterme et al., 1990[a]). In contrast, Butts et al. (1993[b]) has shown that about 70-80% of digesta can be collected in T-cannulated pigs, if a continual collection is done.

PVTC cannulation

van Leeuwen et al. (1988) modified the simple T-cannulation technique to the post-valve T-caecum cannulation technique (PVTC), where a part of the caecum is removed and the one large T-cannula is joined with the remnants of the caecum directly opposite to the ileo-caecal valve. When the cannula is closed the digesta flows from the terminal ileum to the colon and, when the cannula is open, the ileal chyme flows from the ileo-caecal valve into the cannula by the over-pressure from the colon. In addition, the samples from the PVTC cannula can be assumed to be representative because marker recovery is about 100% (van Leeuwen et al., 1991). With this technique, an almost complete collection of ileal contents is possible. The results of the apparent ileal digestibility of dry matter and nitrogen determined in pigs fitted with the PVTC cannula were comparable with that determined in pigs fitted with simple or re-entrant canulas (den Hartog et al., 1988; Köhler et al., 1990). Also, as a modification of the PVTC cannulation, the steered ileo-caecal valve (SISV) cannulation has been reported by Mroz et al. (1991, 1996). Proliferation of fibrous tissue and dilation of the distal ileum by muscular hypertrophy, however, limits application of this technique to about 6-8 weeks (Mroz et al. 1991). Nevertheless, the simple T-cannulation and PVTC cannulation techniques have the distinct advantage that the functional integrity of the small intestine and of the ileo-caecal valve is maintained.

The re-entrant cannulation

The ileo-ileal and ileo-caecal re-entrant cannulation techniques were described by Cunningham et al. (1962) and Easter and Tanksley (1973) to overcome the uncertainties associated with obtaining representative samples via a simple T-cannula and the shortcomings of a digestibility marker (i.e. uniformity of markers in the diet and digesta, uncertainties concerning the markers absorbability). Pigs can be fitted with re-entrant cannulas either approximately 30 to 40cm anterior to the

sphincter (ileo-ileal) or in the ileum (5 to 10cm anterior to the sphincter) and caecum. This technique allows the quantitative collection of ileal digesta via the proximal cannula and the return of the remaining digesta into the animal via the distal cannula after sampling. Due to the total transection of the small intestine, however, this technique interrupts the transmission of the normal migrating myo-electric complex that is necessary for the normal digesta passage. Consequently, blockages of the cannulas and leakages around the cannulas have been reported (Zebrowska, 1978; Sauer and Ozimek, 1986). There seems to be more blockage of digesta in pigs fitted with ileo-ileal rather than ileo-caecal re-entrant cannulas. The comparative study of simple and re-entrant cannulas has found no significant differences in the digestibilities of dry matter, nitrogen or AAs (Taverner et al., 1983). Also, no effect of cannulation on the faecal digestibility was reported in the studies by Buraczewaska et al. (1979), Huisman et al. (1985), and Metz et al. (1985). However, Sauer et al. (1977[a]) and Jorgensen et al. (1985) reported higher faecal digestibilities of AAs, dry matter, nitrogen and lysine in cannulated than in intact pigs.

Darcy et al. (1980) modified the re-entrant cannula to overcome blockage problems of cannulas, the so called ileo-colic post-valve cannulation (ICPV). Since the proximal part of this re-entrant cannulation is formed into the remnants of the caecum, this technique preserves the functional role of the ileo-caecal valve. However, the blockage problems still occur when fed the pigs fibrous diets and surgical procedure is too complex for routine measurements to be feasible (Darcy-Vrillon and Laplace, 1990).

The ileo-rectal anastomosis technique (IRA)

The ileo-rectal anastomosis technique has been proposed as an alternative to the different cannulation techniques (Fuller and Livingston, 1982; Picard et al., 1984[a]; Darcy-Vrillon and Laplace, 1985; Souffrant et al, 1985; Green et al., 1987; Laplace et al., 1989; Green, 1988). This technique involves anastomosis of the terminal ileum to the rectum, thereby allowing the digesta to bypass the large intestine and allows total collection of ileal digesta. This technique assumes that the removal of the large intestine does not interfere with digestive physiology of the pig. Pigs prepared with the ileo-rectal anastomosis technique require much less time and effort to maintain compare to pigs fitted with re-entrant cannulas. Also food intake can be maintained at normal levels and high fibre diets can be tested. Due to the functional destruction of the large intestine, however, there are serious doubts concerning the physical normality of anastomised animals (Picard et al., 1984[a]; Moughan, 1991). In addition,

increased number of goblet cells, hypertrophy of smooth muscle, elongation of the crypts and atrophy of the enterocytes at the villus were observed when investigated the terminal ileum 26 weeks post-surgery (Fuller, 1991), while no changes were observed when conducted eight weeks post-surgery (Souffrant et al., 1985). Furthermore, the comparative study of nutrient digestibility between ICPV and the ileo-rectal anastomosis has shown that the digestibilities of dry matter, nitrogen and total AAs were significantly higher in ICPV pigs than anastomosized pig when fed high fibre diets (Darcy-Vrillon and Laplace, 1985, 1990). More recently, Köhler et al. (1992) found about a 53% reduction of the daily gain in end-to side ileo-rectal anastomosized pigs compared with intact pigs. This fact indicates a strong influence of the IRA technique on the energy metabolism. Furthermore, N-retention was lower in IRA pigs than PVTC or intact pigs due to the higher nitrogen excretion in the digesta and in the urine.

Mobile nylon bag technique

A rapid method for measurement of ileal digestibility of AA using a mobile nylon bag technique has been proposed (Sauer et al., 1983[a]; Leibholz and Gamnon, 1987; Cherian et al., 1988, 1989; Leibholz, 1991; van der Pole et al., 1991). The advantage of this method is the rapid measurement of large number of samples (Sauer et al, 1989[b]). However, due to the isolation of test material from contact with the gut wall, membrane-bound hydrolyses of AAs and the interaction between the antinutritional factors and the digestive tract are interrupted (Huisman et al, 1988; Cherian et al., 1989; Sauer et al., 1989[b]). Therefore, AA digestibility will be underestimated using this technique due to the interruption of hydrolysis of AAs in the intestinal brush border, or overestimated due to the decreased endogenous nitrogen when the test material rich in antinutritional factors which increase endogenous secretion in normal digestion (Kik et al., 1989).

1.4.1.3 Limitations of the ileal digesta collection method

The superiority of the ileal digesta collection method over the faeces collection method is now generally agreed for determination of AAs digestibility in monogastric animals. The digestibility value, however, does not provide complete information about availability of the absorbed nutrient (McNab, 1976; Batterham et al., 1990). Consequently, the ileal method creates inaccurate information for amino acid

availability if certain feedstuffs have undergone the Maillard reaction during heat processing, especially for lysine. This makes these amino acids not available for protein synthesis, and are hence excreted in the urine. Moreover, there may be a generally lowered *in vivo* digestibility of all AAs due to the formation of enzyme resistant cross-linkages and a possible direct effect of the advanced Maillard compounds on the digestive enzymes (Hurrelland Finot, 1985; Öste et al., 1986). However, except for lysine, tryptophan and the sulphur AAs, digestibility values of AAs for processed feeds, and for feeds not having undergone significant heat damage, should be a useful overall criterion of bioavailability. Also, ileal digestibility coefficients for lysine, tryptophan and sulphur AAs may well be satisfactory for practical dietary formulation purposes. Consequently, the relative quantity of unavailable lysine is small and does not affect significantly for digestibility assay (Austic, 1983).

A further limitation of the ileal method is that the microflora in the stomach (Schneider and Bolduan, 1985) and the terminal ileum (Rérat, 1990) may destroy or modify ingested nutrients. However, Dierick et al. (1986[a;b]) observed measurable but a small catabolism of AAs by the flora in the upper digestive tract of the pig.

In addition, digestibility values are affected by the sampling of the ileal digesta when determined by the slaughter or simple T-piece cannulation methods because digestibility measurements depend upon samples being representative and the validity of the marker used in these methods. Nevertheless, the digestibility coefficients determined by ileal method are reasonably accurate in describing the AAs uptake from the gastrointestinal tract (Low et al., 1982; Just et al., 1985; Moughan and Smith, 1985).

1.4.1.4 Factors affecting the accuracy of ileal AA digestibility

Various factors may influence the ileal digestibility of AAs in pigs.

- **Digesta collection method**

Picard et al. (1984[b]) conducted a comparative study to examine differences of apparent AA digestibility values among intact and caecectomised cockerels, cannulated pigs and ileo-rectal anastomised rats, and the results showed no significant variation in apparent ileal digestibility values of AAs. Also Leterme et al. (1990[a]) found no significant differences between anastomised and T-cannulated pigs in ileal AA digestibility values. However, a lower apparent digestibility of total nitrogen

and AAs in ileo-rectal anastomised pigs compared to an ileo-colic post-valve cannulation was reported (Darcy-Vrillon and Laplace, 1990). A further study by Köhler et al. (1991) demonstrated that digestibility values of crude protein and lysine in PVTC cannulated pigs were comparable with the values measured in re-entrant cannulated pigs, simple T-cannulated pigs and ileo-rectal anastomised pigs. Moreover, AA digestibility values determined by the slaughter technique were not significantly different to those determined in cannulation methods (George et al., 1988) and in the T-cannulation method (Moughan and Smith, 1987; van Barneveld et al, 1991).

Given that higher faecal digestibility values of AAs in ileo-caecal re-entrant cannulas (Sauer et al., 1977[a]; Jorgensen et al., 1985) and lower apparent ileal digestibilities of dry matter and nitrogen in ileo-rectal anastomosis (Darcy-Vrillon and Laplace, 1990) have been observed, it appears that PVTC cannulation, simple T-cannulation, or the slaughter method, are likely to yield the most reliable results for AA digestibility (Moughan and Donkoh, 1991).

- **Food intake**

There was no significant effect of feeding level on apparent ileal digestibility of AAs when pigs containing an ileo-caecal re-entrant cannula were fed a barley diet (Sauer et al., 1982[b]) and fed a corn-soybean meal diet (van Leeuwen et al., 1987). It is concluded that the level of feed intake has no effect on nutrient digestibility if highly digestible diets are used. However, there may be a decrease in apparent nutrient digestibility as the level of food intake is increased if less digestible diets are used (Sauer et al., 1982[b]; van Leeuwen et al., 1987).

- **Dietary fibre**

The influence of dietary fibre in digesta transit time is well recognised. The rate of passage generally decreases as the amount of fibre in the diet increases (Fioramonti and Bueno, 1980; Kuan et al., 1983; den Hartog et al., 1985). Dietary fibre also increases the endogenous nitrogen flow by increasing mucus production and sloughing off mucosal cells (Schneemann et al., 1982). Further, the thick cellulosic cell wall of dietary fibre may physically hinder the access of the proteolytic enzymes, thereby decreasing the AA digestibility. In fact, *in vitro* studies revealed that the activity of proteolytic enzymes was reduced when various fibre sources were incubated with pancreatic juice due to the absorption of these enzymes in the fibre structure (Schneeman, 1978). However, and in older pigs (120kg sows), inclusion of

fibre (5-7.5%) did not affect the ileal digestibility of AAs, probably due to sufficient secretion of proteolytic enzymes (den Hartog et al., 1988[a]).

Viscous-forming soluble fibres (NSP) generally obstruct the access of the digestive enzymes. Therefore soluble NSP decrease digestibilities of dry matter, nitrogen and starch at the terminal ileum. This point will be discussed extensively in part 4 of this review.

- **Dietary protein concentration**

The undigested dietary protein fraction increases as the dietary protein concentration increases, while the endogenous fraction remains fairly constant. Consequently, positive relationship between protein concentration in the diet and the apparent ileal digestibility of AAs have been found in many studies (Eggum, 1973; Sauer et al., 1980; Bell et al., 1983; Furuya and kaji, 1989[a]; Keith and Bell, 1991; Donkoh and Moughan, 1994; Fan et al., 1994; Pfeiffer et al., 1995; Angkanaporn et al., 1997). However, no effects of dietary protein level on apparent ileal AA digestibility were found in few studies (Buraczewaska and Haraczynski, 1983; van Leeuwen et al., 1987). It is well agreed that true ileal digestibility is independent of dietary protein concentration (Taverner, 1979; Green, 1987; McNab, 1989; Furuya and Kaji, 1989[a]; Zuprizal et al., 1991; Donkoh and Moughan, 1994; Pfeiffer et al., 1995; Angkanaporn et al., 1997).

- **Anti-nutritional factors**

Anti-nutritive factors which are found mainly in plants affect the digestion and absorption of dietary proteins by reacting with food proteins, thus making them less digestible, or by reacting with gut cells and affecting their secretory, or protective functions. Protease inhibitors (in unprocessed bean and peas etc.) inhibit the digestion of proteins in the small intestine by binding to the proteolytic enzymes. The antinutritive effects of trypsin inhibitors in soybean (Vandergrift et al., 1983; Ozimek and Sauer, 1985) and peas (Leterme et al., 1990[b]) on the ileal digestibilities of AAs have been reported.

The poly-phenolic compound tannins are known to bind to dietary proteins and proteolytic enzymes, thereby forming complexes resistant to digestion and absorption in animals (Eggum and Christensen, 1975; Krogdahl, 1987). It was reported by Cousins et al. (1981) that the apparent AA digestibility at the terminal ileum was lower in high-tannin sorghums than in low-tannin sorghums. However, Elkin et al (1996) found that other components besides tannins were responsible for variations in nutrient digestibilities of sorghum grain cultivars. Moreover, Yu et al. (1995; 1996)

reported that neither true ileal digestibilities of nitrogen and AAs or endogenous AA secretions were affected significantly by the condensed tannins in the cottonseed hull when rats were fed cottonseed-kernel-based diet, while a significant depression of the mean apparent and true ileal AA digestibilities was observed when rats were fed a casein-based diet. Therefore, the effect of condensed tannin on protein digestion may differ with source of protein.

Also, β-glucan and arabinoxylan in cell walls of some grains are known to decrease nutrient digestibility by reacting with digestive enzymes or forming a viscous solution in the digestive tract. This point will be discussed extensively in part 4 of this review.

- **Processing**

The processing of feed such as grinding and heating influences nutrient digestibility. Fine grinding of wheat (Sauer et al., 1977[b]) and decreased particle size of sorghum (Owsley et al., 1981) increased AAs digestibilities in pigs. Moderate heating generally increases nutrient digestibility while over-heating depresses AA digestibilities at terminal ileum by the Maillard reaction between free amino groups of protein and reducing sugars (Schutte et al., 1987).

1.4.1.5 Apparent and true amino acid digestibility

Apparent ileal digestibility is defined as following equation:

$$\text{Apparent ileal digestibility of AA (\%)} = \frac{\text{Dietary AA - Ileal AA flow}}{\text{Dietary AA}} \times 100$$

However, these data do not take into account any endogenous nitrogen excretions. These include undigested AAs from digestive secretions and from cells sloughed from the lining of the gastrointestinal tract during the passage of digesta. Correction of these endogenous losses from apparent ileal digestibility is defined as true ileal digestibility.

$$\text{True ileal digestibility of AA (\%)} = \frac{\text{Dietary AA - (Ileal AA - Endogenous AA)}}{\text{Dietary AA}} \times 100$$

The level of crude protein in the diet influences the apparent digestibility coefficient because the endogenous AAs contribute a high proportion in the low protein diet, while the proportion of endogenous AAs decreases as the protein level in the diet increases, resulting in an increased apparent ileal digestibility (Sauer et al., 1980; Bell et al., 1983; Furuya and Kaji, 1989[a]; Keith and Bell, 1991). Consequently, apparent digestibility is underestimated when it is determined in low-protein diets. Therefore, it is recommended that the diets should contain at least 150-160g crude protein per kg diet to determine apparent ileal AA digestibility (Sauer et al., 1989[a]).

Consequently, true AA digestibility has the advantage over apparent digestibility and it allows feed ingredients to be accurately compared, even if they are ingested in different quantities.

Since true rather than apparent digestibility is more additive (Taverner et al, 1981[b]), true digestibility should be used when calculating the AA digestibility in practical feed formulation (Green et al, 1987; Furuya and Kaji, 1989[b]). In contrast, several early studies recommended the use of apparent values for the formulation of diets (Low 1982[b]; Austic, 1983; Sauer et al, 1983[b]) due to the difficulty of accurately determining endogenous excretion (Kidder and Manners, 1978). Low (1980) proposed the use of apparent digestibility values rather than the true values for practical purpose, because the former indicates the net loss of AAs that results from feeding a test diet. However, true digestibility values provide more accurate coefficients than apparent values for the purpose of evaluating individual feedstuffs for diet formulations.

1.4.1.6 Endogenous nitrogen and ileal AA secretion in monogastric animals

The ileal digesta contains not only undigested nitrogen from exogenous protein but also nitrogen from endogenous origin such as enzymes, mucoproteins, desquamated epithelial cells, urea, AAs produced by cellular catabolism and albumin, bacteria and ingested hair (Rérat et al., 1976). To determine the quantities of endogenous nitrogen and AAs at the terminal ileum, the quantities of nitrogen and AAs secreted and digested are of importance, and hence this field has been studied extensively. Consequently, large amounts of data are available in previous studies even though large variations exist between studies due to experimental factors (live weight, surgical and experimental techniques, diet composition and level of feeding). About 60% of the total amount of nitrogen secreted into the pig gastrointestinal tract per day are derived from the small intestinal mucosa and about 25% of total nitrogen

are from gastric secretion (Butts, 1991). Similar result havs been indicated by Souffrant (1991) who showed that the endogenous nitrogen contributes 38-60% of total nitrogen intake with secretion from small intestine accounting for 22-27% of total nitrogen intake in pigs. The mean daily total AA output from the pancreatic, biliary and intestinal secretions measured at the end of the small intestine of pigs is 83g (70-92g) (Butts, 1991).

Endogenous nitrogen and AA secretions, however, change before reaching the terminal ileum because they are digested and absorbed along with the dietary nitrogen (Snook and Meyer, 1964[b]; Fauconneau and Michel, 1970; Buraczewaski, 1980). Early studies showed that endogenous proteins were digested and absorbed more slowly than dietary proteins in the rat (Ochoa-Solano and Gilter, 1968) and in pigs (Low 1982[a]). Other workers, however, found similar rates of disappearance between these two proteins in the rat (Nasset et al, 1973; Romero and Canolth, 1979). Later, it was realised that the digestibility of individual proteins varies widely (Alpers, 1987). Gastrointestinal mucin is resistant to enzymatic digestion (Hashimoto et al., 1963; Hoskins, 1978). Consequently, the four most abundant AAs in endogenous ileal digesta were proline, glycine, glutamic acid and aspartic acid in order of decreasing abundance (Taverner et al., 1981[a]), which are most abundant in muco-proteins (Horowitz, 1963; Taverner, 1979; Zebrowska, 1982). In fact, 75% of endogenous excretion was likely derived from the intestinal mucosa in the form of muco-protein and the protein present in sloughed epithelial cells (Fauconneau and Michel, 1970).

Digestion of the endogenous protein secretions occurs along the entire length of the intestine and is probably a continuous process. Rérat (1990) estimated that approximately 85% of endogenous protein secreted into the gastrointestinal tract of the pig was digested and absorbed. Also Réret et al. (1976) and Low (1982[a]) reported that 54% of secreted endogenous nitrogen was reabsorbed before it reach the terminal ileum.

It is well known that endogenous secretion is affected by the composition of the diet. Dietary peptides or proteins stimulate pancreatic secretion (Schneeman et al., 1982; Temler et al., 1983; Rérat et al., 1976) and antinutritional factors such as trypsin inhibitors and tannins increase the endogenous excretion of nitrogen (Green et al., 1973; Rostango et al., 1973). Moreover, fibre in the diet increases sloughing of intestinal mucosal cells (Bergner et al., 1975), stimulates mucus production (Schneeman et al., 1982), and increases daily nitrogen secretion in pancreatic juice

(Zebrowska, 1985). In addition, dietary fat has been shown to increase the quantity of nitrogen in the pancreatic juice of the pig (Ozimek et al., 1985).

1.4.1.7 Determination of the endogenous excretion of protein

The methodologies for estimating endogenous nitrogen are recently reviewed by Boisen and Moughan (1996) and Nyachoti et al. (1997). Traditionally the protein-free and regression methods have been used to determine endogenous nitrogen and AA excretions in ileal digesta. Due to the creation of non-physiological metabolism by these methods, however, other methods such as synthetic AA-based diets, guanidated proteins and radioactive isotopes have been developed and evaluated. All these methods are described and discussed below.

