NUTRITIONAL EVALUATION OF GRAIN LEGUMES FOR POULTRY

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The fear of the LORD is the beginning of knowledge: but fools despise wisdom and instruction (Proverbs 1:7)

ABSTRACT

The nutritional value of faba beans (*Vicia faba*), Australian sweet lupins (*Lupinus angustifolius*), white lupins (*Lupinus albus*) and peas (*Pisum sativum*) grown in New Zealand for broilers were evaluated in terms of their nutritional characteristics, protein quality (protein efficiency ratio), apparent metabolisable energy, apparent ileal digestibility coefficient of amino acids and the effects on bird performance. The effects of dehulling and extrusion cooking on the nutritive value of legumes were also investigated.

The first experiment discussed in Chapter 3 evaluated the effect of cultivars on the nutrient profile and protein quality of chickpeas (*Cicer arietinum*), Australian sweet lupins, peas and soybeans (*Glycine max*). With the exception of white lupins, cultivars had no effect on the proximate and fibre composition of grain legumes. Starch was the primary carbohydrate component of chickpeas and peas, whilst non-starch polysaccharides were the major carbohydrates in lupins. The legume proteins were deficient in lysine, methionine, cystine and threonine. No differences were found in protein quality between cultivars of the different grain legume species. The lowest weight gain and protein efficiency ratio, in addition to the highest relative pancreatic weight and mortality rate was found in raw soybeans, suggesting that soybeans contained high a concentration of anti-nutritional factors, such as protease inhibitors. Birds fed chickpeas, lupins and peas had a low mortality rate and relative pancreatic weight, confirming that the level of anti-nutrients in these legume seeds was low.

The apparent metabolisable energy and apparent ileal digestibility coefficient of amino acids of faba beans, Australian sweet lupins, white lupins and peas were determined in the second experiment (Chapter 4). Cultivar effect on the apparent metabolisable energy values was observed only for faba beans and white lupins. Faba beans, white lupins and peas had comparable apparent metabolisable energy values, but these values were higher than those of Australian sweet lupins, and lower than that of soybean meal. No cultivar differences were found in the apparent ileal digestibility coefficient of amino acids of grain legumes. The apparent ileal digestibility coefficient of amino acids of both lupin species was found to be comparable to that of soybean meal.

The effects of feeding diets containing 200 g/kg faba beans, lupins or peas on the performance, digestive tract development and litter quality of broilers were investigated in the third and fourth trials. In the cage trial (Chapter 5), the results showed that the weight gain of birds fed diets containing grain legumes was similar to that of control diet. Feed

intake and feed per gain of birds fed diets containing the majority of grain legume cultivars did not differ from those fed the maize-soy diet. Birds fed diets containing faba beans had more dry and friable excreta compared to other treatment diets. The performance of birds fed diets containing 200 g/kg grain legumes during the 35 d grow-out period, in the floor pen trial (Chapter 6), confirmed the results of the cage trial. In this trial, weight gain and feed per gain of birds fed diets without meat meal were superior to those with meat meal. In cage trials, the modification of some segments of digestive tract development was probably due to the dietary NSP. Whilst in floor pen trial, digestive tract development was not influenced by the inclusion of grain legumes.

The effect of methodology of determination (direct vs. difference method) on the apparent ileal digestibility coefficient of amino acids of wheat, maize, Australian sweet lupins, peas and soybean meal for broilers was evaluated in the fifth study (Chapter 7). The influence of methodology on apparent ileal digestibility coefficient of amino acids was found to vary amongst the feed ingredients. In general, the apparent ileal digestibility coefficient of amino acids of test ingredients determined by the difference method was higher than those determined by the direct method, suggesting that the use of the direct method may underestimate the apparent ileal digestibility coefficient of amino acids in low and medium protein ingredients.

Data reported in Chapter 8 shows that dehulling increased the apparent metabolisable energy values of faba beans and Australian sweet lupins, but it had no beneficial effect on peas. The increase of apparent metabolisable energy values may be attributed to the decrease in non-starch polysaccharides of these legume seeds after dehulling. The removal of hulls increased the amino acid concentrations, but it had no effect on the apparent ileal digestibility coefficient of most amino acids. These results suggest that dehulling of grain legumes would be nutritionally beneficial and, likely to be economical in view of the improved amino acid concentrations and energy values.

The final experiment (Chapter 9) demonstrated that extrusion of peas markedly influenced the content of crude protein, non-starch polysaccharides, starch, and trypsin inhibitors. The soluble non-starch polysaccharides and trypsin inhibitor contents of the majority of extruded pea samples were higher than those of raw peas, but insoluble and total non-starch polysaccharides decreased with extrusion. Extrusion had no effect on the apparent ileal protein digestibility and the apparent metabolisable energy of peas, but it increased ileal starch digestibility.

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List of abbreviations

AA	Amino acid
ADF	Acid detergent fibre
ANOVA	Analysis of variance
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANF	Anti-nutritional factor
AIDC	Apparent ileal digestibility coefficient
BBI	Bowman-Birk Inhibitor
BSE	Bovine spongiform encephalopathy
BW	Body weight
Ca	Calcium
Cys	Cystein
DCP	Dicalcium phosphate
DM	Dry matter
DMSO	Dimethyl sulfoxide
FCR	Feed conversion ratio
GE	Gross energy
HU	Haemaglutinin Unit
IVPD	*In vitro* protein digestibility
IVSD	*In vitro* starch digestibility
LSD	Least significant difference
MBM	Meat and bone meal
MM	Meat meal
NDF	Neutral detergent fibre
NSP	Non starch polysaccharide
PER	Protein efficiency ratio
SCFA	Short- chain fatty acids
SD	Standard deviation
SBM	Soybean meal
SEM	Standard error of mean
Ti	Titanium

TIA	Trypsin inhibitor activity
TIU	Trypsin inhibitor unit

CHAPTER 1

General Introduction

Soybean meal (SBM), fish meal, and meat and bone meal (MBM) are the most common sources of protein used in poultry feed formulations around the world. These ingredients are widely use because of their high protein contents and well balanced amino acid profiles. However, in some parts of the world, the use of animal protein sources are now banned because of the Bovine Spongiform Encephalopathy (BSE) scare associated with MBM. In addition, although SBM is still the preferred protein source, the increasing price of this raw material continues to be a concern. As a result, it has become necessary to evaluate alternative protein sources, which can fully or partially substitute conventional protein sources in poultry feed formulations.