- **Protein-free method**

The most commonly used method for the measurement of endogenous ileal nitrogen flow is the protein-free method this involves feeding a nitrogen-free diet to the animal and determining the nitrogen and AAs in the ileal digesta. However, Low (1980) indicated that feeding a protein-free diet creates a physiologically abnormal metabolism in the animal. Based on previously published literature, the following limitations can be deduced when an animal is fed a protein-free diet; (1) Reduction of protein secretion into gastrointestinal tract (Snook and Meyer, 1964[b]; Fauconneau and Michel, 1970; Schneeman et al., 1977; Buraczewaska, 1979; Buraczewaski, 1980; Rodriguez et al., 1982), (2) Reduction of cell replication and cell protein turnover in the gastrointestinal tract (Munro and Goldberg, 1964; Millward et al., 1976; Simon, 1989; Muramatsu, 1990), (3) Elevation of break-down and re-utilisation of secreted enzymes (Snook and Meyer, 1964[a]; Fauconneau and Michel, 1970). Recently, evidence that a protein-free diet considerably underestimates ileal endogenous AA secretion in monogastric animals has been demonstrated (Moughan and Rutherfurd, 1990; Darragh et al., 1990; de Lange et al., 1990; Butts et al., 1993[a]).

Determination of endogenous ileal AA and nitrogen excretion using the protein-free method has been extensively studied by Green et al. (1987), Leibholz and Mollah (1988), de Lange et al. (1989[a;b]), Furuya and Kaji (1989[a]), Wang and Fuller (1989), Leterme et al. (1990[a]), Furuya and Kaji (1991), Hennig et al. (1991), Moughan and Schuttert (1991), Butts et al. (1993[a]), Mariscal-Landín et al. (1995), Darragh and Moughan (1998) and Viljoen et al., (1998).

Table. 1.1. Summery of literature values for endogenous ileal AA excretion (g Kg^{-1} DM intake) in the pig determined under protein-free alimentation.

AAs	Endogenous flow																Mean
Lys	0.27	0.53	0.25	0.47	0.27	0.48	0.53	0.63	0.26	0.38	0.23	0.46	0.35	0.25	0.24	0.24	0.37
Met	0.06	0.17	0.10	0.10	0.06	0.18	0.16	0.22	0.14	0.11	0.13	0.12	-	-	0.05	0.14	0.12
Cys	-	0.30	-	0.19	0.13	-	-	-	-	0.19	0.12	0.14	-	-	0.13	-	0.17
His	0.14	0.26	-	0.18	0.12	0.18	0.22	0.26	0.15	0.41	0.21	0.13	0.26	0.18	0.11	0.21	0.20
Phe	0.23	0.40	-	0.38	0.23	0.45	0.60	0.79	0.25	0.36	0.57	0.30	0.21	0.18	0.17	0.31	0.36
Tyr	0.13	0.38	-	0.33	0.21	0.36	0.41	0.47	0.30	0.35	0.35	0.19	0.31	0.18	0.89	0.23	0.34
Thr	0.39	0.97	0.53	0.48	0.35	0.43	0.65	0.91	0.46	0.87	0.69	0.34	0.67	0.47	0.49	0.65	0.58
Leu	0.39	0.76	0.34	0.64	0.40	0.72	0.60	0.77	0.41	0.62	0.62	0.49	0.46	0.36	0.40	0.65	0.54
Iso-Leu	0.21	0.79	0.19	0.34	0.24	0.60	0.36	0.47	0.23	0.28	0.31	0.33	0.22	0.22	0.23	0.41	0.34
Val	0.31	0.94	0.33	0.51	0.35	0.67	0.48	0.65	0.34	0.45	0.45	0.37	0.34	0.31	0.35	0.43	0.46
Ala	0.42	0.69	0.44	0.46	0.36	-	0.59	0.73	0.42	-	1.01	0.50	0.95	0.36	0.45	0.43	0.56
Asp	0.56	1.17	0.70	0.79	0.48	-	1.01	1.24	0.50	-	0.90	0.62	0.78	0.64	0.52	0.65	0.76
Arg	0.49	0.43	0.48	0.41	0.25	0.34	0.73	0.62	0.37	0.45	0.39	0.30	1.79	0.40	0.21	0.28	0.50
Ser	0.38	1.56	0.50	0.42	0.30	-	0.70	0.85	0.46	-	0.58	0.30	0.66	0.54	0.49	0.55	0.59
Glu	0.71	2.26	0.72	0.89	0.64	-	1.16	1.39	0.61	-	1.11	0.82	0.86	0.95	0.55	0.68	0.95
Gly	1.39	1.02	1.51	0.62	0.46	-	1.94	1.44	1.23	-	0.57	0.45	4.15	1.21	1.03	1.20	1.30
Pro	4.74	1.66	2.29	0.41	0.35	-	6.22	3.64	-	-	0.55	0.79	14.07	1.22	1.09	0.42	2.88
Total AA	10.95	14.47	8.38	7.58	5.20	-	16.36	15.08	6.13	-	9.28	-	-	-	-	-	10.38
Nitrogen	2.05	2.97	1.81	1.36	-	1.12	3.17	2.96	1.71	-	1.38	1.35	-	1.50	1.31	1.61	1.87
Ref.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

References

1. Sauer et al. (1977[a]), n=6, 45-70kg
2. van Weeden et al (1980), n=8, 45kg
3. Taverner et al. (1981[a]), n=5, 86kg
4. Darcy et al. (1982), n=5, 59kg
5. Green et al. (1987), n=4, 20-25kg
6. Leibholz and Mollah (1988), n=6, 25kg
7. de Lange et al. (1989[a]), n=8, 60kg
8. de Lange et al. (1989[b]), n=4, 55kg
9. Furuya and Kaji (1989[b]), n=4, 42kg
10. Wang and Fuller (1989), n=8, 25-30kg
11. Leterme et al. (1990[a]), n=6, 50kg
12. Henning et al. (1991), n=8, 144kg
13. Moughan and Schuttert (1991), n=6, 13.8kg
14. Butts et al. (1993[a]), n=8, 20kg
15. Mariscal-Landín et al. (1995), n=10, 35kg
16. Darragh and Moughan (1998), n=6, 2.3kg

From the above studies, the values of endogenous AA excretion vary between studies, especially for proline (see Table 1.1). Sauer (1982) demonstrated that the true digestibilities of proline and arginine were over estimated when determined under protein-free method.

Also, higher values of serine, threonine, proline, glycine, aspatic and glutamic acids in endogenous ileal AA excretions were reported when this method was used. These AAs are major constitutions of mucus glycoproteins (Hashimoto et al., 1963; Nemoto and Yosizawa, 1969; Bell and Kim, 1972; Cetta et al., 1972; Neutra and Fostner, 1987). Aspartic, glutamic acid and leucine are high in pancreatic and intestinal secretions (Corring and Jung, 1972; Buraczewaska, 1979; Pöhland et al., 1993), while glycine is a major constitution of bile secretion (Juste, 1982; Souffrant, 1991). Proline secretion, however, is relatively lower than other AAs in all the digestive organs except mucus (Buraczewaska, 1979; Just, 1982).

The high proline and glycine secretion measured under the protein-free method is probably due to low transport of proline and other AAs from the gut under protein-free alimentation which causes an increase in muscle break down and release of large quantities of glutamine which is metabolised to proline in the intestinal tract (Nagchaudri and Sharma, 1972; Karasov et al., 1986).

Dietary fibre in the protein-free diet affects the endogenous loss at the terminal ileum. Endogenous excretion of AA increases as the dietary fibre contents increase in the diet, due to increased cell sloughing and increased mucus and pancreatic secretions (Sauer et al., 1977[c]; van Weerden et al., 1980; Taverner et al., 1981[a]; Green et al., 1987; de Lange et al., 1989[a]). The absence of protein in the small intestine evokes physiologically abnormal metabolism (Low 1980) because the protein-free diet induces negative body nitrogen balance, therefore decreases body protein synthesis (Millward et al., 1976). Consequently, endogenous protein excretions and mucus production are decreased.

Evidence that the normal levels of digestive enzyme secretion, particularly the pancreatic enzymes, were affected by the presence of dietary peptide can be found (Snook, 1965; Fauconneau and Michel, 1970; Schneeman, 1982). In fact, a protein-free diet induces decreased mucus protein secretion and epithelial cell turnover (Munro and Goldberg, 1964; Fauconneau and Michel, 1970, Snook, 1973; Buraczewaska, 1979). It has been suggested that decreased endogenous AA excretion under protein-free alimentation is probably due to the reduced epithelial cell losses, reduced mucoprotein and digestive enzyme secretion, and elevated enzyme reutilisation by the host animal (Moughan and Rutherfurd, 1990).

- **Regression method**

The regression method is another traditionally used approach in determining endogenous nitrogen and AA loss in the distal ileum.

Table. 1.2. Summery of literature values for endogenous ileal AA excretion (g Kg^{-1} DM intake) in the pig determined by the regression method.

	INGREDIENT										
AA	Wheat	Barley	Ove -rall	Milk	Cotton-seed meal	Casein	Milk protein	Barley	Soy-bean meal	Soy-bean meal	Mean
Arg	0.62	0.58	0.53	0.29	0.40	0.37	1.44	0.25	0.64	0.68	0.58
His	-	-	-	0.15	0.13	0.14	0.33	-	0.23	0.24	0.21
Iso-Leu	0.15	0.14	0.15	0.61	0.59	0.23	0.80	0.28	0.46	0.45	0.39
Leu	0.33	0.43	0.33	0.64	0.67	0.41	1.14	0.58	0.69	0.69	0.60
Lys	0.33	0.28	0.32	0.37	0.37	0.26	1.05	0.32	0.47	0.48	0.42
Met	0.14	0.16	0.13	0.16	0.12	0.14	0.30	0.08	0.13	0.13	0.15
Phe	-	-	-	0.36	0.40	0.25	0.54	0.39	0.31	0.38	0.38
Thr	0.58	0.51	0.60	0.45	0.39	0.42	1.06	0.33	0.69	0.71	0.58
Val	0.55	0.28	0.42	0.56	0.69	0.33	1.41	0.35	0.54	0.56	0.57
Cys	-	-	-	-	-	-	0.30	-	0.20	0.21	0.24
Ala	0.62	0.59	0.58	-	-	0.41	1.22	0.28	0.59	0.56	0.61
Asp	0.72	0.84	0.82	-	-	0.47	1.18	0.47	0.90	0.90	0.86
Glu	0.82	0.91	0.85	-	-	0.55	2.24	1.07	1.06	0.98	1.06
Gly	2.27	2.12	1.71	-	-	1.23	2.76	0.25	1.17	1.19	1.57
Pro	3.67	4.26	1.73	-	-	-	-	-	-	-	3.22
Ser	0.62	0.60	0.63	-	-	0.46	1.60	0.33	0.68	0.72	0.71
Tyr	-	-	-	0.34	0.36	0.29	0.46	0.29	0.36	0.36	0.35
Total AA	11.42	11.69	8.80	-	-	5.96	18.46	5.27	9.12	9.24	9.98
Nitrogen	2.51	3.00	2.29	0.94	0.89	1.67	3.44	-	2.67	2.62	2.24
Reference	1	1	1	2	2	3	4	5	6	7	

References

1. Taverner et al. (1981[a]), n=2,2,7, 86kg
2. Libholz and Mollah (1988), n=6,6, 25kg
3. Furuya and Kaji (1989[b]), n=4, 42kg
4. Leibholz (1982), n=20, 4kg
5. Moughan et al. (1987), n=11, 26kg
6. Fan et al. (1995), n=6, 35kg
7. Fan and Sauer (1997), n=6, 35kg

This method involves feeding a range of diets with increasing levels of dietary protein, measuring the distal ileal AA flows, and determining endogenous nitrogen and AA loss by extrapolation to zero protein intake using linear regression (Carlson and Bayley, 1970; Tavemer et al., 1981[a]; Leibholz, 1982; Moughan et al., 1987; Leibholz and Mollah, 1988; Furuya and Kaji, 1989[a]; Fan et al., 1995; Fan and Sauer, 1997). This method is thought to be superior over the protein-free method because it

takes into account the influence of dietary protein and fibre. Due to the assumption that there is no change in the amount of endogenous AA excretions, however, the increased AA flow with increasing protein level is attributed entirely to increased amounts of undigested food

protein in the regression estimation. Also, several studies have shown that endogenous protein secretion from the pancreas was increased when dietary protein content was increased (Partidge et al., 1982; Hee et al., 1988). Moreover, several studies have shown that intestinal excretions of nitrogen and AAs vary with increasing levels of dietary protein (Snook and Meyer, 1964[a;b]; Lavau et al., 1974; Temler et al., 1983; Ozimeck et al., 1984). The concept that endogenous loss remains constant with increasing levels of protein intake is based on the finding that the absorptive capacity of the small intestine may increase as the dietary AA content is increased (Karasov and Diamond, 1987; Scharrer, 1989).

Therefore, the efficiency of AA and small peptide absorption may increase linearly in both of dietary and endogenous origin. Consequently, increased AA flow with increasing protein level is the result of elevated undigested protein and enhanced secretion of endogenous protein as well. Also, it is unlikely that a linear relation exists between feed intake and endogenous nitrogen or AAs in digesta or faeces because increased protein level is always associated with changes in dietary composition of diets, which hinders the interpretation of the results regarding cause and effect (Souffrant, 1991; Boisen and Moughan, 1996).

Literature values for endogenous AA flows at the distal ileum of the pig determined by the regression method are almost identical with that obtained by the protein-free method (See Table 1.2) (Darragh et al., 1995; Boisen and Moughan, 1996). This is clearly demonstrated in those studies where both methods have been used to determine the endogenous nitrogen and AA loss at the distal ileum of the pig (Taverner et al., 1981[a]; Leibholz and Mollah, 1988). Furthermore, and using rats and pigs, Moughan et al. (1987) demonstrated that the estimated endogenous loss, namely intercept values, were not significantly different from zero when using the regression method.

- **Enzyme hydrolysed casein (EHC)/ Ultra-filtration**

The EHC/Ultra-filtration method, which was developed recently by Moughan and Rutherfurd (1990) and Darragh et al. (1990), involves feeding pigs a semi-synthetic diet containing peptides and free AAs as the sole source of nitrogen, collection of digesta at the terminal ileum, centrifugation and ultrafiltration of the digesta, and then measurement of endogenous AA flow by adding the precipitate and retentate. During ultra-filtration, unabsorbed free AAs and peptides are discarded. Using this new method, significantly increased endogenous ileal protein loss was observed than under protein-free or a synthetic free AA feeding in rats (Moughan et al., 1990; Darragh et al., 1990; Butts et al., 1991) and in pigs (Butts et al., 1993[a]). Further, significantly higher endogenous flow of lysine at the terminal ileum of the rats fed a diet containing guanidinated protein than that of rats fed a protein-free diet was reported by Moughan and Rutherfurd (1990). More recently, a comparative study among EHC/Ultra-filtration, nitrogen-free and regression methods was conducted by Donkoh et al. (1995), and higher endogenous flows in the rat under EHC/Ultra-filtration method was observed (11.7, 6.9 and 6.4mg/g DM intake, respectively).

The determination of endogenous AA flow using the EHC/Ultra-filtration method, however, relies on the assumption that the dietary peptides and AAs are completely absorbed by the distal ileum of the rat. Consequently, the presence of dietary peptides and free AAs in the ileal digesta would result in the overestimation of endogenous ileal AA loss (Darragh et al., 1990; Moughan and Rutherfurd, 1990). In order to overcome this limitation, Moughan et al. (1990) used trichloroacetic and perchloric acids to separate endogenous protein from the dietary peptides and free AAs in the ileal digesta of rats. The nitrogenous fraction was separated physically using large volume disposable Centriprep-10 ultrafiltration devices (Amicon, W.R. Grace and Co., Danvers, Massachussetts). The endogenous AA flow is measured from the fraction, which contained the high molecular weight fraction (M.W. >10,000 Daltons). The low molecular weight fraction may contain non-protein nitrogen, endogenous free AAs and small peptides, and unabsorbed dietary AAs and peptides. However, the unabsorbed dietary AAs and peptides are expected to be at low concentration (Moughan et al., 1990; Butts et al., 1991). Nevertheless, removal of the low molecular fraction creates some under estimation of endogenous AA flow in the terminal ileum.

- **Homoarginine method**

The homoarginine method has been proposed by Hagemeister and Erbersdobler (1985) as a novel approach to determine the endogenous nitrogen loss at the terminal ileum, and has been applied in several studies (Siriwan and Bryden, 1987; Siriwan et al., 1987; Moughan and Rutherfurd, 1990). The homoarginine method, which is able to discriminate between endogenous and dietary protein, involves guanidation of dietary lysine by treatment with O-methylisourea that is transformed to homoarginine. It is assumed that guanidated and unreacted lysine are equally absorbed. Due to the absence of homoarginine in the mammalian body and endogenous secretions, and to the extremely low incorporation into endogenous protein, this method was considered as a more accurate technique for determination of endogenous nitrogen flow. This technique, however, has assumptions that the guanidation process does not influence the digestibility of protein, that homoarginine does not affect protein metabolism in the animal, and that arginase activity should be low enough within the gastrointestinal tract.

With regard to the first assumption, Moughan and Rutherfurd (1990) demonstrated that the degree of guanidation of gelatin had no significant effect on the lysine flows determined at the terminal ileum of the rat although the lysine in the dietary protein is unable to be completely converted into homoarginine. However, decreased milk protein digestibility following guanidation process has been reported recently (Drescher et al., 1994). Furthermore, the possibility of different digestibility between guanidated and unreacted lysine is claimed by Boisen and Moughan (1996). Also, Caine et al. (1998) demonstrated that guanidation of defatted soy flour changed the AA composition of the test meals, and ileal recoveries of endogenous AAs were increased in pigs fed guanidated protein test meals.

The homoarginine is absorbed in the intestine and part of the absorbed homoarginine reconverted to lysine by arginase in the liver, releasing urea. With regard to the latter assumption that arginase activity should be below enough within the gastrointestinal tract, an *in vitro* experiment by Schuttert et al. (1991) detected no arginase activity in the small intestine of the growing rat.

Nevertheless, the superiority of this method over protein-free and regression methods has been demonstrated by the finding that 90% of the AAs appearing in the ileum were of endogenous origin (Hagemeister and Erbersdobler, 1985; Siriwan and Bryden, 1987; Siriwan et al., 1987), and that endogenous lysine loss from the terminal ileum is considerably enhanced above that found with protein-free alimentation.

The major limitation of the method is that it provides direct information only for endogenous lysine flow. Other AAs are based on endogenous lysine flow, assuming the constancy of endogenous AA composition. Another limitation of this method is that guanidated protein cannot be fed for prolonged periods because homoarginine may accumulate in the body due to the slow rate of conversion of homoarginine to lysine, and may interfere with the urea cycle leading to an accumulation of ammonia in the body. In fact, decreased feed intake was observed with time over the experimental period (Moughan and Rutherfurd, 1990; 1991).

- **Isotope dilution techniques**

Another approach using radioactive isotopes or tracers (^{15}N, ^{14}C, ^{13}C, ^{35}S, ^{75}Se) to determine endogenous protein flow at the terminal ileum has been studied extensively (Nasset and Ju, 1961; Ochoa-solano and Gilter, 1968; Buraczewaska et al., 1979; Bergner et al., 1980; 1983; 1984; de Lange et al., 1990; Huisman et al., 1992; Moughan et al., 1992; Schulze et al., 1995[a;b]). This method involves labelling either the food or body protein using radioactive or stable isotopes and determining endogenous protein from the ileal digesta. Among the several isotopes, ^{15}N dilution method is most successful and commonly used method (Bergner et al., 1983; 1984; de Lange et al., 1990; Krawielitzki et al., 1990; Huisman et al., 1992; Moughan et al., 1992; Schulze et al., 1995[a;b]; Boisen and Moughan, 1996).

Using the ^{15}N stable isotopes, de Lange et al. (1990) and Schulze et al. (1995[b]) observed considerably enhanced endogenous nitrogen flow at the terminal ileum with increased dietary fibre. This method, however, has some difficulties for routine use because it requires further critical analysis include the method of labelling the animal's N pool, and the selection of the pool with a labelling level equal to that of total endogenous nitrogen (Souffrant et al., 1982; Souffrant, 1991). Also, the choice of the precursor pool has a significant effect on the dilution factor (Moughan et al., 1992).