Amongst the potential candidates, grain legumes such as peas (*Pisum sativum*), chick peas (*Cicer arietinum*), faba beans (*Vicia faba*), Australian sweet lupin (*Lupinus angustifolius*) and white lupin (*Lupinus albus*) have the greatest potential for further evaluation in New Zealand. Grain legume crops play an important role in both human and animal nutrition. The seeds of these legumes contain moderately high levels of protein and their amino acid profiles are generally comparable to that of SBM, with the exception of sulphur-containing amino acids. However, the utilisation of grain legumes in poultry diets remains limited, due to the variability in their nutritional composition and the presence of a variable amount of anti-nutritional factors. It is well documented that feeding poultry with diets containing raw legumes can cause a number of nutritional disturbances (Farrell *et al*., 1999; Olkowski *et al*., 2001; Rubio *et al*., 2003).

According to Ravindran and Blair (1991), three main factors relating to nutritional, technical and socio-economic aspects, limit the use of new ingredients in practical formulations. The nutritional aspects include limited published information on nutrient contents, variability in nutritive value, the presence of anti-nutritional factors and the need for supplementation (energy and/or amino acids). The technical limitations include seasonal and unreliable supply, bulkiness, physical characteristics, need for processing and, limited research and development efforts. With regard to socio-economic aspects, factors that should be taken into consideration include competition as human foods, poor prices relative to other

arable crops, the cost per unit of protein (or limiting amino acids) relative to SBM and the cost of processing.

In New Zealand, comprehensive information about locally grown grain legumes will give the feed industry more stability and also add flexibility to diet formulations. The usefulness of grain legumes in diets for poultry has been extensively researched elsewhere and a wealth of information is available on their nutritive value (see Chapter 2). However, whilst such information could serve as a guide, it may be inadequate for accurate feed formulations under local conditions. It is known that differences in cultivars, soil, climate and agronomic factors can cause appreciable variations in the nutrient profiles and nutrient utilisation between locally grown feed ingredients and those available in other parts of the world. Thus a research programme undertaken to characterise various aspects of the feeding value of grain legumes is warranted. The focus of this thesis research will be on selected grain legumes that were deemed to have the agronomic potential under local conditions. On this basis, chickpeas, faba beans, lupins (Australian sweet and white lupins) and peas were chosen for detailed evaluations. Chickpeas were selected for the initial evaluations, but they were not considered in subsequent studies because of low grain yields.

Most published data on the amino acid digestibility in ingredients for poultry was determined at the excreta level. This method, however, does not accurately reflect the excretion of unabsorbed dietary and endogenous amino acids from the birds due to the modifying effects of hindgut microbes, which metabolise protein leaving the small intestine. Currently, the accepted method for determining the amount of amino acids lost to the animal is to collect and analyse digesta from the terminal ileum (Payne *et al*., 1968; Ravindran *et al*., 1999).

The apparent ileal digestibility coefficient of amino acids can be determined by three different methods, namely, the direct, difference and regression methods (Lemme *et al*., 2004). In a study conducted by Fan and Sauer (1995a,b) with pigs, it was found that the amino acid digestibility values of high-protein feedstuffs can be determined with either direct or the difference methods, whilst direct or the regression methods can be used in determining the digestibility in low-protein feedstuffs. However, Ravindran and Bryden (1999) stated that the use of the direct method to determine the apparent ileal digestibility coefficient (AIDC) of amino acids in low-protein feedstuffs will result in the underestimation of AIDC because of the relatively greater proportion of endogenous amino acids in the digesta.

The digestibility of a feedstuff by the animal depends not only on the animal factors and environmental conditions, but also on the physical and nutrient characteristics of the feedstuff. The presence of anti-nutritional compounds in feed legumes including phytic acid, condensed tannins, polyphenols, protease inhibitors, α-amylase inhibitors and lectins can have negative effects on nutrient digestibility. Thus, some form of processing is needed in order to improve nutrient digestibility and therefore utilisation of legumes.

Several reports have shown that dehulling and heat processing, such as extrusion cooking, could be used to reduce or even eliminate the negative effects of the anti-nutritional factors found in legume seeds and to improve the digestibility of individual feed components (Alonso *et al*., 2000a,b; Brenes *et al*., 2003). The principle aim of dehulling is to remove the undigestible fibre component of the seed, mostly cellulose and hemicellulose, with the remaining kernel having higher energy and protein content, whilst the aim of extrusion is to achieve a high level of starch gelatinisation and disruption of the grain structure. However, inappropriate heating temperature (both under-heating and over-heating temperatures) applied during processing can produce negative effects in nutritional quality. Underheating will fail to eliminate heat-labile anti-nutritional factors, whilst overheating may destroy or lower the availability of essential amino acids such as lysine and cystine.

The main issue that was addressed in this research project was that "*What is the nutritional value of the selected grain legumes for poultry, what are the major limitations and how could the nutritional quality of these ingredients be improved?"* In order to answer these questions, a series of experiments were conducted. The specific aims of the research reported in this thesis were to:

1. characterise the nutrient content, in terms of proximate and fibre components, minerals, amino acid profile, and protein quality of cultivars of grain legumes grown in New Zealand,
2. determine the apparent metabolisable energy (AME) and ileal amino acid digestibility of grain legumes for broilers,
3. investigate the influence of methodology on the determination of ileal amino acid digestibility of grain legumes, cereals and soybean meal,
4. evaluate the growth performance, digestive tract development and litter quality of broiler chickens fed practical diets containing grain legumes,
5. explore possible ways of improving the nutritional value of grain legumes through dehulling and extrusion technology.

This thesis consists of ten chapters. The first two chapters provide a framework for the experimental research, with Chapter 1 providing the rationale for the focus of the research. A review of current literature covering various aspects of the use of grain legumes as protein sources in poultry diets is presented in Chapter 2 which forms the basis for subsequent experimental chapters. In the first experiment (Chapter 3), locally available cultivars of chickpeas, Australian sweet lupins, white lupins and peas were screened for their proximate and fibre components, mineral and carbohydrate contents, amino acid profile and protein quality. Chickpeas were excluded in subsequent evaluations and replaced by faba beans. The AME and AIDC of amino acids in grain legumes for broilers were determined and reported in Chapter 4. The effects of feeding diets containing 200 g/kg grain legumes on the performance and digestive tract development of broiler chickens housed in cages and floor pens are reported in Chapters 5 and 6, respectively. In the study reported in Chapter 7, the influence of methodology on the determination of apparent ileal amino acid digestibility of selected grain legumes, cereals and soybean meal is compared. The sixth experiment (Chapter 8) examined the effects of dehulling on the AME and ileal amino acid digestibility of grain legumes. The influence of extrusion on the nutritive value of peas is reported in the last experiment (Chapter 9). Peas were chosen because the nutritional value of this ingredient was not improved with physical processing (dehulling). The final chapter of the thesis provides a general discussion relating to the major findings of all experiments and it highlights the practical implications and possible areas for future research.