Using an isotope technique, a differentiation can be made between non-digested dietary and endogenous protein (Souffrant et al., 1981; de Lange et al., 1990). However, the isotope dilution technique has been criticised because it can only be used to estimate total endogenous protein, not the levels of each of the AAs. Therefore the constancy of endogenous AA composition is assumed determined by the protein-free method. Nevertheless, Schulze et al. (1995[a]) demonstrated that the values of endogenous ileal protein loss determined under the EHC/utrafiltration

method and ^{15}N approach were similar in the growing pig. However, the usage of this technique may be limited by considerable expense and specialised equipment.

1.5 Chemistry and anti-nutritive effect of cereal non-starch polysaccharides (NSP) in monogastric animal nutrition

In the following section, the definition and chemical structure of major NSP present in wheat and barley will be described with the relationship between the chemical structure and the anti-nutritive effects. Also, the anti-nutritive effects of β-glucan and arabinoxylan and their different impact on pigs and poultry will be discussed.

1.5.1 Chemical characteristics of barley

Starch, dietary fibre and protein are the main components of barley grain, but it also contains low molecular weight sugars, fat and ash. For example, average chemical composition of barley in the study by Bach Knudsen et al. (1987) was: starch, 58.4%, dietary fibre 22.3%, protein 12.0%, fat 3.4%, low molecular weight sugar 2.2%, and ash 2.2%. In addition, barley contains both water-soluble and insoluble polysaccharides (NSP) which constitute between 2 and 16% of the dry matter (Åman and Graham, 1987[a]; Bhatty et al., 1991). Typical contents (% DM) of major NSP are: 7.9% arabinoxylan (0.8% soluble fraction), 4.3% β-glucan (3.6% soluble fraction), and 3.9% insoluble cellulose. Furthermore, barley contains about 0.2% each of mannose, galactose and uronic acid (Smits and Annison, 1996; Choct, 1997).

Due to the inverse relationship between starch and fibre components, however, considerable variation in the chemical composition of barley has been reported, particularly with regard to starch and dietary fibre. Åman et al. (1985) showed that the contents of Swedish hulled barleys (n=92) ranged from 53-67% starch, 14-25% dietary fibre, and 9-14% crude protein.

The crude fat, ash and low molecular weight sugars contents varied from 3-4%, 2-3% and 1-7%, respectively. Also, polysaccharide contents of barley varied from 4-11% arabinoxylans and 3-7% mixed-linked β-glucan (Salomonsson et al., 1984; Åman and Newman, 1987; Åman and Graham, 1987[b]).

The β-glucans are mainly present in the starch endosperm cell walls. Barley endosperm cell walls consist of about 20% of arabinoxylan and approximately 75% of β-glucan (Fincher, 1975). Waxy barley types contain more β-glucan than other barley types (Fincher, 1975; Klopfenstein, 1988). Part of the β-glucan is extractable in water (Åman and Graham, 1987[b]), and extractable high-molecular weight β-glucans give rise to high viscosity which limits the value of feed barley, especially in poultry diets (Campbell et al., 1989). In contrast to β-glucans, a large proportion of arabinoxylans are found in the husk and aleurone layer cell walls (McNeil et al., 1975; Bacic and Stone, 1981). The walls of barley aleurone cells are composed of 85% of arabinoxylan and 8% cellulose. Considerable amounts of xylose residues are substituted with α-L-arabinofuranosyl residues only at O2 of arabinoxylans in aleurone cell walls and in water unextractable arabinoxylans from dehusked barley (Vietor et al., 1992). However, a high degree of arabinose substitution in water extractable arabinoxylans from rye flower has been found to give more viscous solutions (Bengtsson et al., 1992). It has been shown that the enzymic degradation of arabinoxylans by endoxylanase is restricted by the presence of arabinoxyl substituents on the xylan backbone, and xylose substitution at O2 and O3 particularly hamper the action (Vietor et al., 1994). This may be of importance for the degradation of arabonoxylans in barley used as feed.

1.5.1.1 Factors influencing the chemical composition of barley

The contents of β-glucan and chemical composition in barley varies according to sample, genotype, environment and stage of maturity at harvest are known to play a role (Hesselman and Thomke, 1982). Hot and dry conditions during the ripening period and early harvest result in barleys with significantly increased soluble β-glucan contents (Hesselman et al., 1981; Hesselman and Åman, 1986). This is due to β-glucan synthesis in barley increasing the water-holding capacity, or to the inhibition of the formation or action of endogenous β-glucan hydrolysing enzymes. Moisture stress, brought on by drought conditions during kernel filling, elevates both acid-soluble and total β-glucan deposition. Also, the genetic background of barley is important for the variation in β-glucan contents since there are differences between cultivars, although the climatical factors and the stage of ripeness at harvest seem to be of great importance. Therefore, barley grown under warm and arid conditions, leading to an early harvest, increases β-glucan contents.

The nutrient content of barley grain varies with different varieties (Bhatty et al., 1974; Bach Knudsen et al., 1987) and with cultivar within varieties (Bhatty et al., 1975). The spring melting varieties contain higher starch and protein and lower fibre and total and soluble β-glucan than the spring feeding varieties.

In addition, locality is a one of the factor influencing chemical composition of barley. Bach Knudsen et al. (1987) reported that barley grown on clay soil 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% DM protein, 57.2% DM starch and 22.7% DM total dietary fibre).

1.5.1.2. Nutritional characteristics of barley

Barley is used in monogastric animal rations to provide energy and protein. Barley, on average, contains 11.5% crude protein, 1.9% crude fat, 5.0% crude fibre, 12.6MJ DE/kg, 0.08% calcium, 0.42% phosphrous and 2.5% ash (English et al., 1988). The digestible energy (DE) content of barley is significantly correlated with protein content and gross energy content (GE). The digestion coefficient of barley is positively correlated with bulk weight, plumpness, and ether extract, and is negatively correlated with fibre (Bhatty et al., 1974). Hull content is a major factor affecting the DE content of barley. Hulls and crude fibre content are highly negatively correlated with energy digestibility in barley ($r = -0.9$ for both factors) (Bell et al., 1983). However, since the hull content of barley varied with variety, the digestibility of energy and digestible energy content varied among varieties (Bhatty et al., 1975).

The DE 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 animal. Graham et al. (1986) showed that less than 5% of dietary starch escapes digestion in the small intestine, and Åman et al. (1985) and Bach Knudsen et al. (1987) demonstrated that cereal fibre (NSP and lignin) escaped digestion in the small intestine of the pigs. The net energy of carbohydrates hydrolysed and absorbed as monosaccharides from the small intestine is appreciably higher than that of carbohydrates fermented and absorbed as short chain fatty acids from the hindgut, although a significant amount of NSP is broken down by microbial enzyme in the hindgut. Both the composition of the NSP-fraction and the degree of lignification influence the extent of microbial breakdown of NSP in the hindgut. Therefore a higher dietary fibre content reduces DE and ME in feedstuffs and the utilisation of ME is lower as the DE derives from short chain fatty acids. Fincher and Stone (1986)

demonstrated that barley is less digestible and yields less energy when fed to poultry than when fed to pigs or ruminants. Thus, it is concluded that the actual nutritive value also varies with the animal to which the barley is being fed.

Similar to the energy component, the digestibility of crude protein varies with different varieties of barley and different species of animal. Also, the digestibility of crude protein is dependent on the fibre and hull content (Bell et al., 1983).

Barley had a feeding value 91% to that of maize (Morrison, 1956) although it contains more total protein and higher levels of lysine, tryptophan and the sulphur-containing amino acids (See Table 1.3) (English et al., 1988). This reduced feeding value of barley is due to its relatively higher crude fibre content and the apparent inability of the pig to consume enough net energy to gain at a maximum and efficient rate. Barley hull fibre acts as a diluent of available nutrients and may also contain factors which physically and chemically inhibit nutrient digestion, absorption or utilisation (Larsen and Oldfield, 1960). The removal of the hull through pearling greatly improves the nutritive value of barley (Dinusson et al., 1956). It has been shown that barley hulls added to diets based on either pearled barley or maize significantly depressed growth rate and efficiency of feed conversion more than a similar level of fibre added from purified cellulose (Larsen and Oldfield, 1960). Consequently, hulless cultivars of barley lower in fibre and higher in energy have been developed. The hulless variety has a higher crude protein and starch content and a lower fibre level.

Table. 1.3. Typical amino acids contents of barley and corn (English et al., 1988)

	Amino Acids (%)											
Cereal	Met	Cys	Lys	Try	Thr	IsoLeu	His	Val	Leu	Arg	Phe	Gly
Barley	0.18	0.25	0.53	0.17	0.36	0.42	0.23	0.62	0.80	0.50	0.62	0.36
Corn	0.17	0.13	0.22	0.09	0.34	0.37	0.19	0.42	1.00	0.52	0.44	0.33

When these cultivars are given to chicks (Anderson et al., 1961) or swine (Newman et al., 1968; Mitchall et al., 1976), however, the feeding value is not superior compared to hulled barley. This is due to the higher β-glucan contents in hulless barley cultivars compare to hulled cultivars. The anti-nutritive effects of barley in poultry and the higher feed conversion rate of barley in pigs than corn or wheat was due to the presence of mixed-linked β-glucan in the endosperm and aleurone

cell walls (White, et al., 1981; Hesselman et al., 1981; Hesselman and Åman, 1986; Henry, 1987; McNab and Smithard, 1992; Miller et al., 1994).

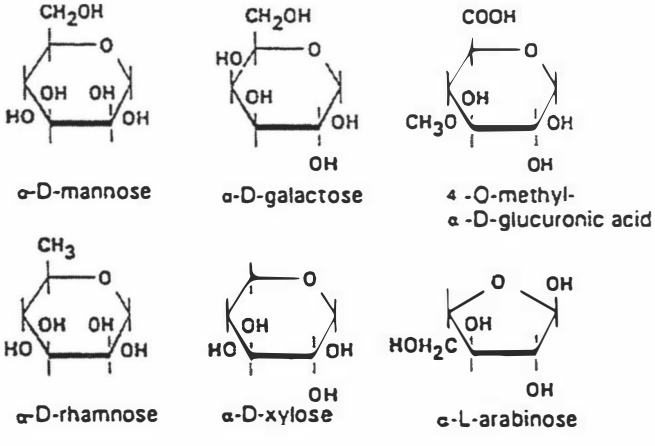
1.5.2 Definition and classification of the non-starch polysaccharides (NSP) found in wheat and barley

Cereal carbohydrates can be classified as mono-, di-, oligo- and poly-saccharides. Nutritionally, sugars usually mean mono- and di-saccharides (Asp, 1996).

Monosaccharides commonly present in cereal cell walls are (1) hexoses such as D-glucose, D-galactose, D-mannose (2) pentoses such as L-arabinose, D-xylose, and (3) acidic sugars such as D-galacturonic acid, D-glucuronic acids and its 4-O-methyl ether (see Fig. 1.1) (Choct, 1997).

Polysaccharides are defined and classified by the following structural components: (1) present monosaccharide, (2) the ring forms of monosaccharide (six-membered pyranose or 5-membered furanose), (3) position of glucosidic linkage, (4) configurations of glucosidic linkages (α- or β-), (5) the sequence of the monosaccharide components in the chain, and (6) presence or absence of non-carbohydrate components (Choct, 1997).

Fig.1.1. Monosaccharides commonly found in plant NSP (Annison and Choct, 1994)



The polysaccharides can be divided into starches, which are linear (amylose) or branched (amylopectin) homopolymers of glucose with α-glucosidic bonds (α-glucans), and non-starch polysaccharides (NSP). NSP consist of cellulose, which are

a linear β-glucan, and a range of heteropolysaccharides without α-glucosidic linkages. Plant cell walls are the main source of dietary NSP (Asp, 1996).

In starch, the glucose molecules are joined mainly by α-(1→4) bonds with a small number of α-(1→6) bonds. These bonds and the α-(1→2) links in sucrose, the β-(1→4) link between glucose and galactose and the α-(1→1) link of trehalose are cleaved by endogenous avian or mammalian enzymes. All other glucosidic bonds are resistant but microbially-derived enzymes (Annison and Choct, 1994) may cleave them. NSP are resistant to hydrolysis by monogastric animal enzymes, but are highly susceptible to degradation by the bacteria in the hind-gut (see Table 1.4).

Table 1.4. Carbohydrate content (g/kg DM) of a barley based diet and resultant digesta (Graham et al., 1986)

Site	Fructose + Glucose	Sucrose	Maltose	Starch	NSP
Feed	3	12	-	510	185
Duodenum	50	10	30	375	173
Ileum	Trace	Trace	trace	70	356
Faeces	-	-	-	trace	331

NSP do not generally exist as completely separate components in cereals. Most NSP are part of the cell wall and are closely associated with other polysaccharides or non-carbohydrate materials such as protein and lignin. These associations are of importance because its negative influence on the digestion of bound nutrients in the gut of monogastric animal (Smits and Annison, 1996).

NSP, together with lignin, are equivalent to dietary fibre (Trowell et al, 1976). The structural complexity and confusion in the nomenclature have made it almost impossible to draw a clear classification of NSP. NSP were originally classified by the methodology used for extraction and isolation of polysaccharides. The soluble fraction by alkali was called hemicellulose and the fraction remaining after a series of alkaline extractions was called cellulose (Choct, 1997). Graham (1991) and Longland and Low (1995) classified plant cell wall NSP as structural or storage polysaccharides. According to their classification, the cell wall storage polysaccharides include mannans, galactans, and xyloglucans, and the cell wall structural polysaccharides include cellulose, hemicellulose and pectins. Also, NSP can be classified by differences in solubility. Crude fibre (CF) is the insoluble fraction following extraction with acid and alkali and includes variable portions of the insoluble

NSP. Neutral detergent fibre (NDF) is a portion of insoluble NSP and lignin. Acid detergent fibre (ADF) refers to a portion of insoluble NSP comprised largely but not exclusively of cellulose and lignin (Choct, 1997). Moreover, NSP are classified sometimes by contents of uronic acid in the chain. For example, those rich in uronic acids are known as the pectin fraction and those poor in uronic acids are defined as the hemicelluloses (Southgate and Englyst, 1985; Wisker et al, 1985; Schneeman, 1986). However, the main groups of NSP can be classified as cellulose, non-cellulosic polymers (arabinoxylans, mixed-linked β-glucans, mannans, galactans, xyloglucan) and pectic polysaccharides (polygalacturonic acids, which may be substituted with arabinan, galactan and arabinogalactan) (Choct, 1997).

1.5.3The structure and chemistry of the major NSP present in wheat and barley

Mixed-linked β-glucans and arabinoxylans in the endosperm cell walls are the major NSP in barley and wheat, respectively (see Table 1.5.) However, the variation in contents of major NSP in wheat and barley varieties is wide (see Table 1.6.).

Table 1.5. Typical contents (% DM) of NSP in wheat and barley (Smits and Annison, 1996; Choct, 1997)

Cereal	Arabinoxylan	β-glucan	Cellulose	Mannose	Galactose	Uronic acid	Total
Wheat							
Soluble	1.8	0.4	-	Trace	0.2	Trace	2.4
Insoluble	6.3	0.4	2.0	Trace	0.1	0.2	9.0
Barley							
Soluble	0.8	3.6	-	Trace	0.1	Trace	4.5
Insoluble	7.1	0.7	3.9	0.2	0.1	0.2	12.2

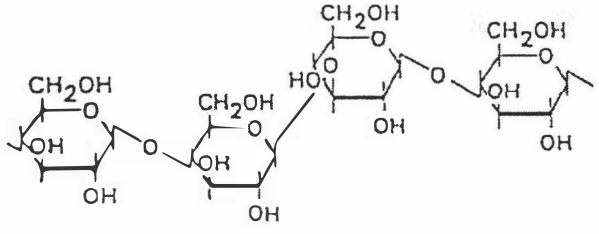
Table. 1.6. Variation in content and composition of major NSP (% DM) in Swedish barley (n=16) and wheat (n=24) samples (Graham, 1991)

NSP		Barley	Wheat
β-glucans	mean	3.4	0.8
	range	2.4 – 4.2	0.7 - 1.0
	CV(%)	18	10
Arabinoxylans	mean	7.0	6.0
	range	6 – 11	5 - 7
	CV(%)	25	7

1.5.3.1 β-glucans

β-glucans are found in most cereals, being particularly high in barley. β-glucans are linear polymers of glucose with β-(1→3),(1→4) glucosidic linkes (see Fig.1.2.) (Smits and Annison, 1996).

Fig. 1.2. Major soluble NSP of barley: β-(1→3),(1→4) D-glucan (Smits and Annison, 1996)



The endosperm cell walls of barley consist of an amorphous matrix in which microfibrillar structures are dispersed (Bacic and Stone, 1981). The matrix polymers consist mainly of β-glucans and arabinoxylans in the case of barley endosperm. Some of the matrix polymers are cross-linked and the others are loosely held on the surface of the walls. Approximately 70% of barley endosperm cell walls are composed of mixed-linkage β-glucans (Selvendran et al., 1987). Among these β-glucans approximately 70% of β-glucans are (1→4) linked and 30% of β-glucans are (1→3) linked in both warm water-soluble (glucan-I) and alkali soluble (glucan-II) fractions (Fincher and Stone, 1986; Selvendran et al., 1987; Choct, 1997).

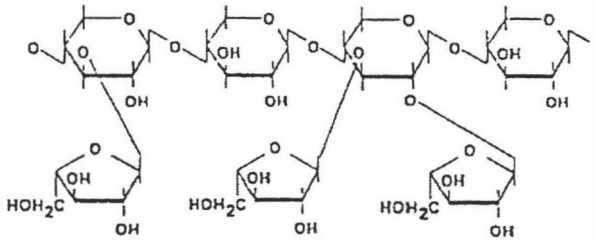
In barley, the alkali-soluble β-glucan (glucan II) fraction is covalently linked to other cell wall components via protein and/or phenolics (Selvendran et al., 1987). This association influences the solubility of β-glucan in aqueous media. The molecular weight of water-soluble β-glucans ranges from 200,000 to 300,000 corresponding to degrees of polymerisation of 1,200 - 1,850 monomers (Choct, 1997). Incorporation of the β-(1→3) linkages in the β-glucan molecules breaks the regular structure of β-(1→4) linkages and the interruptions are spaced at irregular intervals preventing close packing of the chains to give a more soluble polymer (Fincher and Stone, 1986; Choct, 1997).

1.5.3.2 Arabinoxylans (Pentosans)

The chemical structure of arabinoxylans is more complex being composed of two sugars, arabinose, and xylose in a branched structure (Smits and Annison, 1996).

Barley arabinoxylans generally have a (1→4)-β-xylopyranosyl backbone that carries single α-L-arabinofuranosyl residues mostly through O3 but also through O2 of the xylosyl residues (MacGregor and Fincher, 1993). The arabinose originates from the pectin fractions whereas xylose comes from hemicellulose (Longland and Low, 1995) (see Fig. 1.3.)

Fig. 1.3. Major soluble NSP of wheat: arabinoxylan (Smits and Annison, 1996)



The molecular structure of arabinoxylan consists of a linear (1→4)-β-xylan backbone to which substituents are attached through O2 and O3 atoms of the xylosyl residues. The major substituents are single arabinose residues and hexoses, and hexouronic acids exist as minor substituents. Phenolics and proteins also exist as side chains (Choct, 1997).

The predominant polysaccharides of wheat endosperm cell walls are arabinoxylans (88%), of which one-third are soluble in water; alkali agents are needed to dissolve the remaining two-thirds (Mares and Stone, 1973). The water-insoluble arabinoxylans are anchored in the cell walls by alkali-labile, ester-like cross-links rather than by a simple physical entrapment. In contrast, the water-soluble arabinoxylans are not bound to the cell walls and can form highly viscous solutions. They can absorb about ten times their weight of water (Choct, 1997). In the presence of peroxidase (H_2O_2), arabinoxylans accelerate the formation of gel networks as a result of the re-establishment of covalent cross-linking reactions (Geissman and Neukom, 1973; Choct, 1997).

In addition, arabinoxylans can form intermolecular hydrogen bonding between unsubstituted regions, "Junction zone", of the xylan backbone and this non-covalent interaction affects solubilities of arabinoxylans (Fincher and Stone, 1986; Choct, 1997).