CHAPTER 2

Literature Review

2.1. Introduction

Soybean meal, fish meal, and meat and bone meal are the commonly used sources of dietary protein in poultry feed formulations around the world. In recent years, however, future availability and the increasing cost of these ingredients are becoming serious threats to the continued expansion of the poultry industry. As a result, it has become necessary to evaluate alternative protein sources which can fully or partially substitute the conventional protein sources in poultry feed formulation.

Grain legumes, such as chickpeas (*Cicer arietinum*), faba beans (*Vicia faba*), peas (*Pisum sativum*) and lupins (*Lupinus* spp.), are widely available in many parts of the world. These legumes play an important role as protein sources in both human and animal nutrition. However, their use in the poultry feed industry remains limited because of the uncertainty over their nutritional value and the presence of anti-nutritional factors which interfere with nutrient utilisation resulting in poor animal performance.

In general, grain legumes are moderate to good sources of protein, containing 150 to 400 g/kg crude protein (Boulter, 1980; Hedley, 2001). The predominant protein fraction in legume seeds is made of globulins (60 – 90 %), which are storage proteins rich in arginine, glutamic acid, aspartic acid and their amides. However, legume seeds are deficient in sulphur-containing amino acids (Wang *et al*., 2003). The deficiency of these amino acids, however, does not pose a problem in commercial feed manufacturing because of the availability and low cost of crystalline methionine. The deficiency of methionine and cystine could also be overcome, in part, by mixing the legume seeds with cereal proteins (Shewry and Tatham, 1999).

The usefulness of grain legumes in diets for poultry and pigs have been extensively researched elsewhere and considerable information is available on their nutritive value. The aim of this chapter is to review the published data on nutritional composition, including anti-nutritional factors, and the feeding value of grain legumes for poultry. The focus of the review will be on the following four legume species: chick peas (*Cicer arietinum*), faba beans (*Vicia faba*), lupins (Australian sweet and white lupins) and peas (*Pisum sativum*).

2.2. Anti-nutritional factors

Anti-nutritional factors are defined as naturally occurring substances that interfere with nutrient intake and/or availability in the animal (Saini, 1989). Their biological effects can range from a mild reduction in animal performance to death. Enneking and Wink (2000) reported that, based on their chemical and physical properties, anti-nutritional factors in grain legumes can be divided into 10 groups, namely non-protein amino acids, quinolizidine alkaloids, cyanogenic glycosides, isoflavones, tannins, oligosaccharides, saponins, phytate, lectins and protease inhibitors.

Studies with pigs and poultry have demonstrated that the anti-nutritional factors in raw, unprocessed grain legumes produce adverse physiological effects when ingested and, lower nutrient utilisation and animal performance. However, it must be noted that different species and age groups respond differently to a particular type and level of anti-nutritional factors. Also, each legume species or cultivar is likely to have a variable level of anti-nutritional factors and may have different biological effects (Gatel, 1994). Of the 10 groups of anti-nutritional factors identified above, protease inhibitors, lectins, tannins and non-starch polysaccharides (NSP) are considered more relevant in the legume species of interest in this thesis and discussed below in detail.

2.2.1. Protease Inhibitors

Protease inhibitors (trypsin and chymotrypsin inhibitors) are proteins of wide distribution in the plant kingdom, and are common constituents of legume seeds. They are grouped into Kunitz (Kunitz, 1947) and Bowman-Birk (Bowman, 1946; Birk *et al*., 1963) inhibitor families. The former is ‘single headed’ structure, consist about 180 amino acid residues and mostly active against trypsin, whilst the latter is ‘double headed’ structure, consisting of approximately 80 amino acid residues including 7 disulphide bridge and inhibit both trypsin and chymotrypsin (Habib and Fazili, 2007). Fernandez *et al*. (2007) showed that the most important interactions in the Bowman-Birk Inhibitor-trypsin inhibition complex were salt bridges and hydrogen bonds, whereas in the Bowman-Birk Inhibitor -chymotrypsin inhibition complexes, the most important interactions were hydrophobic.

Published values for trypsin inhibitor activity values in raw legumes range from 5.3-19.0 Trypsin Inhibitor Unit/mg for chickpeas (Wiryawan, 1997; Guillamón *et al*., 2007), 0.2-15.0 Trypsin Inhibitor Unit/mg for peas (Valdebouze *et al*., 1980; Griffiths, 1984; Morrison *et al*., 2005), 2.5-11.8 Trypsin Inhibitor Unit/mg for faba beans (Wiryawan, 1997; Guillamón

et al., 2007), <1 for *Lupinus* species (Wiryawan, 1997) and 43-84 Trypsin Inhibitor Unit /mg for soybeans (Guillamón *et al*., 2007).

Liener and Kakade (1980) showed that protease inhibitors can cause growth depression, and pancreatic hypertrophy and/or hyperplasia in rats and chickens, when fed plant products containing high levels of these detrimental constituents. Kakade *et al*. (1974) reported that protease inhibitor was responsible for 40 % of growth depression and pancreatic enlargement in rats fed raw soybeans. The inhibition of endogenous proteases in the small intestine would stimulate, by feed back control mechanism, pancreatic enzyme secretions (Liener, 1989). Since pancreatic enzymes are rich in sulphur amino acids, this stimulation would cause a loss of methionine and cysteine for body tissue synthesis.

It is known that when trypsin is inhibited by active trypsin inhibitors, proteins are poorly digested and the availability of amino acids is reduced (Wang *et al*., 1998). Kakade *et al*. (1969) reported that active trypsin inhibitors 'lock in' an appreciable proportion of cysteine which is already relatively deficient in legume seeds. As a consequence, the problem of meeting the requirements of sulphur-containing amino acids is made worse.

Protease inhibitors are readily denatured by heat, acid, alkali and enzymes or their concentrations can be reduced through plant breeding technology. Asao *et al*. (1991) demonstrated that a Bowman-Birk type proteinase inhibitor from faba bean was most stable in acidic conditions, but it loses its activity upon heat treatment (100 ^{o}C) at alkaline pH ($\geq$ 9). Meijer and Spekking (1993) evaluated the use of enzymes produced by certain strains of fungi and bacteria to inactivate proteinaceous protease inhibitors. The results of their study indicated that enzymes produced by fungi and bacteria could very quickly inactivate Kunitz trypsin inhibitor.

2.2.2. Lectins

Lectins (or haemaglutinnins), which are found in most legumes, are proteins with specific binding affinities for carbohydrate containing glycoproteins which are present in the plasma membrane of cells. Dietary lectins require, as a first step, binding to epithelial cells in the gut in order to elicit changes in cellular and body metabolism and the binding of lectins to cell surface glycoproteins may cause agglutination, mitosis, or other biochemical changes in the cell. Different lectins have different levels of toxicity, although not all lectins are toxic (Pusztai et al., 1990; Grant and van Driescche, 1993). Haemagglutinating activity in raw faba beans has been determined to be 49.3 Haemaglutinnin Unit/mg DM (Alonso *et al*., 2000b) and 6000 Haemaglutinnin Unit /kg DM in peas (Alonso *et al*., 2000a).

Soybean lectins have been stated as being responsible for about 25 % of the growth inhibition attributable to the ingestion of raw soybeans by rats (Liener 1953; Sullivan, 2000), but it has been concluded by some researchers that soybean agglutinin does not play any major role as a determinant of the nutritional quality of soybean protein (Liener, 1980).

Unlike most other proteins, legume lectins are highly resistant to digestive breakdown and substantial quantities of ingested lectins may be recovered intact from the faeces of animals fed legume-based diets (Liener, 1989; Pusztai, 1991; D'Mello, 2000). Apart from the high degree of resistance to proteolysis, the capability of lectins to bind brush border cells can cause damage to microvillus membrane, shedding of cells, and decrease in the absorptive capacity of the small intestine (Pusztai, 1991).

The anti-nutritional effects of legume lectins can be lowered by processing such as soaking, sprouting and fermenting (Pusztai, 1991; Gatel, 1994). Soaking legumes overnight does appear to remove or inactivate many of the lectins. Heating seems to remove lectins in some feeds, but both the duration of heating and the temperature are important. Steam heating also seems more efficient than dry heating. However, there is limited data to prove that any of these methods completely remove lectins.

2.2.3. Tannins

Tannins are water soluble polyphenolic compounds of varying molecular masses which have the ability to react with proteins, polysaccharides and other macromolecules, and to precipitate proteins from aqueous solutions (Butler, 1989; Mansoori and Acamovic, 2006). Proanthocyanidins (condensed tannins) and hydrolysable tannins are the two major classes of tannins. Proanthocyanidins are flavonoid polymers, whereas hydrolysable tannins are polymers of gallic or ellagic acid esterified to a core molecule, commonly glucose or a polyphenol such as catechin.

It has been well documented that feeding poultry with diets containing tannic acids depress bird performance through adverse effects on nutrient digestibility (Longstaff and McNab, 1991; Ortiz *et al*., 1993), increased endogenous amino acid excretion (Mansoori and Acamovic, 2006; Gabriel *et al*., 2007a), damage to the mucosal lining of the digestive tract (Ortiz *et al*., 1994), impaired immune function (Marzo *et al*., 1999), and alteration in the excretion of certain cations (Hassan et al., 2003). High concentrations of tannic acid (30 to 70 g/kg) could be toxic to poultry (Vohra *et al*., 1966; Joslyn and Click, 1969).

The effects of tannins on protein and amino acid digestion in monogastrics have been investigated by several researchers (Longstaff and McNab, 1991; Jansman, 1993a,b, Jansman *et al*., 1993a,b; Ortiz *et al*., 1993; Gabriel *et al*., 2007a,b). All these authors reported that protein and amino acid digestibilities were decreased by tannins. Ortiz *et al*. (1993) reported that there was a significant negative correlation between the dietary level of condensed tannins and amino acid digestibility coefficients. The decrease in amino acid digestibility in diets containing tannins is attributed to the binding of dietary tannins and feed proteins, and the complexation of tannins with digestive enzymes (Bressani *et al*., 1988; Salunkhe *et al*., 1990).

2.2.4. Non-starch polysaccharides

Polysaccharides are linear or branched chains of glycosidically linked sugar units, synthesized from a few types of hexoses, deoxy hexoses, pentoses and uronic acids (Englyst and Hudson, 1996). It is difficult to present a general description of the plant polysaccharides, partly because they are complex heterogeneous compounds and partly because they have been classified in a variety of ways, depending on the interests of the investigators (Englyst and Hudson, 1996).

Englyst and Hudson (1996) grouped all polysaccharide components other than starch as non-starch polysaccharides. Non-starch polysaccharides are further classified into cellulose and non-cellulosic polysaccharides, the latter containing hemicelluloses, β-glucans, pectic substances in addition to the storage polysaccharides such as inulin, gums and mucillages.

Non-starch polysaccharides contain components that are insoluble in aqueous media and those which are soluble. Many investigators, for analytical purposes, divide NSP into soluble and insoluble, based on solubility or extractability. This distinction, however, does not sufficiently differentiate between properties, but interestingly corresponds, in someways, to distinguishable physiological functions in the gut (Englyst and Cummings, 1990).

Hemicellulose, pectins, β-glucans and galactomannan gums are the examples of soluble dietary fibres (Cho *et al*., 1997). These fibre fractions are found to dissolve in buffer and enzyme solutions. Hemicellulose is the fibre fraction which is insoluble in cold and hot water and dilute acid, but soluble in dilute alkali. Pectin, which is the most widespread soluble dietary fibre structure in foods, is a polygalacturonic acid and β-glucans are linear polymers of glucose with β-(1→ 3), (1→ 4) glucosidic linkages (Smits and Annison, 1996). The insoluble fibre fraction refers to the fibre components that do not dissolve in various

solvents such as water, alkali and acid solutions. The insoluble fractions include cellulose, lignin, and some hemicelluloses.

It is generally accepted that the adverse effects of soluble NSP are primarily associated with the viscous nature of these polysaccharides and, their resultant effects on gastrointestinal physiology and morphology, and the interaction with gut microflora. The other modes of action include altered intestinal transit time, and changes in hormonal regulation due to a varied rate of nutrient absorption (Choct, 2001).

The soluble fraction of NSP increase the gut viscosity by directly binding water molecules, and the NSP molecules themselves interact and become entangled in the network (Smits and Annison, 1996). This increase in gut viscosity reduced the mixing of digestive enzymes and substrates in the intestinal lumen (Choct, 1997). Combined with increased mucus production, NSP can also increase the resistance of the unstirred water layer at the intestinal surface (de Lange, 2000). Furthermore, NSP in cell walls physically inhibit the access of digestive enzymes to nutrients that are encapsulated within cell walls. Soluble NSPs, in particular, may stimulate microbial growth and increase the amounts of microbial protein and fat at the terminal ileum. Certain NSP may also stimulate the growth of toxin producing microbes, which may affect gut health and digestive function (de Lange, 2000). In addition, endogenous secretions, such as bile acids, may be bound by the viscous NSP and consequently reduces the extent of recycling. All of the above could eventually lead to a reduction in nutrient digestion and utilisation.

Water-holding capacity is another characteristic of NSP that may influence its anti-nutritional properties of NSP (de Lange, 2000). The ability to absorb large amount of water and maintain normal motility of the gut becomes one of the most important attributes of insoluble NSP in monogastric nutrition (Stephen and Cumming, 1979). Choct (2004) reported that insoluble NSP can affect not only the digesta transit time and gut motility, but it can also act as a physical barrier leading to lowered nutrient digestion. However, Jorgensen *et al.* (1996) reported that insoluble NSP (cellulose) has only minor effects on the performance in poultry. Only at high levels, digesta retention time and nutrient digestibilities are adversely affected.