1.5.4 Relationship between chemical structure and anti-nutritive effects of NSP in monogastric animals

1.5.4.1 Viscosity and water holding capacity

NSP can roughly be separated into soluble and insoluble fractions (see Table 1.5.). Soluble refers to solubility in water or weak alkali solutions. NSP have hydrophilic properties due to their free hydroxyl groups. In fact, all NSP that exhibit anti-nutritive properties are water-soluble. Thus, it seems that water solubility is an important property of anti-nutritive NSP (Annison, 1993).

The soluble NSP increase the viscosity at low concentrations by directly interacting with the water molecules. As the concentration increases, the molecules of the NSP themselves interact and become entangled in a network (Smits and Annison, 1996). This process can cause great increases in the viscosity and is dependent on the formation of junction zones of the polysaccharide molecules (Annison and Choct, 1994). Because of the formation of the networks with water the viscosities and water holding capacities of soluble NSP are relatively high compared with those of insoluble NSP (Smits and Annison, 1996). Viscous formation is increased by the inter-molecular hydrogen bonding and by the re-establishment of cross-links between molecules (Choct, 1997). It was demonstrated that soluble NSP, that have higher water holding capacity, delay gastric emptying and increase intestinal viscosity (Johansen et al., 1996).

The viscosity caused by NSP depends on their solubility and molecular weights. The solubility of NSP depends on the chemical structure of the NSP and association of the NSP with the other cell wall components. However viscosity is not specific to the sugar composition or linkage types exist in the NSP (Annison and Choct, 1994; Smits and Annison, 1996; Choct, 1997). Vohra and Kratzer (1964) have indicated that polysaccharides with branched structures tended to display a greater anti-nutritive activity. Branching introduces irregularities into the structure of polysaccharides, which prevent the chains from interacting closely and allow water to penetrate easily. Thus, in general, the greater the degree of branching, the higher the solubility (Annison, 1993). Also, Bedford and Classen (1992) found a strong relationship between the luminal concentration of soluble high-molecular weight carbohydrates (>500kDa) and intestinal viscosity in broiler chicks. Consequently, NSP which have a high molecular weight, a high water holding capacity, have a

highly branched structure, and are the water-soluble fraction, may have strong anti-nutritive activity in monogastric animals.

Many soluble NSP give rise to highly viscous aqueous solutions even when present at very low levels. There is evidence that the ability of the dietary cereal NSP to increase the viscosity of the digesta of chickens is major factor in the mechanism of their anti-nutritive action. For example, when barley β-glucan was added to the diet of chickens, the viscosity of the intestinal contents increased three-fold. Moreover, the addition of β-glucanase to barley diets ameliorates the growth depression of chickens and allows barley to be used optimally in the small intestine (White et al., 1981).

Viscosity enhancing and gel forming properties of NSP are important for four main reasons: (1) viscous components can delay gastric emptying, (2) viscous components possibly reduce absorption rates in the small intestine (Selvendran et al., 1987), (3) viscous components can increase endogenous secretion (Low, 1989), and (4) proliferation of microorganisms in lower digestive tract (Choct, 1997). Generally, high gut viscosity decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction at the mucosal surface. Soluble NSP interacts with the glycocalyx of the intestinal brush border and thickens the rate limiting unstirred water layer of the mucosa, which reduces the efficiency of nutrient absorption through the intestinal wall (Choct, 1997). Increase in digesta viscosity may inhibit nutrient digestion simply by impeding the diffusion of digestive enzymes and their substrates and products (Annison, 1993). Proliferation of microbes in the lower part of the small intestine can lead to production of toxins and deconjugation of bile salt (Choct, 1997). Thus, the soluble NSP act as a physical barrier to nutrient digestion and absorption by increasing gut viscosity (Choct, 1997).

1.5.4.2 Physiological effects of NSP

It is well recognised that the soluble NSP depress the activity of certain pancreatic enzymes *in vitro,* namely, amylase, lipase, trypsin and chymotrypsin. Dunaif and Schneeman (1981) found that cellulose and xylan reduced the activity of amylase, lipase, trypsin and chymotrypsin to less than half their original activity. The reduction in enzyme activity is due to non-specific binding of the enzymes by the NSP polymers. Selvendran et al. (1987) indicated that the inhibitory effects of NSP on the activity of the intestinal enzymes may not have significant effects on the digestibility of food in the small intestine, because of vary large excess of enzyme

activity present in pancreatic secretions. However this action of NSP, at least in part, would affect on nutrient digestibility.

Low (1989) found that larger amounts of water are found within the gut when the content of dietary NSP is increased, and this is due not only to hydrophilic nature of many forms of NSP, but also to increased endogenous secretions. For example, wheat bran increased secretion of pancreatic juice by 115%, protein by 40%, chymotrypsin by 59%, trypsin by 53%, lipase by 78% and amylase by 70%. Moreover Low (1989) indicated that NSP elevate secretory output from the salivary glands, stomach, liver, pancreas and intestinal wall. This results in an increased excretion of water, proteins, lipid and electrolytes. Low (1989) explained that NSP, especially those known to be particularly hydrophilic, entrap large volume of water and increase the volume of food. This process will increases intestinal secretion because the greater the volume of food elevates the intestinal secretion. The observations that increases in the NSP content of diets may stimulate the secretion of pancreatic enzymes under some circumstances may be due to the inhibition of enzyme activity in human pancreatic juice by NSP, as found *in vitro* for xylan and cellulose (Dunaif and Schneeman, 1981; Low, 1989).

Thus, prolonged intake of soluble NSP is associated with significant adaptive changes in the digestive system. The changes in the gut are characterised by enlargement of the digestive organs and increased secretion of digestive juices (Choct, 1997; Low, 1989). As evidence of adaptive changes, Southon et al. (1985) and Younosjai et al. (1978) demonstrated that a high NSP diet given to rats induced higher rates of protein synthesis in the jejunum and ileum, and more rapid mucosal cell division. These changes in the digestive system are accompanied by a decrease in nutrient digestion (Choct, 1997). The effect of NSP on nutrient digestibility will be discussed in brief in the following section.

- **Anti-nutritional effects of NSP on starch digestion**

Smits and Annison (1996) have indicated that the convective transport of glucose was impaired in an *in vitro* viscous environment. Fengler and Marquardt (1988) have demonstrated that an arabinoxylan-rich extract from rye decreased the rate of dialysis of glucose, as well as glucose being enzymatically released from starch. Further evidences come from *in vivo* studies (Choct and Annison, 1992[a,b]) of decreased starch digestion using wheat pentosan in poultry. In these studies, the starch digestibility was decreased as the wheat arabinoxylan contents were increased.

- **Anti-nutritional effects of NSP on protein digestion**

Choct and Annison (1990,1992[a]) demonstrated that wheat arabinoxylans depressed protein digestion, and Annison (1993) found that the arabinoxylans increase the secretion of endogenous protein in the gastrointestinal tract of broiler chickens. These effects were demonstrated with very low levels of arabinoxylans (1.5 - 3.5%) whereas much higher levels (9.2%) of insoluble cellulose (Solka-floc) and powdered polyethylene (Alkathene) had no effects. These results, in turn, support the notion that increased nitrogen secretion may partly responsible for depressed protein digestion when fed soluble NSP to rats (Larsen et al., 1993; 1994) and to pigs (Mariscal-Landin et al., 1995). In these assays, ileal mucus excretion was increased in soluble-fibre fed rats and pigs. Furthermore, Gee et al. (1996) indicated that a high viscosity stimulates epithelial cell proliferation and is may contributes to some loss of epithelial cell when the animal given soluble NSP.

Also, Angkanaporn et al. (1994) observed significantly depressed overall digestibility of AAs and increased endogenous AA losses when wheat soluble arabinoxylans included in the broiler chicken diets.

It was found that soluble NSP with high water holding capacity disrupt protein digestion and absorption in pigs, while insoluble NSP with high water holding capacity had no influence on protein digestion and absorption (Leterme et al., 1998).

- **Effects of NSP on nutrient absorption**

As nutrients move from the bulk water phase of the intestinal contents into the intestinal epithelial cell they essentially must pass through two membranes in series, the unstirred water layer (UWL) which is adjacent to the intestinal mucosa, and the lipid membrane of the microvillus surface (Wilson et al., 1971, 1974; Smithson et al., 1981).

It has been shown that gel-forming gums and pectin give rise to an increase in the thickness of the UWL (Johnson and Gee, 1981; Flourie et al., 1984) The mucus produced by goblet cells participates in the formation of the UWL by increasing the volume of the adherent mucosal fluid and its viscosity. Also, Satchithanandam et al. (1990) indicated that dietary fibre might increase the secretion of mucus. In *in vitro* studies using inverted sacs of rat jejunum, guar gum and carboxymethyl cellulose have been shown to interact with the glycocalyx of the intestinal brush border to produce thickening of the unstirred water layer. Glucose transport was reduced in

preparations, and it was suggested that the increase in the viscosity of the fluid film surrounding the villi increased resistance to the passive diffusion of nutrients resulting in their decreased absorption (Johnson and Gee, 1981).

Thus, viscosity caused by soluble NSP induces a secretory response of mucus. It may increase the resistance for transport of nutrients through the UWL adjacent to the epithelial surface by increasing the thickness of the mucus layer (Smits and Annison, 1996). Furthermore, increased proliferation rate of the enterocytes and changes in the morphology of the villi and microvilli can decrease mucosal uptake of end products of nutrient digestion. Johnson et al. (1984) demonstrated that various gelling agents increased the proliferation rate of the enterocytes of the jejunum and distal ileum and decreased the activity of specific epithelial surface enzymes in the rat.

Also, viscous NSP may decrease the apparent digestibility of nitrogen at the end of the ileum by increasing endogenous losses. Larsen et al. (1993) have demonstrated that endogenous nitrogen loss was significantly increased with increasing fibre viscosity, and that the extra endogenous nitrogen secretion found in the rat was derived from mucus.

Therefore, ileal digestibility coefficients of nutrients may be decreased by viscous NSP due to increased retention time of digesta, increased thickness of the UWL adjacent to mucosa, decreased diffusion and convective transport, increased proliferation rate of enterocytes, decreased mucosal uptake of digestion end products and/(or) increased endogenous secretions (Smits and Annison, 1996).

- **Interaction between viscosity and gut microflora**

Non-viscous water-holding NSP may diminish the overall bacterial activity in the intestinal tract by decreasing the time available for fermentation in the gut. Moreover, bacteria may adhere to the insoluble NSP structure (Smits and Annison, 1996). However, viscous soluble NSP significantly elevate fermentation in the terminal part of the small intestine. Soluble NSP increase the average retention time of digesta in the gastro intestinal tract (Choct, 1997; Smits and Annison, 1996). It is likely that this creates an excellent environment for anaerobic microflora due to the decreased oxygen tension. As the flow of digesta is reduced, the amount of undigested material in the small intestine is increased. This gives the anaerobic microflora more time and more substrate to colonise the proximal small intestine. Consequently, this gives more chance for enhanced bacteria adhesion to the mucosal surface, which sometimes may cause bacterial disease (Annison, 1993; Smits and Annison, 1996).

Production of toxins and deconjugation of bile salts, which are essential for the digestion of fat, may be increased by proliferation of some anaerobic organisms (Choct, 1997; Smits and Annison, 1996). Moreover, most of the soluble NSP sources are fermentable. An increase in bacterial activity in the small intestine may cause a systemic effect on the gut secretions and morphology of the small intestine. As a result, poor digestibility coefficient values of nutrients may be observed by the reduced nutrients absorption through the affected gut walls. In addition, digestible carbohydrates such as starch and glucose can be converted through microbial action to volatile fatty acids which represents the inefficiency of nutrient utilisation by monogastric animals (Choct, 1997; Smits and Annison, 1996).

1.5.5 Anti-nutritive effects of NSP in the pigs

1.5.5.1 The anti-nutritive effects of arabinoxylans (Pentosans) from wheat in pigs

The arabinoxylans consist of β-1,4-linked xylopyranosyl residues with terminal 1,2 and 1,3 arabinofuranosyl substitutions. Arabinofuranosyl substitution reduces the ability for hydrogen bonding between carbohydrate chains and consequently results in fractions that are water soluble and highly viscous. The soluble fractions are considered of major importance in determining the nutritional value of cereal grains for monogastric animals. After ingestion, arabinoxylans become soluble resulting in increased digesta viscosity (Classen and Bedford, 1991). The viscous nature of the NSP is the primary cause for their anti-nutritive effect in monogastric animals, because the increased bulk and viscosity of the intestinal contents decrease the rate of diffusion of substrates and digestive enzymes and hinder their effective interaction at the mucosal surface (Ikegami et al., 1990). The arabinoxylans might also directly complex with digestive enzymes and reduce their activity (Ikeda and Kusano, 1983). However, wheat arabinoxylans have different anti-nutritive effects between chickens and pigs.

The anti-nutritive effects of wheat pentosans in pigs have received little attention due to their weak, or lack of deleterious, effects in pig nutrition. Available information is very little.

An early study with wheat bran showed that its inclusion increased the sloughing of intestinal mucosal cells and enhanced mucus production in rats (Schneeman et al., 1982). Also the *in vitro* study by Schneeman (1978) observed adsorption of

proteolytic enzymes to fibre and a decrease in the activities of these enzymes. Murray et al. (1977) demonstrated that addition of 6% methylcellulose in a barley-soybean meal diet decreased apparent ileal N digestibility in pigs, and it was suggested that when gel-forming polysaccharides are given, digestion of protein rather than the absorption of products of digestion is impaired. Pals and Ewan (1978) reported that the apparent faecal digestibility coefficients (AFDC) for dry matter, nitrogen, and energy decreased linearly in piglets as the level of wheat middlings (NDF 43% DM) increased. The reduction in the AFDC for dry matter and energy was probably because the young pig cannot utilise the fibre present in wheat middlings, and the reduction in the AFDC for nitrogen may be due to the increased metabolic faecal nitrogen excretion by increased fibre intake. Moor et al. (1986) observed decreased faecal apparent nitrogen digestibility and apparent digestible energy content of the diets when wheat bran was added in a soybean meal and corn-based grower diet. It was suggested that decreased faecal nitrogen digestibility was due to increased bacterial protein synthesis and endogenous nitrogen losses. Graham et al. (1986) observed that inclusion of wheat bran increased faecal water contents and output due to water holding capacity of dietary fibre components. However, wheat bran did not influence the apparent ileal and faecal digestibilities of starch, nitrogen and fat. Sauer et al. (1991) observed that the inclusion of 10% powdered cellulose or barley straw affected ileal AA digestibility, but only slightly. However faecal AA digestibility was decreased significantly due to increased bacterial protein synthesis and increased bacterial nitrogen excretion. An increase in the purified NDF from wheat bran resulted in decreased apparent ileal protein digestibility due to increased ileal losses of both endogenous and exogenous protein. Increased losses of endogenous nitrogen were due to increased secretions (pancreatic juice, bile, mucus and sloughed epithelial cells) and decreased re-absorption.

Fibre components may also adsorb AAs and peptides and withhold them from absorption. Moreover, water-holding capacity of the fibre reduces the diffusion of the products of digestion toward the mucosal surface (Schulze et al., 1994). However, Lenis et al. (1996) concluded from their study with purified wheat bran NDF that purified NDF did not significantly affect the absorption of ileally digested AAs.

As mentioned before, there is little information available about the anti-nutritive effects of wheat arabinoxylans in pigs. However, from the above experimental results it appears that wheat arabinoxylans may reduce ileal nutrient digestibility and increase endogenous excretion of nitrogen and amino acids. Faecal nitrogen

digestibility may be reduced due to increased bacterial nitrogen synthesis and bacterial nitrogen voided in faeces.

The anti-nutritive effect of pentosans in the pig seems much weaker than in poultry. Thacker (1988) reported that pentosanase supplementation to rye-based pig diets slightly improved liveweight gain and feed conversion ratio.

Moreover, Bedford et al. (1992) found that pentosanase supplementation of barley or rye-based diets for weanling pigs did not provide any benefit in terms of animal performance or nutrient digestibility. Also, Thacker et al. (1991;1992[a]) concluded that pentosanase supplementation of diets for starter and growing pigs gave only minor benefit. Therefore, in contrast to the poultry, arabinoxylans in cereal grains may have only little anti-nutritive effects on pig nutrition.

1.5.5.2. The anti-nutritive effects of β-glucans from barley in pigs

Monogastric animals cannot synthesise β-glucanase and the amount of β-glucanase derived from barley grain and bacteria in the gastrointestinal tract is insufficient for complete hydrolysis of β-glucans. Thus β-glucans in barley diets create a viscous environment in the digestive tract causing poor absorption of dietary nutrients and reduced growth rate (Wang et al., 1992). However, barley β-glucans have different anti-nutritive effects between poultry and swine.

As evidence of the secretory response to β-glucan in pigs, Sambrook (1981) and Zebrowska et al. (1983) observed significantly higher outputs of gastric, biliary and pancreatic secretions in pigs fed a barley-based diet compared to pigs fed a starch, casein and cellulose based diet. Rainbird and Low (1983) found that soluble dietary fibre delayed the rate of digesta emptying. However, unlike the chick (Hesselman and Åman, 1986), β-glucanase supplementation (0.5%) in a barley-based diet did not increase digesta and faecal dry matter content in 80kg pigs (Graham et al., 1989). Supplementation of barley-based diets with 0.1% β-glucanase in 50kg growing pigs afforded relatively small improvements in productive value (Graham et al., 1986). In this study, however, significantly increased solubility of the β-glucans in the diet at both the duodenum (34 to 46%) and terminal ileum (18 to 24%) was observed, demonstrating that the enzyme preparation was active within the gastrointestinal tract. In spite of the normal enzyme activity in the gastrointestinal tract the apparent digestibilities of any dietary component at duodenum, terminal ileum or faeces were not significantly affected by enzyme supplementation.

Also, attempts by other researchers to increase the performance of pigs fed barley-based diets through enzyme supplementation have not produced improvements to those observed with poultry. Thacker et al. (1988) observed no significant differences in growth rate, feed intake or feed conversion efficiency between 20kg pigs fed hulless barley diets supplemented or unsupplemented with 0.25% β-glucanase, however, increases of 1-3% units in the digestibility of dry matter and energy as a result of enzyme supplementation of grower diets were observed. These increases arise primarily from enzymatic degradation of the β-glucans present in the feed rather than from a reduction in intestinal viscosity impacting on the digestibility of other nutrients. Graham et al. (1989) have also observed that 0.5% β-glucanase supplementation increased the ileal apparent digestibility of starch from 92.6 to 94.3% and the ileal digestibility of mixed-linked β-glucans from 95.7 to 97.1% in 80kg growing pigs. These small improvements were due to disruption of the endosperm cell wall by enzyme and increased pre-ileal digestibility of the endosperm cell wall component. This disruption lead to a greater pre-ileal digestibility of starch, which is partly responsible for the improvement in feed conversion efficiency sometimes observed in enzyme supplemented barley-based diets. Therefore, β-glucanase supplementation will most likely result in only limited improvement in the nutritive value of barley-based diets for growing pigs.

Thacker et al. (1992[b]) also observed little benefit from 0.25% β-glucanase supplementation of barley-based diets for 25kg growing pigs. In this study, the authors observed increased dry matter digestibility coefficient but crude protein and energy digestibilities for growing pigs were not affected by enzyme supplementation. A study conducted by Bedford et al. (1992) showed no reduction in intestinal viscosity in 12kg starter pigs fed barley-based diets supplemented with 0.2% β-glucanase.

Interestingly, most studies which mentioned above have used older pigs which are known to have large duodenal lactobacilli populations capable of degrading β-glucans (Graham et al., 1986). However, several studies that used young pigs shown improved digestibility with supplementation of β-glucanase.

Newman et al. (1983) observed increased faecal energy and protein digestibility in 18kg pigs with supplementation of 0.1% bacterial diastase in a hulless barley diet. Bedford et al. (1992) observed increased weight gain (0.9kg in 10 days) and ileal nitrogen digestibility in 12kg pigs when 0.2% β-glucanase was added in a hulless barley diet. Also, Thacker et al. (1992[b]) observed increased growth rate and feed efficiency in 8kg piglets with 0.25% β-glucanase supplementation in a barley diet.

Moreover, improved ileal nitrogen digestibility has been observed in 12kg (Bedford et al., 1992), 19-25kg (Graham et al., 1988), and 40kg pigs (Thacker et al., 1988) with β-glucanase supplementation in barley based diets, while no improvements were found in 80kg (Graham et al., 1989), and 50kg pigs (Graham et al., 1986).