Legume NSPs are more complex in structure than those in cereals, containing a mixture of colloidal polysaccharides called pectic substances (Choct, 2006). Pectic substances are mainly found in the cotyledon of legume seeds, whilst cellulose and xylans, which are the major NSP in cereal grains, are only found in the hulls or husks of most

legume seeds. Periago *et al*. (1997) reported that the major constituents of the total NSP of chickpeas were cellulose, arabinose, and uronic acids.

The level of soluble, insoluble and total NSP of grain legumes are summarised in Table 2.1. The highest total NSP content was found in Australian sweet lupin, followed by white lupin, soybean meal, peas, faba beans and chickpeas. Carré *et al*. (1985) reported that lupin kernels contain pectic-like substances with the major polysaccharides being β-1,4-galactan consisting of a mixture of D-galactose, L-arabinose, L-rhamnose, and galacturonic acid. The cotyledon of lupin seeds also contains considerable amount (50-80 g/kg) of oligosaccharides of the rhamnose family (Saini, 1989). Gdala *et al*. (1997) showed that Australian sweet lupin NSP is primarily comprised of galactose (349 g/kg) and glucose (315 g/kg). Uronic acid, arabinose and xylose were found at intermediate levels (120, 107, and 83 g/kg, respectively).

The main components of NSP in peas and faba beans were glucose (476 g/kg and 453 g/kg, respectively), arabinose (194 and 164 g/kg, respectively) and uronic acid (150 g/kg and 146 g/kg, respectively) (Gdala and Buraczewska, 1997). According to Selvendran (1984) most of the arabinose in faba beans and peas is present as arabinose-containing pectin substances in the cell walls of the cotyledons.

Table 2.1. Soluble, insoluble and total NSP contents (g/kg DM) of some grain legumes and soybean meal

Legume	Soluble NSP	Insoluble NSP	Total NSP	References[1]
Chickpeas	20-33	74-76	96-107	1,5
Faba beans	50	140	190-209	3,8
Australian sweet lupin	22- 40	229-340	251-392	1,2,8
White lupins (cotyledon)	14-134	170-244	244-280	1,2,4
Peas	25-59	129-322	173-347	1,3,4,6,7,8
Soybean meal	63-139	154-164	217-303	1,4

[1] Smits and Annison (1996); 2. Van Barneveld (1999); 3. Knudsen (1997); 4. Knudsen (2001); 5. Periago *et al*. (1997); 6. Anguita *et al*. (2006); 7. Englyst and Hudson (1996); 8.Gdala *et al*. (1997).

2.3. Grain legume species

2.3.1. Chickpeas (*Cicer arietinum*)

Chickpeas are grouped into two types, namely '*Desi'* or *Kabuli'*, which are based partly on seed size, colour and the thickness and shape of the seed coat. Reisselman and Miller (2001)

reported that *Desi* types produce smaller seeds, generally 400 or more seeds per 100 grams. These seeds have a thick, irregular-shaped seed coat which can range in colour from light tan to black. *Kabuli* cultivars, which are also called 'garbanzo beans', produce larger seeds that have a thin seed coat. The *Kabuli* cultivars produce seeds with colours that range from white to a pale cream coloured tan.

Nutrient composition: Published data on the nutrient composition of chickpeas are summarised in Table 2.2. The crude protein content of chickpea seed is moderately high, ranging between 182 and 290 g/kg. The primary carbohydrate in chickpea is starch, ranging between 300 and 584 g/kg, with *Desi* cultivars containing less starch and more fibre than *Kabuli* types.

The lipids in chickpeas comprise mostly polyunsaturated fatty acids, with linoleic and oleic acids as the primary constituents (Carnovale, 1999; Reisselman and Miller, 2001). The moderate content of fat and high starch content (480 g/kg) make chickpeas excellent sources of available energy. In human nutrition, the high concentration of unsaturated fatty acids and high fibre levels (particularly in *Desi* types) make this legume useful a cholesterol reducer (Carnovale, 1999; Reisselman and Miller, 2001).

Table 2.2. The nutritional composition (g/kg) of chickpeas

Nutrient	Average	Standard Deviation	Range	References[1]
Dry matter	853	20	875-966	1, 2, 3, 5,6, 7, 8, 9
Crude protein	228	27	182-290	1, 2, 3, 4, 5,6, 7, 8, 9, 10
Crude fat	47	9.3	29-64	4, 6, 9,10
Crude fibre	52	23	30-97	7, 8, 9
Acid detergent fibre	90	44	44-164	7, 8, 9,10
Neutral detergent fibre	198	53	127-295	6, 7, 8,10
Total fibre	278	0.0	278	1
Soluble fibre	43	0.0	43	1
Insoluble fibre	235	0.0	235	1
Ash	33	3.5	26-43	1, 2, 4, 5,6, 7, 9,10
Starch	480	74	300-584	3, 8, 9
Calcium	1.5	0.29	1.1-2.2	2, 7, 9,10
Phosphorus	3.8	0.69	2.3-4.7	2, 7, 9,10

[1] References: Candela *et al*. (1997); 2. Iqbal *et al*. (2006); 3. Jood *et al*. (1998); 4. Milán-Carrillo *et al*. (2000); 5. Nestares *et al*. (1996); 6. Perez-Maldonado *et al*. (1999); 7. Racz and Thacker (1998); 8. Ribeiro (1990); 9. Viveros *et al*. (2001); 10. Thacker *et al*. (2002).

The amino acid composition of chickpeas is presented in Table 2.3. There is considerable variation between published reports, which probably reflect the differences in

cultivars and growing conditions. Glutamic acid is found in highest concentrations in chickpeas, followed by aspartic acid and arginine. Chickpeas are a good source of lysine, but they are deficient in methionine and cystine.

Table 2.3. Amino acid content (g/kg) of chick peas

Amino acid	References				
	1	2	3a	3b	4
Indispensable					
Arginine	17.6	25.6	23.2	22.4	14.4
Histidine	5.1	6.9	8.9	6.9	4.4
Isoleucine	8.5	11.4	11.4	9.1	6.6
Leucine	14.9	18.3	20.6	16.3	12.0
Lysine	11.8	15.2	3.8	12.4	9.4
Methionine	2.6	3.0	16.8	2.6	na*
Phenylalanine	11.4	13.8	16.8	13.2	10.3
Threonine	7.3	8.8	11.6	8.4	8.3
Valine	8.9	11.5	11.5	9.4	8.8
Dispensable					
Alanine	8.2	10.2	11.4	8.9	6.8
Aspartic acid	22.0	26.8	29.5	23.1	15.7
Cystine	3.3	3.5	na	na	na
Glycine	7.9	9.25	11.2	7.9	7.9
Glutamic acid	31.3	38.9	54.5	43.6	24.9
Proline	8.1	na	na	na	12.3
Serine	10.2	13.2	14.2	11.8	9.4
Tyrosine	5.8	6.6	8.5	6.7	7.9

References: 1. Perez-Maldonado *et al*. (1999)-cv. Amethyst; 2. Ravindran *et al*. (2005); 3 a,b. Viveros *et al*. (2001) a:cv *Kabuli*; b:cv *Desi*); 4. Rubio (2005); *na : not available.

Apparent metabolisable energy: Published data on the apparent metabolisable energy of chickpeas are limited. Viveros *et al*. (2001) determined the AME values of chickpea cv *Kabuli* and cv *Desi* to be 12.6 and 10.5 MJ/kg, respectively. The lower energy availability of the *Desi* types was attributed to its higher fibre content (97 g/kg) compared to Kabuli types (34 g/kg). The AME value of chickpeas (cv *Amethyst*) to layers has been reported by Perez-Maldonado (1997) to be 10.6 MJ/kg.

Amino acid digestibility: Studies reporting the digestibility of amino acids of chickpeas are scanty. Ravindran *et al*. (2005) reported that the apparent ileal digestibility coefficient of amino acids ranged from 0.58 for cystine to 0.84 for arginine (Table 2.4). The poor digestibility of cystine is probably related to the lowest concentration of this amino acid in chickpea. The mean amino acid digestibility coefficient was determined to be 0.74.

Table 2.4. Apparent ileal amino acid digestibility coefficients of chickpeas for broilers

Amino acid	
Indispensable	
Arginine	0.84
Histidine	0.77
Isoleucine	0.70
Leucine	0.70
Lysine	0.76
Methionine	0.72
Phenylalanine	0.78
Threonine	0.70
Valine	0.73
Dispensable	
Alanine	0.73
Aspartic acid	0.73
Cystine	0.58
Glycine	0.68
Glutamic acid	0.78
Serine	0.74
Tyrosine	0.72

References: 1. Ravindran *et al*. (2005).

Feeding value: Based on the nutrient composition, chickpeas are potential sources of protein and energy in poultry diets. However, its feeding value may be limited due to the variation in nutrient profile, high fibre content and the presence of anti-nutritional factors. Further processing may be needed in order to destroy or lower the levels of anti-nutritional components to improve the nutritive value of chickpeas.

Viveros *et al*. (2001) concluded from their experiments that the dietary inclusion of chickpea cv. *Kabuli* (up to 450 g/kg) and chickpea cv. *Desi* (up to 150 g/kg) lowered the performance of growing chickens and increased the relative weight and length of the intestinal tract. They also found that the inclusion of *Kabuli* chickpea resulted in lowered nutrient digestibilities, intestinal enzyme activities and AME compared to the control diet. Autoclaving of *Desi* chickpeas, but not *Kabuli* chickpeas, improved the performance of the birds.

Perez-Maldonado (1997) investigated the nutritional value of chickpeas (cv. *Amethyst*) for broilers. Weight gain and feed conversion ratio (FCR) of young birds (starter phase) were inferior on diets with chickpeas compared to other grain legumes, excluding sweet lupin. Birds fed chickpea diets had lower digesta viscosity, but the heaviest pancreatic weights. No differences were found in growth rate, feed intake or FCR between older birds

(finisher phase) fed chickpeas and those fed other grain legumes. In another broiler experiment with the recommended inclusion level based on the previous study, it was found that, over a 42-day trial period, the growth of broilers receiving diets containing 150-220 g/kg chickpeas was poorer than those fed peas at the same level of inclusion. It was concluded that the maximum inclusion level of chickpeas in broiler starter and finisher diets was 100 g/kg.

Farrell *et al*. (1999) demonstrated that broilers fed balanced diets containing different inclusion levels (120, 180, 240, 300 and 360 g/kg) of chickpeas (cv. *Amethyst*) had similar weight gain, feed intake, feed per gain, digesta viscosity, and excreta scores. Relative pancreatic weights were observed to numerically increase with the level of inclusion, which was attributed to the presence of anti-nutritional factor(s) in this cultivar. The performance of broilers fed diets containing chickpeas was comparable to those fed faba bean and pea diets. In contrast, Ruiz *et al*. (1996) found that broilers fed diets containing chickpeas at 240 g/kg or more grew poorly and were less efficient, and attributed this to the adverse effects of saponins in chickpeas.

Perez-Maldonado et al. (1999) concluded from their experiments with layers, that egg production was still satisfactory at an inclusion rate of 250 g/kg chickpeas. However, it was suggested that it is safer to use lower inclusion levels because of pancreas enlargement in hens fed the chickpea diet, indicating the presence of trypsin and chymotrypsin inhibitors.

2.3.2. Faba beans (*Vicia faba*)

Faba beans (also known as horse bean or tic bean) rank sixth in world production among the legume crops after soybean, peanut, beans, peas, and chickpeas (Milner, 1972; Thacker 1990). Breeding varieties with tannin-free seeds and (more recently) with low vicine–convicine contents offer new perspectives for the feed use of faba beans (AEP, 2007).

Nutrient Composition: Published data on the nutrient composition of faba beans are summarised in Table 2.5. The large variation in the nutritional composition of faba beans probably reflects differences in variety, environment, growing conditions and year of harvest (Rubio *et al*., 1992; Hughes and Choct, 1999). Chavan *et al*. (1989) and Muehlbauer and Tullu (1997) reported that the crude protein content of faba bean can vary between 200 and 410 g/kg. Faba bean proteins are made largely of globulins (600 g/kg), albumin (200 g/kg), glutelin (150 g/kg), and prolamins (Larralde, 1991). Legumin is the predominant globulin and has a larger proportion of arginine, threonine and tryptophan (Hulse, 1994).

Starch content in faba beans is moderately high, ranging from 413 to 420 g/kg. Cerning *et al*. (1975) reported that faba bean seeds contain 510 g/kg to 680/kg of total carbohydrates, the major proportion of which is constituted by starch (410–530 g/kg). The main soluble sugars in faba beans are α-galactosides, including raffinose, stachyose and verbascose (Sosulski and Cadden, 1982). Raffinose, stachyose and verbascose are not polysaccharides. These oligosaccharides stimulate inappropriate fermentation leading to gas production and, consequently, the presence of these sugars in faba beans limits its use in human nutrition (Christofaro *et al*. 1972; Price *et al*., 1988).