Unlike older pigs (Graham et al, 1986; 1989) where no effect of β-glucanase supplementation to a barley-based diet on the ileal digestibilities of gross energy, crude protein and NSP was seen, Li et al. (1996) observed increased ileal digestibilities of gross energy, crude protein, β-glucans and majority of the Aas, and faecal digestibilities of gross energy, crude protein and Aas, with supplementation of 0.2% β-glucanase to a hulless barley-based diet in young pigs (7.3kg). This is most likely due to the increase in fibre-degrading capacity with age in the distal part of small intestine of the pig (Graham et al., 1988). Due to young pigs having a less mature digestive tracts and lower endogenous enzyme secretions than in mature pigs, the age of the pig influences its response to enzyme supplementation (Lindemann et al., 1996). Therefore, it is concluded that the anti-nutritive effect of β-glucan is greater in young pigs than older pigs.

1.5.6 Different responses to anti-nutritive effect of NSP between species

Pigs are less affected by soluble NSP such as β-glucan or pentosan (Honeyfield et al., 1983) than chicks. A hulless barley containing high soluble β-glucan that has consistently given substantial growth depression in young chicks gave comparatively good results when fed to pigs (Bhatty et al., 1979). β-glucanase supplementation in grower and finisher pig diets produced little or no improvement in digestibility of nutrients (Graham et al., 1986; 1988; Thacker et al., 1988), whereas improvements in growth and nutrients digestibility of chicks were remarkable (Rexen, 1981; Campbell et al., 1984; Hesselman and Åman, 1986). Only in weanling pigs were there significant improvements in weight gain and nutrient digestibility by β-glucanase supplementation (Bedford et al., 1992; Li et al., 1996).

The failure to obtain a comparable improvement in pigs as in the fowl may be due to the denaturation of the enzymes by acid in the pig stomach and the presence of a large population of lactobacilli with known β-glucanase and pentosanase activity in the small intestine, even in the young pig (Graham et al., 1986; Jonsson and Hemmingsson, 1991).

There are anatomical differences between swine and poultry, which can explain why pigs do not appear to suffer from digestive disturbances attributable to dietary β-

glucan. The crop in poultry provides a comparatively ideal environment for enzyme activity, at least for enzymes with pH optima in the 4-5 range. Therefore, solubilisation of NSP and viscous digesta development occur in this organ of poultry (Annison, 1993). However, the pH of pig stomach is lower and most fungal enzyme activities would be sub-optimal. The presence of proteolytic enzymes and the lengthy residence time in the pig's stomach would reduce the survivability of most enzymes (Campbell and Bedford, 1992).

Furthermore, digestion of NSP and other nutrients in the chick small intestine is less than that in the pig in spite of the rich microflora present in the upper gastrointestinal tract of poultry. The higher nutrient digestibility in pigs than chicks fed barley-based diets may be due to the longer small intestine, and greater mean digesta transit time to the terminal ileum in the pig (Graham et al., 1986; 1989; Åman and Graham, 1987[a]; Classen and Bedford., 1991). Thus exogenous feed enzymes and microbial activity in the stomach and small intestine of the pig can substantially degrade dietary fibre, and consequently facilitate the more complete digestion and absorption of other nutrients in the fore-gut (Graham et al, 1986; 1989; Åman and Graham, 1987[a]).

Physiological differences were suggested as another reason by Campbell and Bedford (1992) for the different nutritional response to NSP. Pigs differ physiologically from young chicks in that the digesta has a higher water content. Since β-glucan-induced viscosity is logarithmically related to concentration, simple dilution can easily eliminate the viscosity problem and the associated constraints on luminal diffusion. Bedford et al. (1992) observed no effect on digesta viscosity from β-glucanase supplementation in barley-based weanling pig diets and viscosity did not significantly increased as digesta moved through the digestive tract. The 100-fold less viscosity in the pig's digestive tract than in the rye-fed poultry was observed in the experiment. This may be due to the fact that digesta dry matter in the pig small intestine is much lower than in the chicken, which would significantly dilute the viscous-forming NSP.

1.6 Conclusion

Cereal polysaccharides are classified as mono-, di-, oligo-, and poly-saccharides. Polysaccharides are divided into starches and non-starch polysaccharides (NSP). NSP are not hydrolysed by monogastric animal enzymes but are degraded by the hindgut and ileal bacteria. Major NSP in barley and wheat are β-glucan and

arabinoxylan, respectively. β-glucans are linear polymers of glucose with β-(1→3),(1→4) glucosidic links and arabinoxylans are composed of two arabinose and a xylose in a branched structure. Soluble NSP but not insoluble NSP exhibit anti-nutritive properties in both pigs and chickens due to their hydrophilic properties, highly branched structure and association with the other cell wall components. Soluble NSP increase the viscosity of the digesta, hence delay gastric emptying, reducing digestion and absorption and increase endogenous secretion. However, animal responses to NSP differ between species. Generally, swine are less responsive to the anti-nutritive effects of NSP than poultry. This is due to the physiological and anatomical differences between swine and poultry.

In the present work, firstly, the anti-nutritional effect of barley β-glucan on ileal and faecal energy, true ileal amino acid digestibility and NSP digestibility at ileal and faecal samples will be evaluated with a range of barleys grown in Australia. Secondly, the relationships between chemical composition and ileal and faecal nutrient digestibility will be studied for quick and reliable *in vitro* prediction of digestibility coefficients in barley.

Chapter 2

Effects of β-glucan and NSP contents of Australian barley on ileal and faecal energy, nitrogen, ileal apparent and true amino acid digestibility in growing pigs.

2.1 Introduction

The feeding value of hulled barley for monogastric animals is generally inferior to that of corn or wheat, mainly due to a relatively high fibre content and cell wall non-starch polysaccharides (NSP) resulting in a lower level of available energy (Hollis and Palmer, 1971).

Several workers have identified the soluble β-glucans in barley as an anti-nutritional factor that, at least in chickens, impedes digestion (Antoniou and Marquardt, 1981; White et al., 1983). Suggested mechanisms of anti-nutritive activity by soluble NSP are: (1) reduction of gastrointestinal enzyme activity (Forman and Schneeman, 1980; Shah et al., 1986; Ikegami et al., 1990) through the adsorption and immobilisation of enzymes and nutrients, (2) reduction of the rate of nutrient absorption due to decreased gastric emptying (Leeds et al., 1979; Tadess, 1986; Johansen et al., 1996), (3) decreased small intestinal transit time (Jenkins et al., 1978; Leeds, 1982; Salih et al., 1991) and increased thickness of the small intestinal unstirred water layer (Elsenhans et al., 1980; Leeds, 1982), and (4) decreased nutrient diffusion by increasing intestinal viscosity (Fengler and Marquardt, 1988).

It is well recognised that soluble, viscous, high molecular-weight NSP depress the digestibility of protein, starch and fat in the chicken (Choct and Annison, 1990; 1992[a;b]). The major NSP, namely β-glucan in barley and oats and arabinoxylan in wheat and rye, have received more attention in poultry nutrition as a result of the recognition of the anti-nutritive effects such as low AME and wet droppings. In poultry, the result of previous studies on the anti-nutritional effects of β-glucan are

largely in agreement with significantly depressed nutrient digestibility (White et al., 1981; Campbell et al., 1989; Wang et al., 1992).

Due to the physiological and anatomical differences between swine and poultry, however, β-glucan of barley seems to have different effects on nutrient digestibility in pigs. In the pig, significantly decreased energy and NSP digestibility was observed when a 6% β-glucan-enriched fraction was added to wheat flour-based diets (Bach Knudsen et al., 1993[a]). Also, a slightly increased energy and NSP digestibility was seen when barley-based diets were supplemented with β-glucanase (Newman et al., 1983; Graham et al., 1989; Li et al., 1996), suggesting that β-glucans contributed to reduced nutrient digestibility. Moreover, Miller et al. (1994) demonstrated that total β-glucans were negatively correlated with the DE content of barley for swine.

Initial investigations by Graham et al. (1986) observed that β-glucanase supplementation of barley-based diets did not influence the apparent duodenal, ileal or faecal digestibility of energy, crude protein, starch, and NSP in 30-50kg pigs. Later, research by Thacker et al. (1988) demonstrated that β-glucanase supplementation in hulless barley diets resulted in moderate improvements in the digestibility of dry matter, crude protein, and digestible energy, even though these differences were not reflected in significant differences in average daily gain, average daily intake, feed efficiency and carcass traits. Graham et al. (1988) indicated that β-glucanase supplementation disrupted the endosperm cell walls in barley and increased the apparent ileal digestibility of starch and mixed-linked β-glucans. Baidoo et al. (1999) demonstrated increased ileal digestibility of energy and protein with supplementation of β-glucanase in hulless barley.

More recent research has also focused on the effect of β-glucan on nutrient digestibility in pigs. Bach Knudsen et al. (1993[a;b]) showed that β-glucan in oat bran depressed the ileal digestibility of fat, protein and energy in pigs. Moreover, and to investigate the effect of NSP on nutrient digestibility in pigs, van Barneveld et al. (1995[a,b,c,d]) conducted a series of experiments with lupin kernels, a rich source of soluble NSP. The results showed negative relationships between NSP contents in the diets and amino acid, energy, and NSP digestibilities. More recently, Li et al. (1996) demonstrated the inhibitory effects of protein digestion by β-glucan in weanling pigs, and Meads et al. (1997) found a strong negative relationship between *in vitro* extracted β-glucan and *in vivo* apparent protein digestibility in rats.

Given the relative lack of studies investigating the influence of β-glucan from barley on nutrient digestibility in pigs, the aim of the present study was to determine the nutritive value of a range of Australian barleys. The other objective was to elucidate the effect of dietary NSP on ileal and faecal nutrient digestibility in pigs. The present study aimed to establish correlations between contents of β-glucan and (or) NSP in barley diets and *in vivo* digestibility of energy, nitrogen, and apparent and true amino acid digestibility in the growing pig, with special emphasis on the soluble β-glucan fractions.

2.2 Materials and Methods

2.2.1 Animals, Housing and Surgery

Twenty-one Large White X Landrace entire male pigs of mean (±SE) body weight 36.6 (± 0.7) kg were obtained from a commercial supplier (Wairiki Farms, Foxton, New Zealand) and housed at the Massey University Animal Physiology Unit. A medicated grower diet based on barley and meat and bone meal was supplied during the seven-day pre-surgery adaptation period.

For surgery, the pigs were sedated with a mixture of xylazine/zoletil (Xylaze 100, 100mg/kg, Parnell Laboratories, Tamaki, NZ; Zoletil 100, 100mg/ml, Virbac Laboratories, Auckland, NZ) and placed in left lateral recumbency under halothane/oxygen (Fluothane, Imperial Chemical Industries Ltd, Cheshire, England), with anesthesia administered through an intra-tracheal tube with a low volume, high pressure cuff. The cuff was intended to seal the airway and maximise the quality of inspired gas and minimise the chance for aspiration of foreign matter. A 5-6cm vertical incision was made into the body wall, 3-4cm behind the last rib just above the midline. The small intestine was exteriorised via blunt dissection. A 2-2.5cm incision was made along the anti-mesenteric side of the small intestine approximately 15cm anterior to the ileo-ceacal junction. A simple T-piece cannula (of PVC construction) was inserted through this incision, and a Murphy's purse string suture was made around the incision. The free ends of the purse string suture were gently pulled and secured tightly around the barrel of the cannula. A further purse string suture placed 2-3mm below the Murphy's suture secured the cannula. The cannula was

exteriorised via a stab wound approximately 1cm in diameter, 3-4cm ventral to the initial incision. The intestine was secured to the peritoneum and fascia with continuous sutures. The initial incision was closed with continuous sutures in the deep muscle layer and peritoneum, discontinuous sutures in the subcutaneous muscle, and discontinuous mattress sutures in the skin.

To promote healing of the wound and to prevent leakage around the cannula, skin barrier for use around stoma in human ileostomy patients (Stomahesive® System 2 with 70mm flange; Bristol-Myers Squibb, Princeton, NJ, 08543-4000 USA) was incorporated between the flange of the cannula and the skin. The cannulated pigs were given a long-term antibiotic after surgery (Terramycin, 5ml), and penicillin if required thereafter.

The pigs regained consciousness 2-3 hours following surgery and their appetites were normal within 48 hours of surgery. After surgery, the animals were individually housed in metabolic crates. Room temperature was kept constant at around 21±1°C. The cannulated pigs were given a grower diet for 7 days before the start of the five-week experimental period. At the end of the experimental period, the liveweight of pigs was 55.6 ± 1.61 kg (mean ±SE).

2.2.2 Diet and Experimental Design

The ingredient composition of the experimental diets is given in Table 2.1, and the determined nutrient composition of the diet is given in Table 2.2.

Eleven Australian barleys (including one control barley) were tested in this study: *Skiff Hart Junee, Lindwall Esdaile Moree, Schooner Nokes Forbes, Arapilies Ladlow Horsham, Grimmet PB1 Narrabri, Schooner Hart Junee, Galleon Ladlow Horsham, Sloop Nokes Forbes, Chebec Golder Brim, and Mundah Nokes Forbes, Tantangara Hart Junee (Control barley).*

The experimental was based on a Latin Square design, with three replicates conducted at Massey University and two replicates conducted at the South Australian Research and Development Institute (SARDI). There were five pigs per experimental diet. Each Latin Square consisted of a control barley diet, 2-3 test barley diets, and the EHC diet. As a result, a total of 10 barley varieties (plus one control) was studied.

Table 2.1 Composition of experimental diets

Ingredient	Diet		
	Control Diet	Test Diet 1-10	EHC Diet
Control barley	94.505		
Test barley 1-10		94.505	
Enzymically-hydrolysed casein			12.500
Raw sugar			30.000
Starch			52.005
Dicalcium phosphate	3.000	3.000	3.000
NaCl	0.275	0.275	0.275
Minerals*	0.070	0.070	0.070
Vitamins**	0.050	0.050	0.050
Choline chloride	0.100	0.100	0.100
Celite	2.000	2.000	2.000
Total	100	100	100

* Mineral Mix supplied the following per kg diet: 60mg Iron; 100mg Zinc; 30mg Manganese; 5mg Copper; 2mg iodine; 0.15ppm Selenium.

** Vitamin mix supplied the following per kg diet: 3200IU vitamin A; 480IU vitamin D_3; 20IU vitamin E; 1.5ppm thiamin; 3ppm riboflavin; 14ppm nicotinic acid; 10ppm pantothenic acid; 2.5ppm pyridoxine; 15mg cyanocobalamin; 0.2ppm folic acid; 10ppm ascorbic acid; 0.1ppm biotin; 2ppm vitamin K3.

In order to avoid interaction between dietary ingredients in the digestibility trial, the control barley and ten test barleys were included as the only source of energy and protein in the diet. The enzymically-hydrolysed casein diet was prepared for use in the determination of endogenous AA losses. Acid insoluble ash (added Celite) was included as an indigestible marker. All diets were mixed at SARDI and cold-press pelleted to enhance appetite and to ensure an even distribution of the premix and marker material through the diet.

This study was a collaborate project with SARDI. Five of the barley varieties (plus a control barley) were examined in South Australia (*Grimmet PB1 Narrabri, Schooner Hart Junee, Galleon Ladlow Horsham, Skiff Hart Junee, Schooner Nokes Forbes, and Tantangara Hart Junee* (Control barley)), and five of the barley varieties (plus a control barley) were measured in New Zealand under similar conditions (*Lindwall Esdaile Moree, Arapilies Ladlow Horsham, Sloop Nokes Forbes, Chebec Golder Brim, Mundah Nokes Forbes, and Tantangara Hart Junee* (Control barley)).

Table 2.2 Chemical composition* of the experimental diet

Component / Diet**	TA[a,b]	CH[a]	AR[a]	LW[a]	SL[a]	MU[a]	GA[b]	SH[b]	SK[b]	SN[b]	GR[b]	EHC[a]
Dry matter (%)	90.50	89.48	89.34	89.22	89.93	90.36	89.83	91.26	91.13	89.03	89.36	93.1
Gross energy (MJ/kg)	17.66	17.29	17.25	17.89	17.74	17.98	18.81	17.73	17.54	17.74	17.93	16.7
Crude Protein (%)	11.10	7.69	7.57	9.74	8.17	10.77	7.65	12.57	12.00	10.38	14.23	10.6
NDF (%)	14.93	15.55	14.08	12.00	15.52	15.87	18.81	17.28	17.14	21.09	15.42	-
ADF(%)	5.02	5.26	4.84	3.41	5.17	5.19	6.52	5.74	6.08	6.07	4.04	-
Lignin (%)	0.82	1.03	0.79	0.53	0.82	0.80	0.89	1.13	1.20	1.45	0.87	-
Cellulose (%)	4.21	4.24	4.04	2.88	4.76	4.39	5.36	4.61	4.89	4.62	3.17	-
Hemicellulose (%)	9.92	10.28	9.23	8.60	10.35	10.68	12.29	11.53	11.06	15.03	11.38	-
Total NSP (%)	15.32	11.76	11.65	13.18	12.15	17.40	-	-	-	-	-	-
Arabinose	3.08	2.59	2.38	2.78	2.76	3.14	-	-	-	-	-	-
Xylose	6.09	4.64	4.35	4.51	4.97	5.97	-	-	-	-	-	-
Mannose	0.41	0.39	0.34	0.33	0.40	0.45	-	-	-	-	-	-
Galactose	0.62	0.36	0.35	0.57	0.44	0.80	-	-	-	-	-	-
Glucose	5.13	3.78	4.23	5.00	3.58	7.04	-	-	-	-	-	-
Soluble NSP(%)	7.50	2.66	3.10	5.38	2.80	6.40	-	-	-	-	-	-
Arabinose	1.48	0.53	0.50	0.81	0.72	0.70	-	-	-	-	-	-
Xylose	2.64	0.58	0.63	1.04	0.73	0.77	-	-	-	-	-	-
Mannose	0.19	0.12	0.10	0.11	0.16	0.19	-	-	-	-	-	-
Galactose	0.40	0.04	0.06	0.30	0.18	0.48	-	-	-	-	-	-
Glucose	2.80	1.39	1.81	3.12	1.01	3.91	-	-	-	-	-	-
Insoluble NSP(%)	7.82	9.10	8.55	7.80	9.35	11.35	-	-	-	-	-	-
Arabinose	1.60	2.07	1.88	1.97	2.03	2.45	-	-	-	-	-	-
Xylose	3.45	4.06	3.73	3.46	4.24	5.19	-	-	-	-	-	-
Mannose	0.22	0.27	0.24	0.21	0.24	0.27	-	-	-	-	-	-
Galactose	0.22	0.31	0.29	0.27	0.27	0.32	-	-	-	-	-	-
Glucose	2.33	2.39	2.42	1.88	2.57	3.13	-	-	-	-	-	-
Total β-glucan (%)	3.15	2.91	2.81	3.87	2.00	4.33	2.56	2.99	3.75	2.95	3.51	-
Soluble β-glucan	0.34	0.25	0.23	0.25	0.22	0.32	0.24	0.33	0.30	0.21	0.34	-
Amino Acids (mg/100g)												
Aspartic acid	0.684	0.603	0.664	0.734	0.672	0.829	0.501	0.776	0.768	0.573	0.684	0.944
Threonine	0.385	0.312	0.336	0.396	0.351	0.453	0.220	0.348	0.345	0.202	0.253	0.468
Serine	0.475	0.342	0.384	0.461	0.378	0.498	0.377	0.605	0.619	0.428	0.549	0.721
Glutamic acid	3.019	1.854	2.177	2.685	1.990	3.190	1.753	3.174	3.376	1.715	2.618	2.921
Proline	1.347	0.852	1.000	1.144	0.862	1.391	0.653	1.142	1.130	0.929	1.464	1.136
Glycine	0.419	0.393	0.385	0.493	0.437	0.540	0.210	0.307	0.312	0.167	0.212	0.203
Alanine	0.463	0.412	0.590	0.485	0.438	0.539	0.259	0.385	0.414	0.394	0.454	0.305
Valine	0.617	0.453	0.525	0.610	0.507	0.658	0.385	0.592	0.493	0.426	0.534	0.769
Isoleucine	0.451	0.310	0.376	0.416	0.337	0.449	0.263	0.401	0.444	0.217	0.279	0.596
Leucine	0.859	0.645	0.947	0.811	0.681	0.912	0.508	0.794	0.782	0.455	0.562	1.064
Tyrosine	0.357	0.295	0.343	0.362	0.305	0.408	0.173	0.201	0.249	0.130	0.203	0.293
Phenylalanine	0.642	0.440	0.520	0.576	0.458	0.637	0.332	0.559	0.573	0.373	0.571	0.549
Histidine	0.256	0.183	0.213	0.263	0.210	0.268	0.138	0.226	0.232	0.355	0.421	0.297
Lysine	0.399	0.358	0.334	0.446	0.411	0.478	0.291	0.407	0.388	0.280	0.333	0.904
Arginine	0.558	0.459	0.432	0.596	0.526	0.651	0.298	0.450	0.554	0.286	0.407	0.365
Cysteine	0.212	0.141	0.143	0.173	0.146	0.197	0.181	0.318	0.316	0.221	0.244	0.032
Methionine	0.246	0.161	0.176	0.217	0.175	0.221	0.139	0.207	0.192	0.790	0.100	0.390
Total Amino Acids	11.39	8.21	9.54	10.87	8.88	12.24	6.68	10.89	11.19	7.94	9.89	11.96

*All data are on a dry matter basis

**TA: Tantangara (Control barley: mean value across the replicate of Latin Squares), CH: Chebec, SL: Sloop, LW: Lindwall, MU: Mundah, A
Arapilis, GR: Grimmet, SH: Schooner Harts, SN: Schooner Norkes, SK: Skiff, GA: Galleon.

[a]: Analysed in New Zealand, [b]: analysed at SARDI

2.2.3 Experimental procedures

Following recovery, the pigs were allocated to each experimental diet. Daily feeding rates were adjusted to three times maintenance [3X(0.5MJ DE kg^{-1} body weight $^{0.75}$)/diet DE]. Daily rations were halved and fed twice daily (0800 and 1630). Water was freely available at all times throughout the experiment. The experimental diets were introduced over three days prior to a five-day feeding period. Then, the digesta was collected through the T-cannula over two consecutive days at 2-hour intervals (0800, 1000, 1200, 1400, and 1600). The digesta samples were collected via a plastic collection tube, which was placed on ice to prevent further digestion of digesta by enzymes and microorganisms. The collected ileal samples were stored at -20°C immediately after collection until required for chemical analysis. Faeces sub-samples were collected as voided on each collection day, and stored at -20°C immediately until required. Following collection of digesta and faeces, diets were re-allocated and above procedure was repeated until all pigs had received their required number of diets.

There are considerable quantities of endogenous amino acids in ileal digesta, and these must be quantified to allow correction of apparent amino acid digestibility value to true digestibility. Several methods are available for measurement of endogenous amino acid flow (Boisen and Moughan, 1996; Nyachoti et al., 1997). In the present work, the EHC/Ultrafiltration method (Moughan et al., 1990) was used to determine the endogenous ileal loss of amino acids.

2.2.4 Chemical analysis

All diets, ileal and faecal samples were analysed in duplicate using same method either in South Australia or in New Zealand. The diets, ileal digesta and faeces were analysed in duplicate for total nitrogen (N) using the Kjeldahl method (AOAC, 1980) on a Kjeltech 1030 auto-analyser (Tecator, Sweden). Dry matter was determined by the method of AOAC (1984). The content of acid insoluble ash in each sample was determined by the method of McCarthy et al. (1974) by hydrolysing the samples in 4M HCl solution. Gross energy was determined by the method of AOAC (1984) using an adiabatic bomb calorimeter (Gallem Kamp and Co. Ltd., London).

All eleven barley varieties including the control barley, which was the same in both South Australia and New Zealand) were analysed for total and soluble β-glucan

in Christchurch, New Zealand. Levels of total (1-3), (1-4)-β-D-glucan in all barley samples were determined by the procedures of Jørgensen and Aastrup (1988) using fluorescence-enhancement Flow Injection Analysis. For hydrolysable (Gastro-Intestinal Soluble) β-glucan, duplicate 4g grain samples were wet with 0.5ml aqueous ethanol (80% v/v), then suspended in 19.5ml of 0.1M HCl/55mM NaCl buffer (pH 1.6). The samples were incubated in a 37°C water bath for two hours. This acidic solution was then neutralised by the addition of 0.8ml 10% NaOH and incubated at 37°C for a further 3.5 hours. Upon cooling, the samples were centrifuged at 1000 rpm for 10 min. and the β-glucan content in the supernatant determined by fluorescence-enhancement Flow Injection Analysis.

The neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin contents were determined in duplicate for all eleven barley varieties using the method described by Robertson and van Soest (1981).

The five barley varieties (plus one control) examined in Massey University were analysed for total NSP and their constituent sugars as alditol acetates by gas-liquid chromatography for neutral sugars using the procedure of Englyst et al. (1994). Starch was gently removed by incubation (100°C, 30 min; 50°C, 16h) with a thermostable α-amylase (Termamyl®; Chemcolour Industries (NZ) Ltd, Glenfield, Auckland, NZ, Novo Nordisk A/S), the β-glucanase-free amyloglucosidase (Boehringer Mannheim GmbH, Germany), and with the porcine pancreatic α-amylase (Sigma Chemical Co. St. Louis, Mo, USA.). The polysaccharides in the starch-free residues were allowed to swell in the presence of sulfuric acid (12 mol/L, 35°C, 30 min), hydrolysed with 1 mol/L H_2SO_4 (100°C, 2h), reduced with potassium borohydride to alcohols and acetylated using 1-methylimidazole (Sigma Chemical Co. St. Louis, Mo, USA.) to catalyse the reaction. Allose was used as internal standard. Soluble NSP in the starch-free residue was extracted using a phosphate buffer at neutral pH (100°C, 30 min, pH 7.0) and the neutral and acidic sugars in the insoluble NSP were analysed as described previously. Content of soluble NSP was calculated as

Soluble NSP = Total NSP – Insoluble NSP.

Amino acids were determined following acid hydrolysis using a Beckman 119 BL amino acid analyser (Beckman Instruments, Palo, Alto, CA, U.S.A). Duplicate samples of diet and digesta were hydrolysed in 500μl of 6M HCl with 1% added

phenol, for 24 hours at 110±1°C in glass tubes sealed under vacuum. For the determination of methionine and cysteine, separate duplicate samples were oxidised with performic acid prior to hydrolysis.

To remove any undigested amino acids which originate from diet, the ileal digesta samples obtained from the pigs fed the EHC diet were centrifuged at 7500 rpm for 10 min. at 4°C. The supernatant was gently poured off into a separate container. The precipitate was washed with 1-2ml of deionised water and centrifuged again at 7500 rpm for 10 min. at 4°C. The second supernatant was gently poured off and added to the first supernatant. The supernatants were ultrafiltered in Centriprep-10 concentrators (Amicon, W.R. Grace Company, Danvers, Massachusetts, U.S.A) by centrifuging the ultrafiltration tube at 3000g X 20 min. at 4°C several times. The high molecular weight fraction (M.W.>10,000 Daltons) following ultrafiltration was added to the precipitate. The precipitates were frozen immediately, freeze-dried, and then analyzed for amino acids.

2.2.5 Data analysis

The apparent digestibility of X (energy and nitrogen) was calculated relative to the Acid insoluble ash (AIA) concentration:

$$\text{Apparent digestibility of X (\%)} = \frac{\frac{X_D}{AIA_D} - \frac{X_{I/F}}{AIA_{I/F}}}{\frac{X_D}{AIA_D}} \times 100$$

Where, X_D and $X_{I/F}$ are the concentrations of specific nutrients in the diet (D) and in ileal (I) and faecal (F) materials. AIA and X are in % DM.

Endogenous ileal amino acid flows related to the ingestion of 1g dry matter for the EHC-fed pigs were calculated based on the amino acid and acid insoluble ash concentrations of the precipitate plus high molecular weight fraction following centrifugation and ultrafiltration. The endogenous N and amino acid flows at the terminal ileum related to the ingestion of 1g dry matter (DMI) were calculated using the equation:

$$\text{EAAF } (\mu g\ g^{-1}\ DMI) = AA_I\ (\mu g\ g^{-1}\ DM) \times \frac{AIA_D\ (mg\ g^{-1}\ DM)}{AIA_I\ (mg\ g^{-1}\ DM)}$$

Here, EAAF is endogenous amino acid flow, DMI is dry matter intake, AA_I is the concentration of amino acid in ileal digesta and AIA_I and AIA_D are the concentration of acid insoluble ash in (I) ileal and (D) diet samples.

The apparent and true ileal amino acid digestibility (%) were calculated using the following equation:

$$\text{Apparent ileal AA digestibility (\%)} = \frac{\frac{AA_D}{AIA_D} - \frac{AA_I}{AIA_I}}{\frac{AA_D}{AIA_D}} \times 100$$

Where, AA_D and AA_I are the concentrations of amino aicds in the diet (D) and in ileal (I) materials. AIA is in % DM, AA is in mg/100g DM.

$$\text{True ileal AA digestibility (\%)} = \frac{AA_D\ (\mu g\ g^{-1}\ DMI) - (AA_I\ (\mu g\ g^{-1}\ DMI) - EAAF\ (\mu g\ g^{-1}\ DMI))}{AA_D\ (\mu g\ g^{-1}\ DMI)} \times 100$$

Here, AA_I and AA_D are the concentration of amino acid in ileal digesta (I) and diets (D), EAAF is endogenous amino acid flow, and DMI is dry matter intake.

Statistical analyses were carried out using the computerised statistic package Minitab. All data were subjected to pearson's correlation analysis and linear regression analysis using Minitab. Also, the data were subjected to GLM procedure in Minitab for prediction of *in vivo* apparent ileal digestible lysine content from lysine content in Australian barley varieties and ileal and faecal nitrogen digestibility. The GLM procedure was used to test any difference between replicates of control barley data. Mean value across replicate was used for control barley because difference

between replicates was statistically non-significant. Paired t-test was used to test any difference between endogenous amino acid flow of present study and previous study.

2.3 Results

The pigs remained healthy and consumed their daily allowances throughout the experiment.

2.3.1 Chemical composition of Australian barley

The chemical composition of Australian barley-based diets is presented in Table 2.2. The range in CP (N X 5.83) was considerable (6.66%) with a mean value of 10.17%

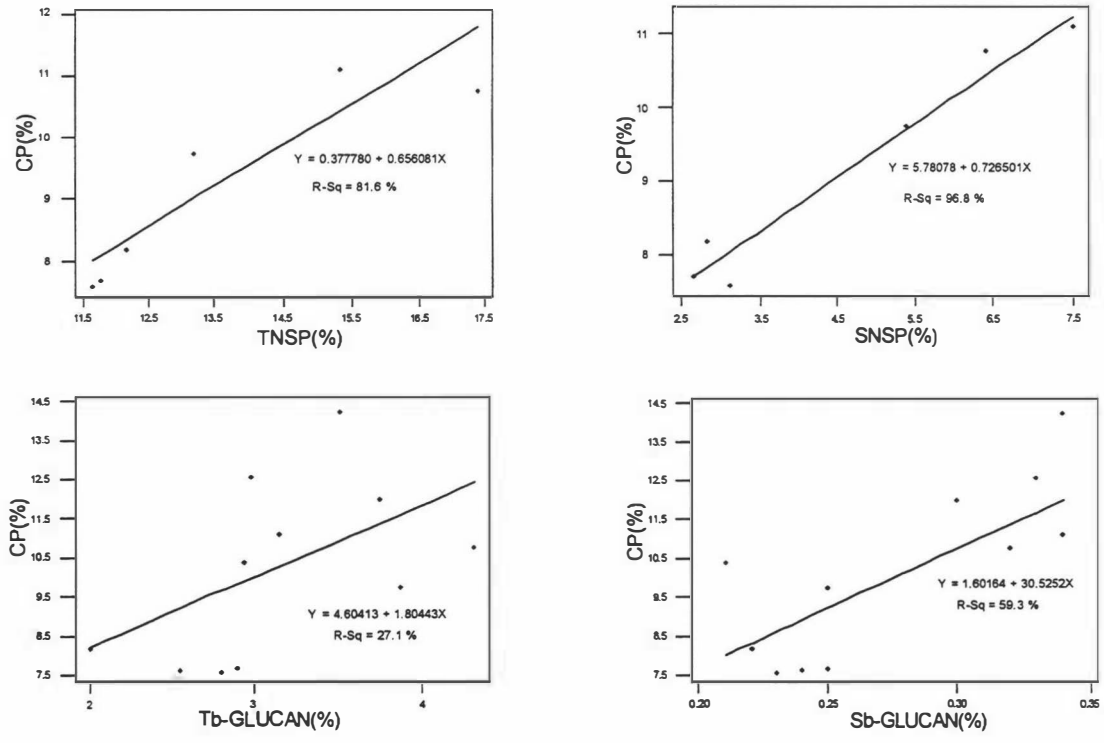


Figure 2.1 The relationships between total NSP (TNSP), soluble NSP (SNSP), Total β-glucan (Tb-glucan), and soluble β-glucan (Sb-glucan), to crude protein (CP) content in Australian barleys.

The range in GE was quite narrow (1.56 MJ/kg DM) with a mean value of 17.76 MJ/kg DM (15.88 MJ/kg on as fed basis). Among the fibre contents, NDF had the widest range at 9.09% units. Acid detergent fibre and lignin contents were much narrower in their range at 3.11 and 0.29% units, respectively.

The soluble β-glucan contents were consistently lower than the total β-glucan levels (0.21-0.34% compared to 2-4.33%). The mean values of soluble β-glucan and the total β-glucan were 0.29 and 3.16%, respectively (Table 2.2).

Gross amino acid compositions (mg/100g DM) in diets were also determined. On average, Cysteine and Histidine had the lowest concentrations and Glutamic acid the highest. The total amino acid concentrations ranged from 6.68 to 12.24 (mg/100g DM), and the mean value was 9.97 mg/100g DM.

Table 2.3 Mean[1] ileal and faecal digestibility of energy (%) of Australian barleys determined in growing pigs.

Diet	Digestibility of energy	
	Ileal	Faecal
Control barley[2]	66.4 (±1.54)	79.9 (±1.21)
Chebec	63.3 (±1.26)	79.9 (±0.24)
Arapilis	65.3 (±0.52)	81.1 (±0.48)
Lindwall	64.2 (±1.14)	82.5 (±0.31)
Sloop	65.2 (±1.79)	79.7 (±0.41)
Mundah	61.3 (±1.86)	79.2 (±0.27)
Galleon	60.8 (±1.78)	ND[3]
Schooner(Harts)	65.6 (±1.55)	ND
Skiff	71.00 (±1.09)	ND
Schooner(Norkes)	57.7 (±0.93)	ND
Grimmet	53.6 (±3.54)	ND
SEM*	0.67	0.32

1. Mean values of 5 observations ( ) Standard error in parentheses.
2. Control barley is expressed as a mean value of 15 observations across the Latin Squares.
3. ND: Not determined.
*SEM: Standard error of mean of 11 barleys for ileal and mean of 6 barleys for faecal.

For the six barleys examined at Massey University (*Lindwall Esdaile Moree, Arapilies Ladlow Horsham, Sloop Nokes Forbes, Chebec Golder Brim, Mundah Nokes Forbes, and Tantangara Hart Junee (Control barley)*) for NSP, the most abundant monosaccharides were: xylose, glucose, and arabinose in the total and insoluble NSP fractions (in decreasing order). However, glucose was the most

abundant monosaccharide in the soluble fraction. About one third of total NSP was the soluble fraction except glucose, in which soluble NSP contents were approximately half of total NSP contents. The range in total and soluble NSP was quite narrow (5.75 and 4.84%, respectively), and the mean values were 13.58 and 4.64%, respectively (Table 2.2).

Investigations between chemical components of the barleys found significant positive relationships between total NSP (r=0.903, p=0.005), soluble NSP (r=0.984, p<0.001), Total β-glucan (r=0.520, p=0.068), and soluble β-glucan (r=0.770, p=0.002) with crude protein content (Figure 2.1).

2.3.2 Ileal and faecal energy digestibility

The mean *in vivo* ileal and faecal digestibility of energy are given in Table 2.3. Ileal digestibility values were ranged from 53.6 to 71.0% with a mean digestibility of 63.9%.

Table 2.4 Mean[1] ileal and faecal digestible energy contents (MJ/kg as fed) of Australian barleys determined in growing pigs.

	Digestible energy (MJ/kg as fed)	
Diet	Ileal	Faecal
Control barley[2]	10.6 (±0.18)	12.8 (±0.16)
Chebec	9.8 (±0.22)	12.3 (±0.04)
Arapilis	10.1 (±0.09)	12.1 (±0.48)
Lindwall	10.3 (±0.20)	13.2 (±0.06)
Sloop	10.4 (±0.32)	12.7 (±0.07)
Mundah	9.9 (±0.34)	12.8 (±0.05)
Galleon	10.2 (±0.33)	ND[3]
Schooner(Harts)	10.6 (±0.27)	ND
Skiff	11.3 (±0.19)	ND
Schooner(Norkes)	9.1 (±0.19)	ND
Grimmet	8.6 (±0.71)	ND
SEM*	0.11	0.10

1. Mean values of 5 observations ( ) Standard error in parentheses.
2. Control barley is expressed as a mean value of 15 observations across the Latin Squares.
3. ND: Not determined. *SEM: Standard error of mean.

Only six barleys that examined at Massey University were measured for faecal energy digestibility, with a range of 79.2 – 82.5% and a mean digestibility of 80.3%.

The mean ileal and faecal digestible energy contents (MJ/kg as fed) of Australian barleys are presented in Table 2.4. The average ileal and faecal digestible energy of eleven Australian barleys were 10.2 and 12.5 MJ/kg as fed, respectively.

All data were subjected to Pearson's correlation analysis, and correlation coefficients between the chemical components of the barleys and *in vivo* ileal and faecal digestibility of energy are given in Table 2.5. Generally, the correlation coefficients were low. However, several of the correlation coefficients were statistically significant ($p>0.05$).

Table 2.5. Correlation coefficients[1] between various chemical characteristics of Australian barleys and apparent ileal and faecal digestibility of energy

	Energy digestibility	
	Ileal	Faecal[a]
CP	-0.154	-0.255
GE	-0.399	-0.090
NDF	-0.299	-0.845*
ADF	0.161	-0.783*
Hemicellulose	-0.501	-0.855*
Soluble β-glucan	0.142	-0.446
Insoluble NSP[a]	-0.811*	-0.502
Soluble NSP[a]	0.110	-0.194
Feacal N digestibility[a]	0.639	-0.270

1. Based on 11 barleys, except the rows and columns with superscript [a] which are correlation coefficients based on 6 barleys.

Significance level: values without * are not significant ($p>0.05$), * $p<0.05$.

Insoluble NSP (INSP) was negatively correlated with ileal energy digestibility (IEd) ($p<0.05$), while faecal energy digestibility (FEd) was negatively correlated with NDF, ADF and hemicellulose ($p<0.05$).

Using chemical components of barleys that demonstrated a significant correlation (Table 2.5), the following linear regression equations were generated:

1. IEd = 74.0 - 1.08 INSP

   (r^2=0.66, RSD=1.004)

2. FEd = 94.5 – 1.44 Hemicellulose
(r^2=0.73, RSD=0.625)

2.3.3 Ileal and faecal nitrogen digestibility

The mean *in vivo* ileal and faecal nitrogen digestibilities (mean of five pigs) are given in Table 2.6. Ileal nitrogen digestibility values ranged from 52.5 to 76.0%, with a mean digestibility of 65.0%. Only the six barley varieties evaluated in New Zealand were measured for faecal nitrogen digestibility, and the mean faecal nitrogen digestibility of six barley varieties was 69.2% (range from 64.4 to 75.7%).

All data were subjected to Pearson's correlation analysis, and correlation coefficients between the chemical components of the barleys and *in vivo* ileal and faecal digestibility of N are given in Table 2.7. Generally, the correlation coefficients were low. However, several of the correlation coefficients were statistically significant (p>0.05).

Table 2.6 Mean[1] ileal and faecal nitrogen digestibility (%) of Australian barleys determined in growing pigs.

	Nitrogen digestibility	
Diet	Ileal	Faecal
Control barley[2]	70.0 (±2.69)	75.7 (±2.26)
Chebec	52.5 (±2.96)	64.4 (±0.44)
Arapilis	58.0 (±2.52)	65.7 (±1.45)
Lindwall	64.2 (±0.70)	67.7 (±1.16)
Sloop	64.5 (±2.22)	69.1 (±1.98)
Mundah	66.9 (±1.95)	66.0 (±0.83)
Galleon	53.9 (±4.98)	ND[3]
Schooner(Harts)	70.1 (±2.60)	ND
Skiff	76.0 (±1.73)	ND
Schooner(Norkes)	66.0 (±2.02)	ND
Grimmet	61.8 (±3.83)	ND
SEM*	1.12	0.94

1. Mean values of 5 observations ( ) Standard error in parentheses
2. Control barley was expressed as a mean value of 15 observations across the Latin Squares
3. ND: Not determined
*SEM: Standard error of mean of all 11 barleys

Ileal nitrogen digestibility (INd) was positively correlated to crude protein content, soluble β-glucan content, soluble NSP content, and faecal nitrogen digestibility ($p<$ 0.05), while faecal nitrogen digestibility (FNd) was positively (but not significantly) correlated to crude protein and soluble NSP content (Table 2.7).