Rubio *et al*. (1992) reported that the mineral concentration of faba beans was found to vary according to cultivars and seed fractions. Light seed coat cultivars tend to have lower contents of minerals and phytate than those with dark seed coat. In both cultivars, calcium was found mainly in the hulls, while other phytate and minerals were more concentrated in the cotyledon. Chavan *et al*. (1989b) reported that the range of calcium content is from 1.20 to 2.60 g/kg dry matter and that 40-60 % of the phosphorus is present as phytates. These values were lower than those reported by Rubio *et al*. (1992) for light seed coat (3.65 g/kg DM) and dark seed coat (4.00 g/kg DM) cultivars.

Table 2.5. The nutritional composition (g/kg) of faba beans

Component	Average	Standard Deviation	Range	Source
Dry matter	907	20	883-948	1,3,4,5,7,8
Crude Protein	292	39	226-332	1,2,3,4,5,6,7,8
Crude fat	16	5.1	11-26	2,3,4,6,7,8
Crude fibre	156	20	88-244	1,2,3,4,5,6,8
Acid detergent fibre	118	20	83-141	3,4,5,7
Neutral detergent fibre	197	61	142-313	2,3,4,5,7
Total fibre	230	0.0	230	9
Ash	35	6.6	25-51	1,2,4,5,6,7
Starch	418	4.6	413-422	3,4
Calcium	2.9	1.5	1.0-4.0	10,11
Phosphorus	5.3	1.2	4.4-6.8	10, 11

References: 1. Brufau *et al.* (1998); 2. Diaz *et al* (2006); 3. Goelema *et al*. (1999); 4. Hickling (2003); 5. Mariscal-Landin *et al.* (2002); 6. Palander *et al*. (2006); 7. Perez-Maldonado *et al.* (1999); 8. Thacker (1990); 9. Gdala and Buraczweska (1997); 10. Rubio *et al*. (1992); 11. Brand *et al*. (2004).

The amino acid composition of faba beans is presented in Table 2.6. The faba bean is rich in glutamic acid and arginine. Methionine and cystine are the limiting amino acids.

Table 2.6. Amino acid content (g/kg) of faba beans

Amino acid	References		
	1	2	3
Indispensable			
Arginine	23.1	25.1	25.0
Histidine	6.3	8.1	6.3
Isoleucine	11.1	12.4	10.2
Leucine	18.8	21.4	18.1
Lysine	15.9	18.2	13.6
Methionine	2.1	2.2	1.6
Phenylalanine	10.9	11.8	10.7
Threonine	9.0	9.8	8.9
Valine	12.2	13.7	11.4
Tryptophan	2.3	2.0	na
Dispensable			
Alanine	10.6	11.6	10.3
Cystine	3.3	3.5	2.8
Glycine	10.6	12.1	10.4
Glutamic acid	42.9	53.1	38.4
Proline	11.8	11.3	10.6
Serine	13.2	13.6	12.0
Tyrosine	7.5	8.4	7.4

References: 1. Diaz *et al*. (2006); 2. Mariscal-Landin *et al*. (2002) (cv. Alfred); 3. Perez-Maldonado (1997) and Ravindran *et al*. (2005); na: not available.

Apparent metabolisable energy: Published AME values for faba beans range from 10.3 to 11.7 MJ/kg dry matter (Table 2.7), which are comparable to that in soybean meal (11.0-11.5 MJ/kg). Hughes *et al*. (2002) reported that the AME values of faba beans range between 10.6 and 13.7 MJ/kg and attributed this variation to differences in cultivar and experimental conditions. Tannin-free cultivars of faba beans tend to have higher AME values than those containing tannins (Table 2.7). Marquart (1993) similarly found that tannins in faba beans reduced the AME and the apparent protein digestibility by 19 and 7 %, respectively. Metayer *et al*. (2003) reported that the nitrogen corrected AME (AMEn) values of three cultivars of faba beans (Gloria, Devine and EE0T0V) ranging from 11.94 to 12.70 MJ/kg for broiler chickens and 11.78 to 12.57 MJ/kg for cockerels.

Table 2.7. The apparent metabolisable energy (MJ/kg) of faba beans for broilers

Cultivar	AME	Reference
Spring white (tannin-free)	11.25	1
Spring coloured	10.32	1
Winter white (tannin-free)	11.68	1
Winter coloured	11.19	1
Fiord	10.57	2

References: 1. Brufau *et al*. (1998); 2. Perez-Maldonado (1997).

Amino acid digestibility: The apparent ileal digestibility coefficient of amino acids in faba beans is generally poor compared to those in soybean meal. The digestibility is lowest for sulphur-containing amino acids (0.58 for cystine and 0.63 for methionine) and highest for arginine (0.81).

Table 2.8. Apparent ileal amino acids digestibility coefficients of faba beans for broilers

Amino acids	
Indispensable	
Arginine	0.81
Histidine	0.72
Isoleucine	0.68
Leucine	0.70
Lysine	0.76
Methionine	0.63
Phenylalanine	0.72
Threonine	0.68
Tryptophan	na
Valine	0.68
Dispensable	
Alanine	0.71
Aspartic acid	0.71
Cystine	0.58
Glycine	0.67
Glutamic acid	0.75
Serine	0.69
Tyrosine	0.70

Reference: Ravindran *et al*. (2005).

Feeding value: Perez-Maldonado (1997) evaluated the nutritional value of faba bean (cv. Fiord) and Australian sweet lupins in both layer and broiler diets. In the first experiment where the birds were given mash diets with an inclusion level of 250 g/kg, it was found that layers fed the faba bean-based diets produced the smallest eggs. In the second experiment where the diets were steam pelleted, layers fed the faba bean-based diets had higher egg

production than those given the sweet lupin diets, but egg weights in birds fed the lupin-based diet were higher than those fed faba bean diets. The results of these broiler trials showed that birds fed the faba bean-based diets had better weight gain and feed conversion ratio compared to those fed the lupin and pea diets and that the upper level of inclusion of faba beans in broiler starter and finisher diets were 100-150 g/kg.

2.3.3. Lupins (*Lupinus* spp.)

Lupinus is a large genus that has more than 200 species in both the Eastern and Western Hemispheres. Only five species, however, are cultivated: *L. albus, L. angustifolius, L. luteus, L. mutabilis,* and *L. polyphilus*. Of these five species, only the first three are suitable for cultivation as high protein crops (ARC, 2007). Based on the alkaloid content, lupin can be grouped into two categories: those with a-high alkaloid content (up to 54 g/kg), commonly known as bitter lupins, and those with low alkaloid content (less than 0.1 g/kg), referred to as sweet lupins (Olver and Jonker, 1998). Sweet lupins can either be of the white (*Lupinus albus*), yellow (*Lupinus luteus*) or brown seeded (*Lupinus angustifolius*) species (Breytenbach, 2005). In this review, only *L. angustifolius* and *L.albus* are discussed.