Using chemical components of barleys that demonstrated a significant correlation (Table 2.7), the following linear regression equation was generated:

2. INd = 7.4 + 2.42 CP + 0.480 FNd

(r^2=0.795, RSD=2.898)

Table 2.7. Correlation coefficients[1] between various chemical characteristics (% DM) of Australian barleys and apparent ileal and faecal digestibility of N.

	Nitrogen digestibility	
	Ileal	Faecal[a]
CP	0.653*	0.673
GE	-0.203	0.257
NDF	0.007	0.143
ADF	0.043	0.168
Hemicellulose	-0.010	0.232
Soluble β-glucan	0.552*	0.658
Insoluble NSP[a]	-0.100	-0.469
Soluble NSP[a]	0.818*	0.692
Feacal N digestibility[a]	0.770*	-

1. Based on 11 barleys, except the rows and columns with superscript [a] which are correlation coefficients based on 6 barleys.

Significance level: values without * are not significant ($p>0.05$), * $p<0.05$.

2.3.4 Apparent ileal amino acid digestibility

The mean apparent ileal digestibilities of amino acids (%) are presented in Table 2.8. The mean apparent ileal amino acid digestibility of all Australian barleys was 69.7%. On average, Glycine had the lowest digestibility (42.1%) and Glutamic acid the highest (82.2%). The variation in amino acid digestibility between diets was highest in Proline, Glycine, and Alanine (in decreasing order).

Correlation analysis between chemical components of the Australian barleys and apparent ileal digestibility of essential amino acids generally showed poor ($p>0.05$) relationships (Table 2.9).

Table 2.8. Mean[1] apparent ileal digestibility values (%) of amino acids in Australian barleys determined with growing pigs.

Diet*	TA	CH	SL	LW	MU	AR	GR	SH	SN	SK	GA	Mean	SEM[2]
Amino Acids													
Aspartic acid	66.9	62.2	66.5	66.2	67.8	65.8	59.9	71.9	62.2	78.9	62.0	66.6	0.88
Threonine	66.6	57.9	59.3	61.5	64.1	59.9	59.2	68.5	57.9	74.5	51.7	62.8	1.01
Serine	70.4	61.0	63.1	66.9	67.8	64.3	61.0	73.8	61.6	79.3	62.1	67.3	0.95
Glutamic acid	85.9	81.3	78.8	78.8	81.5	82.3	75.9	85.7	75.2	89.8	79.6	82.2	0.62
Proline	61.8	37.4	50.1	66.7	76.1	50.4	62.3	57.8	36.4	64.7	41.6	56.3	2.33
Glycine	43.4	41.2	40.3	49.2	50.5	36.5	33.3	52.8	19.6	63.0	24.3	42.1	1.85
Alanine	61.1	62.	62.2	62.5	64.8	73.1	44.4	55.0	46.6	67.3	29.1	58.1	1.59
Valine	77.7	72.7	72.0	73.8	74.7	75.2	65.7	78.1	67.3	79.5	73.2	74.5	0.63
Isoleucine	78.2	72.0	69.9	71.1	73.1	75.9	60.9	75.0	60.5	83.1	67.7	73.0	0.88
Leucine	79.6	75.5	73.3	73.6	76.1	82.4	67.7	77.2	67.8	82.6	68.0	75.9	0.75
Tyrosine	79.4	76.8	73.0	72.4	74.7	77.3	72.0	68.3	66.0	80.8	67.3	74.6	0.72
Phenylalanine	82.5	78.6	74.3	74.6	77.1	80.6	75.9	82.0	73.3	87.0	74.1	79.0	0.64
Histidine	73.3	64.5	64.3	68.1	69.2	65.1	59.7	80.3	68.3	84.9	71.8	70.4	1.16
Lysine	71.9	68.0	69.7	68.9	68.4	66.1	63.6	72.6	66.4	80.1	69.7	70.1	0.68
Arginine	78.4	74.9	76.1	77.9	78.2	74.1	77.2	82.2	73.8	88.3	78.2	78.2	0.67
Cysteine	76.3	68.8	73.4	73.7	74.3	71.3	60.4	78.4	63.1	81.2	72.0	73.1	0.81
Methionine	84.2	74.9	78.5	77.7	77.3	79.1	80.3	79.6	87.0	85.0	76.7	80.6	0.65
Mean	72.8	66.3	67.4	69.6	71.5	69.4	63.5	71.9	61.9	79.4	62.9	69.7	0.76
SEM[2]	3.39	3.71	3.08	2.22	2.22	3.64	3.55	2.93	4.71	2.37	5.01	3.12	

1. Mean value of 5 observations. Control barley was expressed as a mean value of 15 observations across the Latin Squares
*TA: Tantangara (Control barley), CH: Chebec, SL: Sloop, LW: Lindwall, MU: Mundah, AR: Arapilis, GR: Grimmet, SH: Schooner Harts, SN: Schooner Norkes, SK: Skiff, GA: Galleon.
2. SEM: Standard error of mean

Table 2.9. Correlation coefficients[1] between various chemical characteristics (% DM) of Australian barleys and apparent ileal digestibility of essential amino acids.

Amino Acids	Hemicellulose	S β-glucan	TNSP[a]	SNSP[a]	INd	FNd
Threonine	-0.261	0.614*	0.833*	0.904**	0.873***	0.628
Valine	-0.482	0.413	0.558	0.783	0.522	0.608
Isolucine	-0.586*	0.416	0.392	0.628	0.509	0.593
Lucine	-0.603*	0.357	0.064	0.219	0.471	0.210
Phenylalanine	-0.350	0.596*	0.293	0.531	0.559**	0.580
Histidine	0.077	0.353	0.744	0.854*	0.707**	0.575
Lysine	-0.136	0.309	0.416	0.588	0.673*	0.789*
Arginine	-0.059	0.462	0.661	0.639	0.670*	0.459
Cysteine	-0.442	0.334	0.710	0.870	0.559*	0.853*
Methionine	0.315	0.292	0.388	0.691	0.719**	0.896**
Mean AA Digestibility	-0.468	0.526	0.675	0.747	0.750**	0.475

1. Based on 11 barleys, except the columns with superscript [a] which are correlation coefficients based on 6 barleys.
Significance level: Values without * : Not Significant ($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$
† Sβ-glucan: soluble β-glucan; TNSP: total NSP; SNSP: soluble NSP; INd: apparent ileal nitrogen digestibility; FNd: apparent faecal nitrogen digestibility.

Isoleucine and Phenylalanine had negative relationships ($p<0.05$) with hemicellulose while soluble β-glucan and soluble NSP had positive relationships ($p<0.05$) with Threonine, Phenylalanine, Valine, Histidine, and Cysteine. However, most of the relationships between apparent ileal digestibility of essential amino acids and ileal nitrogen digestibility were significant ($p<0.05 – p<0.001$).

Using ileal nitrogen digestibility values, the following prediction equations were generated from linear regression analysis (Table 2.10).

Table 2.10. Prediction equation of ileal digestibility of essential amino acids from ileal nitrogen digestibility.

Amino acids	Prediction equation	r^2	Significance level
Threonine	Y = 12.5 + 0.772 INd	0.76	***
Valine	Y = 53.6 + 0.318 INd	0.27	NS
Isoleucine	Y = 40.7 + 0.492 INd	0.26	NS
Leucine	Y = 52.3 + 0.359 INd	0.22	NS
Phenylalanine	Y = 55.8 + 0.354 INd	0.31	*
Histidine	Y = 20.1 + 0.772 INd	0.50	**
Lysine	Y = 43.7 + 0.404 INd	0.45	*
Arginine	Y = 53.8 + 0.375 INd	0.45	*
Cysteine	Y = 42.2 + 0.471 INd	0.31	*
Methionine	Y = 53.8 + 0.415 INd	0.52	**
Mean AA digestibility	Y = 33.2 + 0.559 INd	0.56	**

Significance level: NS Not Significant ($p>0.05$), * $p<0.05$, ** $p<0.01$, *** $p<0.001$
† INd: apparent ileal nitrogen digestibility.

Using GLM procedure of Minitab, apparent ileal digestible lysine content of Australian barleys was predicted with the following regression equation:

$$AILYSC = -0.118 + 0.715 \times GLYS + 0.00157 \times FND$$

($r^2 = 0.993$, RSD=0.003)

Here, AILYSC is apparent ileal digestible lysine content (mg/100g DM), IND and FND are apparent ileal and faecal nitrogen digestibility (%), GLYS is gross lysine content (mg/100g DM) in barley.

2.3.5 Endogenous amino acid flows (EAAF)

The mean endogenous amino acid flows (EAAF, μg/g dry matter intake) determined under conditions of EHC/Ultrafiltration are presented in Table 2.11.

Table 2.11. Mean[1] endogenous amino acid flow (μg/g^{-1} dry matter intake) in 35-55kg pigs determined under EHC/Ultrafiltration method, and comparison with literature values.

Reference	1	2	3	Mean[2]	Present Study	SEM[3]
Amino Acids						
Aspartic acid	1276	1531	1878	1404	1111	130
Threonine	993	909	1108	951	792	108
Serine	1378	1383	1287	1381	1291	166
Glutamic acid	2580	3378	3610	2979	2399	280
Proline	2227	1419	1630	1823	1396	229
Glycine	1261	682	762	972	607	81
Alanine	637	485	671	561	620	73
Valine	687	593	1031	640	705	94
Isoleucine	516	504	638	510	638	86
Leucine	744	528	883	636	721	93
Tyrosine	359	244	341	302	369	44
Phenylalanine	386	278	509	332	345	43
Histidine	359	319	436	339	378	70
Lysine	448	461	663	455	472	64
Arginine	510	373	481	442	392	48
Cysteine	ND[4]	ND	432	ND	181	14
Methionine	ND	ND	342	ND	160	20

1. Mean value of 15 observations across Latin Squares (New Zealand and Australia study)
2. Mean of reference 1 and 2. Due to reference 3 using 18% EHC, it was not included for comparison.
3. Standard error of mean for present study
4. ND: not determined

Reference: 1. Butts et al. (1993[a]). 20kg, n=6, 10% EHC
2. Moughan et al. (1992). 15kg, n=5, 10% EHC
3.Schulze et al. (1995). 10kg, n=4, 18% EHC

The mean values of individual amino acid flows were not significantly different from previously reported values (p>0.05, paired T-test between average values). The mean endogenous Lysine flow in 35-55kg pigs was 472 μg/g DMI in the present study.

Regression plots (fitted line plot) of endogenous amino acid flow and endogenous lysine flow (mg/kg DMI) against live weight (kg) are shown in Figure 2.2. Due to the unavailability of live weight data from SARDI, only EAAF and ELF data that were measured in New Zealand (10 pigs) were used for the statistical analyses. Generally, statistically non-significant (p>0.05) relationships were found between live weight (kg) of pigs and EAAF and ELF. The poor relationship seems to be due to the variation

between animals around 40kg pigs. The overall tendency, however, showed decreased EAAF value with increasing live weight (Figure 2.2).

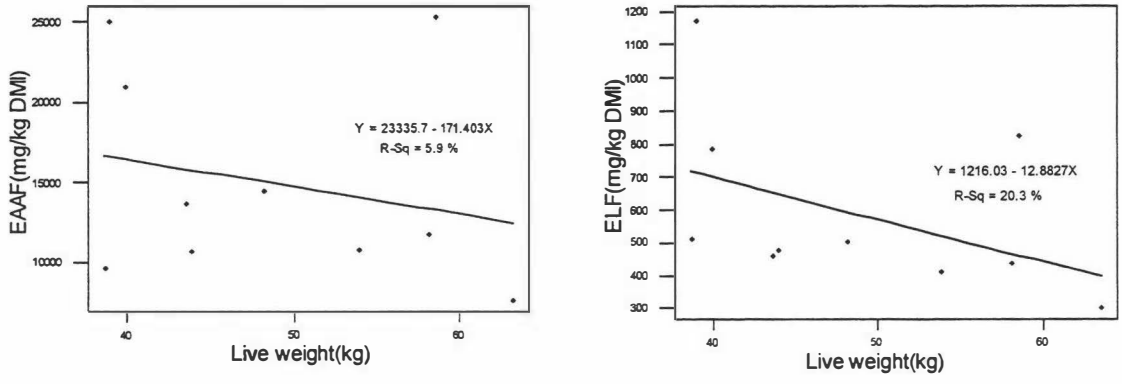


Figure 2.2 Fitted line plot of endogenous amino acid flow (EAAF mg/kg DMI) and endogenous lysine flow (ELF mg/kg DMI) against live weight (kg) of pigs

2.3.6 True amino acid digestibility

Using these EAAF values (the overall mean value of present study including experiment conducted in Australia), the apparent ileal amino acid digestibility values were corrected and the results were expressed as the true ileal amino acid digestibility (%). The mean true ileal amino acid digestibilities of 11 Australian barleys are presented in Table 2.12.

The mean true ileal amino acid digestibility of Australian barleys was 84.1%. The variation of amino acid digestibility between diets was highest in Proline, Glycine, and Alanine (in decreasing order).

Correlation coefficients between the NSP content of barley and true ileal digestibility of essential amino acids are presented in Table 2.13. Generally, statistically non-significant ($p>0.05$) relationships between all chemical components of barleys and true ileal digestibility of essential amino acids were found. In comparison to apparent amino acid digestibility, however, the true digestibility of essential amino acids showed negative relationships ($p<0.01$ – non-significant) with β-glucan and NSP content.

Table 2.12. Mean[1] true ileal digestibility values (%) of amino acids in Australian barleys determined under EHC/Ultrafiltration method with growing pigs.

Diet	TA	CH	SL	LW	MU	AR	GR	SH	SN	SK	GA	Mean	SEM[2]
Amino Acids													
Aspartic acid	83.8	80.6	83.0	81.3	81.2	82.5	76.1	86.2	81.6	93.4	84.2	82.3	0.81
Threonine	88.3	83.3	81.8	81.5	81.6	83.5	90.5	91.2	97.1	97.5	87.6	87.5	1.00
Serine	97.6	98.7	97.3	94.9	93.7	98.0	84.5	95.2	91.7	100	96.3	95.9	0.77
Glutamic acid	94.1	93.9	91.9	87.7	89.2	93.3	85.0	93.3	89.1	96.9	93.3	91.9	0.53
Proline	72.6	55.8	66.3	78.9	86.1	64.3	71.9	70.0	51.4	77.3	63.0	69.5	2.17
Glycine	59.8	55.6	54.2	61.5	61.7	51.9	62.0	72.6	55.9	82.4	53.2	60.9	1.66
Alanine	75.5	77.2	76.4	75.2	76.3	83.6	58.0	71.1	62.3	82.2	53.0	72.8	1.31
Valine	89.3	88.2	85.9	85.4	85.4	88.6	78.9	90.0	83.9	93.8	91.3	87.4	0.72
Isoleucine	92.8	92.6	88.9	86.5	87.3	92.8	83.8	90.9	89.9	97.5	92.0	90.3	0.59
Leucine	88.3	86.7	83.9	82.5	84.0	90.0	80.6	86.2	83.7	91.8	82.2	86.0	0.58
Tyrosine	90.5	89.3	85.1	82.6	83.8	88.1	90.3	86.7	94.3	95.6	88.7	88.8	0.62
Phenylalanine	88.1	88.5	81.8	80.6	82.6	87.2	81.9	88.2	82.3	93.0	84.5	85.7	0.57
Histidine	88.8	82.1	82.3	82.5	83.3	82.9	68.7	97.1	79.0	100	99.1	86.9	1.33
Lysine	84.1	81.2	81.2	79.5	78.3	80.3	77.7	84.2	83.3	92.9	86.0	82.9	0.69
Arginine	85.9	83.5	83.5	84.4	84.2	83.1	86.8	90.9	87.5	95.4	91.3	86.8	0.67
Cysteine	84.9	81.7	85.9	84.2	83.5	83.9	67.8	84.1	71.2	86.9	82.0	82.4	0.79
Methionine	90.6	84.9	87.7	85.0	84.5	88.2	96.3	87.4	100	93.3	88.2	89.9	0.81
Mean	85.5	82.4	82.1	82.0	82.7	83.7	78.9	86.2	81.2	92.4	83.3	84.1	0.62
SEM[2]	2.96	3.49	2.91	2.05	2.02	3.28	3.15	2.40	4.37	1.99	4.16	2.66	

1. Mean value of 5 observations. Control barley was expressed as a mean value of 15 observations across the Latin Squares
2. Standard error of mean

*TA: Tantangara (Control barley), CH: Chebec, SL: Sloop, LW: Lindwall, MU: Mundah, AR: Arapilis, GR: Grimmet, SH: Schooner Harts, SN: Schooner Norkes, SK: Skiff, GA: Galleon.

Table 2.13. Correlation coefficients[1] between various chemical characteristics (% DM) of Australian barleys and true ileal digestibility of essential amino acids.

Amino Acids	T β-glucan	S β-glucan	TNSP[a]	INSP[a]	SNSP[a]
Threonine	-0.250	-0.525	-0.005	-0.482	0.254
Valine	-0.591*	-0.593*	-0.263	-0.462	-0.026
Isolucine	-0.571*	-0.563*	-0.330	-0.361	-0.152
Lucine	-0.541	-0.549	-0.202	-0.323	-0.036
Phenylalanine	-0.317	-0.320	-0.064	-0.422	0.293
Histidine	-0.516	-0.518	0.384	-0.142	0.478
Lysine	-0.393	-0.390	-0.141	-0.610	0.176
Arginine	-0.131	-0.139	0.350	-0.139	0.442
Cysteine	-0.728**	-0.735**	0.295	-0.463	0.540
Methionine	0.250	0.216	0.015	-0.578	0.323
Mean AA Digestibility	-0.528	-0.545	0.162	-0.169	0.260

1. Based on 11 barleys, except the columns with superscript [a] which are correlation coefficients based on 6 barleys.

Significance level: Values without * : Not Significant ($p>0.05$), * $p<0.05$, ** $p<0.01$

† Tβ-glucan: total β-glucan; Sβ-glucan: soluble β-glucan; TNSP: total NSP; INSP: insoluble NSP; SNSP: soluble NSP

2.4 Discussion

2.4.1. Apparent ileal and faecal energy digestibility

The primary function of cereals in monogastric rations is to provide energy for growth and production. Accurate, rapid and inexpensive prediction of *in vivo* energy digestibility from chemical characteristics, therefore, offers possibilities in the routine, cost effective evaluation of feedstuffs. The present work investigated the mathematical relationships between chemical characteristics of barley and *in vivo* ileal and faecal energy digestibiltiy.

The ileal energy digestibility of Australian barley ranged from 53.6 to 71.0% with a mean digestibility of 63.9%. In contrast, Baidoo et al. (1998) reported a range in average digestibility of 64.0 – 72.6% in hulless barley, and Pettersson and Lindberg (1997) reported a range of 70 - 75% in hulled barley. However, the mean apparent ileal energy digestibility observed in this study agreed with the study of Graham et al. (1989), who reported on ileal energy digestibility of 63.3% in a pelleted barley diet. The faecal energy digestibility of Australian barley had quite a narrow range (79.2 – 82.5%) with a mean value of 80.3%. This is in agreement with study of Graham et al. (1989) and Bhatty et al. (1974, 1975).

Correlation analysis revealed negative relationships between apparent ileal energy digestibility and insoluble NSP ($r=-0.811$, $p<0.03$) concentration. Also, faecal energy digestibility was negatively correlated with NDF, ADF, and hemicellulose. These results support those reported previously (Bhatty et al., 1974; King and Taverner, 1975; Bell et al., 1983; Miller et al., 1994; Petterson and Lindberg, 1997) where highly negative correlations ($r=-0.9$) between fibre contents (Crude fibre, NDF, ADF) and energy digestibility in pigs were seen. Also, Beames et al. (1996) found a negative relationship ($r=-0.97$) between insoluble NSP and apparent faecal energy digestibility in 35-48kg pigs. Since the cereal NSP largely escape digestion in the small intestine, especially the insoluble fraction, the concentration of insoluble NSP (or fibre) would directly affect the ileal and faecal energy digestibility of energy (Åmen et al., 1985; Bach Knudsen et al., 1987). Although a significant amount of NSP is broken down by microbial enzyme in the hindgut and distal small intestine, both the composition of NSP fraction and the degree of lignification influence the extent of

microbial breakdown of NSP in the hindgut. Therefore, a higher dietary fibre content reduces DE and ME in feedstuffs and the utilisation of ME is lower as the most of DE derives from short chain fatty acids.

Soluble NSP are known to elevate viscosity in the small intestine of chickens thereby depressing enzyme activity, starch digestion, and convective transport of glucose (White et al., 1981; 1983; Choct and Annison, 1992 [a;b]; Smith and Annison, 1996). However, such antinutritive effects of soluble β-glucan or soluble NSP on energy digestibilities were not found in the present study. This was probably due to the low concentration of β-glucan in barley grain for pigs to obtain a meaningful correlation between soluble NSP and energy digestibility. In the present study, insoluble NSP rather than total NSP negatively affected ileal energy digestibility. This finding supports that of Graham et al. (1989) who showed extensive digestion of soluble NSP fraction (β-glucan) in the upper gut of pigs.

2.4.2. Apparent ileal and faecal nitrogen digestibility

Practical diet formulation requires accurate determination of digestible protein in the single feedstuffs. Therefore, the possibility of predicting *in vivo* protein digestibility from chemical components would offer attractiveness for the cost-effective evaluation of feedstuffs.

The ileal nitrogen digestibilities of eleven Australian barleys ranged from 52.5% to 76.0% with a mean value of 64.9%. The mean faecal nitrogen digestibility was 69.2% (64.4% - 75.7%) in six barleys. These values agree with those reported in the studies of Fan and Sauer (1995) and Graham et al. (1989).

Correlation analysis found positive relationships between crude protein, soluble β-glucan, soluble NSP, faecal nitrogen digestibility and apparent ileal nitrogen digestibility. Faecal nitrogen digestibility was positively (but not significantly) correlated to crude protein and soluble NSP. Bell et al. (1983) reported a positive relationship between CP content of barley and faecal nitrogen digestibility in pigs. Also, Boros et al. (1996) found positive relationships between the CP content ($r=0.80$), total β-glucan content ($r=0.67$), and soluble dietary fibre content ($r=0.33$), of barley to true protein digestibility in rats. However, relationships between ileal or

faecal nitrogen digestibility and soluble NSP or soluble β-glucan in pigs was not examined in any study.

In chicken studies, decreased protein digestibility and increased endogenous protein secretion were demonstrated with increased soluble NSP inclusion (Choct and Annison, 1990; 1992[a]; Annison, 1993). However, such antinutritive effects of barley soluble NSP and β-glucan were not found in the present study using pigs.

Investigations between the chemical composition of barleys found significant positive correlations between total NSP ($p<0.01$), soluble NSP ($p<0.001$), soluble β-glucan ($p<0.01$) and crude protein content. A positive relationships between nitrogen content and total β-glucan content in some barley cultivars was previously reported by Henry (1985). However, relationships between soluble β-glucan, soluble NSP, total NSP, and CP content in barley have not been examined previously in the literature. Given the positive relationship between NSP and CP contents in Australian barleys, such a positive relationship between nitrogen digestibility and NSP can be explained because nitrogen digestibility was positively related to CP content in barley. Therefore, the positive effect of CP on ileal nitrogen digestibility may mask the antinutritive effect of soluble NSP on ileal nitrogen digestibility. Moreover, the (relatively) low concentration of naturally occurring soluble NSP in Australian barleys may have contributed to the above effect.

Also, the use of heavier pigs in the present study may be one reason, at least partly, for the positive relationship between soluble NSP and nitrogen digestibility. Most of studies with pigs weighing more than 20kg failed to improve digestibility coefficients in barley diet by adding β-glucanase (Graham et al., 1986; 1989; Thacker et al. 1988), while several studies that used young pigs showed improved digestibility with supplementation of β-glucanase (Thacker et al., 1992[b]; Li et al.1996). The failure to improve digestibility in pigs more than 20kg may be due to the large duodenal lactobacilli populations capable of degrading β-glucans in the barley diet. Also, Graham et al (1989) reported high digestibility of total NSP (55 - 60%) at the terminal ileum of 80kg pigs.

In addition, the species difference between chicken and pigs may have contributed to the positive relationship between soluble NSP and nitrogen digestibility. The antinutritive effect of soluble NSP was observed in chickens with increased gut viscosity. Bedford and Classen (1992) found significantly decreased

intestinal digesta viscosity with pentosanase supplementation in the rye-fed chicken, while no reduction was found in 12kg pigs fed barley with β-glucanase supplementation (Bedford et al., 1992). This was due to the fact that digesta dry matter in the pig small intestine is much lower than in the chicken, which would significantly dilute the viscous-forming NSP. Also, Johansen et al. (1997) found a very poor correlation between viscosity and the β-glucan concentration in the liquid phase digesta in pigs fed an Oat-bran diet. Moreover, Bedford et al. (1991) found that gut viscosity in rye-fed chickens was only correlated (r=0.93) with high molecular weight carbohydrates (5 X 10^5). Since significant depolymerisation of β-glucan occurs in the gastrointestinal tract of pigs (Johansen et al., 1993), decreased high molecular weight NSP may reduced intestinal viscosity in the pig suggesting possible antinutritive effect of viscous-forming soluble NSP would be low in pigs compared to chickens.

Therefore, positive relationships between NSP fraction in Australian barleys and nitrogen digestibility seems to be due to the depressed antinutritive effect of NSP by the positive effect of CP on nitrogen digestibility. In addition, low concentrations of soluble NSP in Australian barley, the use of growing pigs in the present study, and species difference between chicken and pigs have contributed, at least partly, to the depressed antinutritive effect of NSP on nitrogen digestibility.

2.4.3. Endogenous amino acid flows (EAAF)

Endogenous amino acid flows from the gastrointestinal tract of pigs have been determined previously after feeding the animal a protein-free diet (Furuya and Kaji, 1991; Viljoen et al., 1998). This traditional method, however, is claimed to create a physiologically abnormal state (Low, 1980). The protein-free diet may alter rate of whole body protein synthesis (Millward et al., 1976; Muramatsu, 1990) and may affect the amount of protein entering the gut (De Lange et al., 1990; Chung and Baker, 1992; Moughan et al., 1992; Butts et al., 1992, 1993[a]). Therefore, a new approach, EHC/Ultrafiltration method, for determining endogenous ileal amino acid flows was developed by Moughan et al. (1990). This method involves feeding pigs a semi-synthetic diet containing peptides and free amino acids as the sole source of nitrogen for determination of endogenous amino acids at the terminal ileum of the

pigs. This method was used as a baseline in the present study to determine true ileal amino acid digestibility in Australian barleys. Despite the limitation that it may underestimate EAAF to some degree, because low molecular weight endogenous amino acids and peptides are discarded in the ultrafiltration process, the EHC/Ultrafiltration method was recognised as a valid method for determination of the EAAF. It was demonstrated that the low molecular weight fraction in endogenous amino acid excretion is low (Moughan et al., 1990; Moughan and Schuttert 1991; Butts et al., 1992). Therefore, the degree of underestimation would be small.

The EAAF values of Schulze et al. (1995[a]) were higher than other studies including the present study, probably due to the inclusion of higher amounts of EHC (18% *vs.* 10%) and the use of lighter pigs (10kg *vs.* 35-55kg). These facts were supported by the findings of Hodgkinson et al. (1997) who demonstrated decreased EAAF with low EHC inclusion and increasing pig weight. The correlation between EAAF and live weight in the present study was statistically non significant ($p>0.05$), however, there was an overall tendency for decreased EAAF with increasing live weight, which is in agreement to the study of Hodgkinson et al. (1997).

The EAAF values of the present study were lower than those found by Butts et al. (1993[a]) and Moughan et al. (1992), probably due to the use of higher live weight pigs. The EAAF values of the present study, however, were not significantly ($p> 0.05$) different to these studies. A predominance of non-essential amino acids (Glu, Asp, Pro, and Ser) was found in the present study, contributing about 50% of the total EAAF. This was in agreement with the studies of Moughan et al. (1992), Butts et al. (1993[a]), and Schulze et al. (1995). Glutamic acid, in particular, was present at a high concentration, as found in the other studies (Moughan et al., 1992, Butts et al., 1993[a], and Schulze et al., 1995). Due to their particular resistance to acid and enzyme digestion of mucus glycoproteins (Hashimoto et al., 1963; Hoskins, 1978; Dekker, 1990), Gly, Pro, Thr, Glu, and Asp are the predominat components of ileal endogenous amino acid flows (Bella and Kim, 1972; Cetta et al., 1972; Allen, 1981; Dekker, 1990).

2.4.4. Apparent and true amino acid digestibility

The mean apparent ileal amino acid digestibility of Australian brleys was 69.7%, and the mean true ileal amino acid digestibility was 84.1%. These results are in agreement with the studies of Fan and Sauer (1995) and Baidoo and Liu (1998).

The complete correlation analysis between chemical components of Australian barleys and apparent or true ileal digestibility of essential amino acids showed very poor relationships. However, statistically significant positive relationships were found between apparent ileal digestibility of essential amino acids and apparent ileal and faecal nitrogen digestibility. This finding provides an opportunity to predict *in vivo* ileal apparent digestibility of essential amino acids from a simple determination of apparent ileal nitrogen digestibility. Unlike ileal nitrogen digestibility, faecal nitrogen digestibility was not correlated to apparent digestibility of essential amino acids. This result is most likely due to the extensive bacterial breakdown of exogenous and endogenous nitrogen in the hindgut of the pig and the predominance of bacterial nitrogen (62 – 76%) in the faeces (Stephen and Cummings, 1980; Mason, 1984; Low and Zebrowska, 1989). However, mathemetical investigation was found several prediction equation of apparent ileal lysine content from ileal or faecal nitrogen digestibility along with gross lysine content of barleys (refer to section 2.3.4).

In the present study, the attempt to demonstrate antinutritive effects of soluble NSP on apparent amino acid digestibility could not be supported, most likely due to the relatively small amounts of naturally occurring soluble NSP in the Australian barley lines, and due to a high fibre digestion in the upper gut of the pigs (Åmen and Graham 1987[a]; Graham et al., 1989; Bach Knudsen et al., 1993[b]; Johansen et al., 1997). However, an interesting finding in the present study was the negative (but not significant) relationships between true amino acid digestibility and NSP fraction, while apparent amino acid digestibility showed positive relationships to the NSP fraction.

Apparent amino acid digestibility, but not true amino acid digestibility, is known to be influenced by protein concentration of diets. Its influence was due to a high contribution of endogenous amino acids in the low protein diet, while the proportion of endogenous amino acids decreases as the protein level in the diet increases, resulting in an increased apparent ileal digestibility (Sauer et al., 1980; Bell et al., 1983; Furuya and Kaji, 1989[a]; Keith and Bell, 1991). Therefore, it is recommended that diets should contain at least 15 – 16% crude protein to determine apparent ileal

AA digestibility (Sauer et al., 1898[a]). The diet which was used in the present study (7.6 – 14.2% CP) did not fulfil the above requirement (15 - 16% CP), and thus, apparent AA digestibility would be greatly influenced by crude protein level. Given this, the findings that crude protein in the Australian barleys have significant relationships with NSP fraction can be explained by the lack of antinutritive effects of NSP on apparent AA digestibility.

Moreover, small amounts of naturally occurring NSP in the Australian barleys may have accounted for the lack of antinutritive effect of NSP on apparent AA digestibility. Most of the studies that demonstrated significant antinutritive effect of NSP on nutrients digestibility were designed in such a way that a fibre source was added to the basal diet. For example, van Barneveld et al. (1995[a]) studied to find decreased apparent AA digestibility in the sorghum-based diet by adding 0 to 36% lupin kernel, which is a rich source of NSP. The deleterious effect of NSP was only found when 36% lupin kernels was added to the basal diet. This amount of NSP never occurs in the normal cereal diets. Therefore, the failure to observe any effects of NSP on the apparent AA digestibility in the present study may explained by the greater ability of fibre degradation in the upper gut of pigs and inherently low levels of naturally occurring NSP in the barley.

In contrast, no single chemical characteristics of barleys were correlated with true amino acid digestibility in the present study. Numerous studies have established that the true ileal digestibility coefficients are independent from the protein level in the test diet (Furuya and Kaji, 1989[b]; Zuprizal et al., 1991; Donkoh and Moughan, 1994; Pfeiffer et al., 1995; Angkanaporn et al., 1997). Therefore, and unlike apparent AA digestibility, positive relationships between NSP and true AA digestibility were not found. Inverse, negative (but not significant) relationships were found between most of the NSP fractions and true digestibility of essential amino acids. It is well established that NSP, especially the soluble fraction, and fibre sources have antinutritive effects on nutrient digestibility in chickens and pigs (Bach Knudsen et al., 1993[a;b]; van Barneveld et al, 1995[a;b]). For apparent AA digestibility coefficients the endogenous amino acid flows, which may be influenced by numerous factors such as protein level of diet, and live weight of the pig, may have masked the weak antinutritive effects of NSP on AA digestibility. Due to the true AA digestibility values corrected for endogenous amino acid flows in the ileal digesta, however, even the weak antinutritive effects of NSP on true AA digestibility may be detected.

These results support the concept that NSP contents in the diet have a negative influence on nutrient digestibility in the pigs, however, the naturally occurring NSP contents in the barley diets were not sufficient to obtain meaningful correlations.

2.4.5 Chemical composition of Australian barley

The chemical composition of all Australian barleys was examined to establish correlations between chemical components and ileal and faecal digestibilities of energy, nitrogen, and amino acids.

The chemical analysis showed that the range in CP (7.6 – 14.2% DM, CV 16.3%) for the eleven Australian barleys was wider than previously reported values. Åmen et al. (1985) reported a range in CP of 8.9 – 14% in Swedish 6-row barleys (n=11, CV 15.2%) and 8.6 – 13.4% in 2-row barleys (n=81, CV 9.78%). Also, Åmen and Newman (1986) reported a CP range of 12.2 – 16.8% in Montana 2-row barleys (n=16, CV 9.7%) and 12.2 – 15.8% in 6-row barleys (n=7, CV 9.3%). Meads (1997) reported a range of 8.5 – 13.3% in New Zealand barleys (n=17, CV 12.33%).

The level of β-glucans observed in the Australian barleys (2.0 – 4.33%, CV 18.99%) are consistent with other values reported for barley. Graham (1991) reported 2.4 – 4.2% (n=16, CV 18%) total β-glucan in Swedish barley. Åmen (1986) studied total β-glucan content in springsown Swedish barley (n=92) and found a range of 2.4 - 4.6% (CV 13.8%). Fincher and Stone (1986) reviewed the total β-glucan levels in malting barleys and reported a range from 2.0 – 10.7%. Total β-glucan contents are known to be strongly influenced by the growing climate (Hesselman and Thomake, 1982; van Wijk et al., 1998), which contributes to such a range in concentrations. Soluble β-glucan contents in Australian barleys showed a smaller variation (0.21 – 0.34%, CV 17.24%) compared to New Zealand barleys (0.63 – 3.03, CV 23.3%) (Meads, 1997). Since soluble β-glucan contents are also strongly influenced by the growing climate, ripening period, and harvest time (Hesselman et al., 1981; Hesselman and Thomake, 1982; Hesselman and Åmen, 1986), the difference in soluble β-glucan content between New Zealand and Australian barleys can be explained.

Total, insoluble, and soluble NSP in the Australian barleys were similar to other reported values (Bach Knudsen et al., 1987; Gabert et al., 1995; Oscarsson et al.,

1996; Castanon et al. 1997). In general, the mean determined amino acid concentrations were very close to values for barleys reported in the literature (CSIRO, 1987; Gabert et al., 1995).

The contents of NSP in barley are known to be influenced by the growing climate, and locality (Hesselman and Thomake, 1982; van Wijk et al., 1988). However, the findings that the total and soluble NSP are significantly ($p<0.05$ - 0.001) correlated to CP contents in barley across the locality will provide good information either to plant breeders or to animal nutritionists. It may be considered that if one is selecting barley with high crude protein, the increase of soluble and total NSP may be inevitable.

Chapter 3

General Conclusion

Modern diet formulation requires accurate determination of the nutritive value of each feedstuff to maximise the utilisation of feed. Therefore, the identification of the degree of anti-nutritive effect of some chemical components in feedstuffs is of importance for critical feed evaluation.

The NSP, especially soluble NSP, in the cell walls of barley are known to possess anti-nutritive factors which limit the availability of nutrients by acting on the nutrient themselves or causing a deleterious, physiological effect on the animal. Soluble NSP, which have hydrophilic properties due to their free hydroxyl groups, increase the viscosity extensively in the gut of some monogastric animals such as chickens. Therefore, water soluble NSP and highly branched heavy molecular weight NSP are the main cause of the formation of viscous solution in the gastrointestinal tract of the animal (Annison, 1993; Annison and Choct, 1994; Smith and Annison, 1996; Choct, 1997). The formation of viscous digesta in the gastrointestinal tract may delay gastric emptying, reduce efficiency of endogenous enzyme activity, reduce absorption rates in the small intestine (Selvendran et al., 1987), and increase endogenous secretion (Low, 1989).

The aim of the present study was to determine relationships between chemical characteristics of barley and *in vivo* nutrient digestibility as an indicator of antinutritive effects of NSP concentration on digestibility coefficients.

Ileal and faecal energy digestibilities were negatively correlated ($p<0.05$) to insoluble NSP, NAD, ADF, and hemicellulose contents. This may be due to the dilution effects of fibre component in the barley diet, as found in many other studies (Bhatty et al., 1974; Bell et al., 1983).

Ileal apparent nitrogen and amino acid digestibilities showed positive relationships with most of the NSP fractions, while true amino acid digestibility showed negative (but not significant) relationships with most of the NSP fractions. The positive relationships with apparent digestibility may explained by:

1. The strong, positive correlation between crude protein content and most of the NSP fractions in the barley, thus suppressing the protein effect over any antinutritive effects of NSP.

2. The strong influence of crude protein level in the diet on apparent nitrogen and amino acid digestibility through high contribution of endogenous amino acids in the low protein diet (Furuya and Kaji, 1989[a]; Keith and Bell, 1991).

3. The concentration of NSP in the diet was insufficient to obtain a meaningful correlation between NSP composition and digestibility coefficients.

4. Greater fibre digestion ability, especially soluble NSP, in the small intestine of growing pigs (>20kg) due to greater microorganism activity compared to chickens (Graham et al., 1989).

With respect to the negative (but not significant) relationships between true amino acid digestibility and NSP, even small amounts of NSP in barley may have antinutritive effects on *in vivo* amino acid digestibility. These small effects may be expressed in true amino acid digestibility due to the correction of effect of crude protein content and EAAF on digestibility coefficients.

The prediction of *in vivo* nutrient digestibility either from chemical attributes of the feed or from simple *in vivo* measurements offers the potential to minimise the need for expensive and time consuming animal digestibility trials. It was possible to predict the ileal energy digestibility from the level of insoluble NSP (r^2 = 0.66) and the faecal energy digestibility from the contents of hemicellulose (r^2 = 0.73). Also, ileal nitrogen digestibility was predictable from the level of CP and faecal nitrogen digestibility (r^2 = 0.795). Moreover, the prediction ability of apparent ileal digestible lysine content from faecal nitrogen digestibility and the content of lysine in the barley (r^2 = 0.993) will provide an opportunity to predict apparent ileal digestible lysine content without cannulation of pigs.

In addition, the quantification of endogenous amino acid flows in the evaluation of feed amino acids is extremely important. Using EHC/Ultrafiltration method, 472μg/g DMI of Lysine flow was determined. The mean EAAF of the present study were not significantly different from the previously reported values (Moughan et al., 1992; Butts et al., 1993[a]). The result that statistically not significant but constantly low EAAF values in the present study compared to previous studies was probably due to the use of higher live weight pigs used in the present study (Hodgkinson et al., 1997).

From the above investigation, it is concluded that NSP content in Australian barleys was too low to demonstrate meaningful antinutritive effect in growing pigs, probably due to the extensive fibre digestion in the upper gut of pigs and to the influence of other factors such as age of pigs and crude protein level in the diet. However, inclusion of high amount of NSP in pig diet, especially in the diet of young pigs (>20kg), would most likely affect nutrient digestibility.

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