2.3.3.1. *Lupinus angustifolius* (or narrow-leaf lupin or blue lupin or Australian sweet lupin) is an annual herb that can reach 80 cm or more in height. The flowers are usually blue, but can also range from white to pink. This species contain a single recessive gene which controls the sweetness. Since the 1930s, plant breeders have been developing varieties with lower alkaloid content (called 'sweet lupins'). Culvenor and Petterson (1986) reported that sweet varieties of lupins contain 0.02 g/kg alkaloid. The alkaloid content of bitter lupin is about 1000 times larger than sweet lupin, but bitter plants produce more seeds than sweet plants (ARC, 2007). However, the varieties can cross-pollinate, so mixing seeds will lead to increasing bitterness in each new generation of plants.

Nutritional composition: The nutritional composition of sweet lupins is well documented and commonly acknowledged by feed manufacturers. However, nutritional variability between cultivars and the presence of toxic and bitter alkaloids (Marquardt, 1993; Sipsas and Glencross, 2005) are major limitations in the use of this legume for commercial poultry rations. Reported analysis for the crude protein content of sweet lupins ranges from 223 to 424 g/kg (Table 2.9). This variation is due to differences in cultivars, location and year, and agronomic management (Kingwell, 2005; Sipsas and Glencross, 2005). In addition, Kingwell (2005) reported that the protein and oil contents of sweet lupins are related to seed

size. There was a tendency for a larger seeds to have higher protein and oil contents compared to the smaller seeds within the same variety.

The structure of lupin proteins gives them unique physical and chemical characteristics (Van Barneveld, 1999). Globulins are the major storage proteins, with this fraction being higher in lupins and soybeans than in most other legumes. The globulins themselves are composed of two main proteins characterised by their sedimentation coefficient, which in most cases approaches 7S and 11S. These storage proteins are multimeric and readily undergo association and dissociation reactions, allowing their efficient packing within the protein body in an insoluble form. The ratio of these globulin proteins affects the behaviour of lupin proteins and makes them different from other legume species. In lupin proteins, the 7S-like protein is found in greater proportions than the 11S-like protein, the 7S:11S ratio being 1.3:1. The 11S or legumin type protein in *Lupinus* spp. has been identified as g-conglutinin (Mironenko *et al*. 1978). Similarly, soybean has a 7S:- 11S ratio of 1.6-:1 (Thanh and Shibasaki, 1976). In contrast, faba beans and peas have legumin as the major protein with a 7S:11S (vicilin to legumin) ratio close to 1:2.

According to Van Barneveld (1999), the fact that lupin storage proteins are predominantly globulins suggests that they possibly have poorer emulsion properties (i.e. lower solubility) than a legume with higher levels of albumins. Higher levels of globulins would also indicate that lupin proteins are less viscous compared to legume proteins dominated by albumins, and since globulins have a compact structure, lupin proteins might have a lower buffering capacity in the neutral pH range (Van Barneveld, 1999).

Table 2.9. The nutritional composition (g/kg) of Australian sweet lupins

Nutrient	Average	Standard Deviation	Range	Sources
Dry matter	921	17	896-957	1,2,3,4,6,7,
Crude protein	340	58	223-424	1,2,3,4,5,6,7,9
Crude fat	60	15	29.7-81	1,2,3,4,5,6,7,9
Crude fibre	164	30	118-199	1,3,4,5
Acid detergent fibre	199	40	131-258	1,3,4,6,7
Neutral detergent fibre	258	36	200-307	1,4,6,7
Soluble fibre	34	14	20-48	9
Insoluble fibre	488	10	478-498	9
Ash	36	10	20-52	1,2,3,4,5,6,7,9
Calcium	2.1	0.0	2.1	8
Phosphorus	3.5	0.0	3.5	8

References: 1. Eason *et al*. (1990); 2. Glencross *et al*. (2003); 3. Hickling (2003); 4. Mariscal-Landin *et al.* (2002); 5. Palander *et al*. (2006); 6. Perez-Maldonado *et al*. (1999); 7. Ravindran *et al*. (2002); 8. Rahman *et al*. (1997); 9. Torres *et al*. (2005).

The proportion between hull and kernel, and their nutrient composition differ depending on the species of lupins (Kingwell, 2005; Sipsas and Glencross, 2005). The proportion of seed coat of sweet lupins is approximately 230 g/kg. The seed coat contains mainly cellulosic fibre, whilst the kernels are comprised of 300 g/kg cell wall materials, and pectin like dietary fibres. The cells of the seed consists of protein bodies (400 g/kg), fat bodies (70 g/kg), oligosaccharides (60 g/kg), starch (20 g/kg), phytic acid (10 g/kg) and water (100 g/kg).

The amino acid concentration of Australian sweet lupins is summarised in Table 2.10. Their amino acid profile is similar to other legume proteins in being low in sulphur-containing amino acid and tryptophan. However, the seeds are considered as an excellent source of arginine.

Table. 2.10. Amino acid content (g/kg) of Australian sweet lupins

Amino acids	References			
	1	2	3	4
Indispensable				
Arginine	10.8	32.9	29.9	29.4
Histidine	2.8	7.7	7.6	10.3
Isoleucine	4.8	12.1	11.4	12.9
Leucine	7.3	19.9	20.6	20.5
Lysine	4.7	12.3	13.8	14.0
Methionine	0.8	1.7	2.0	2.4
Phenylalanine	3.9	12.0	10.8	11.4
Threonine	3.5	10.4	10.0	10.8
Valine	4.3	11.7	11.2	12.9
Tryptophan	0.8	na	2.8	na
Dispensable				
Alanine	3.7	10.2	10	10.3
Aspartic acid	10.2	28.1	29.4	28.8
Cystine	1.4	3.2	3.6	2.3
Glycine	4.3	12.3	12.1	12.5
Glutamic acid	24.8	53.6	65.1	60.0
Proline	4.4	12.6	Na	10.9
Serine	5.3	14.5	14.4	15.3
Tyrosine	2.8	9.8	9.5	10.4

References: 1. Mariscal-Landin *et al*. (2002) (cv. Australian); 2.Perez-Maldonado *et al*. (1999) (cv. Gungurru); 3.Ravindran *et al*. (2002); 4. Eason *et al*. (1990); na = not available.

Apparent metabolisable energy: The apparent metabolisable energy (AME) of sweet lupins differs between cultivars (Table 2.11), from 6.53 in to 11.93 MJ/kg. Hughes *et al*. (1998) reported that the AME values of Australian sweet lupins cv Gungurru from three Western Australian sites ranged from 9.8 to 12.3 MJ/kg. This variation within a cultivar may reflect differences in climate, soil and agronomic conditions.

Table 2.11. The apparent metabolisable energy values (MJ/kg) of Australian sweet lupins

Cultivar	AME	Reference
Gungurru	6.53-11.64	2,3,4
Danja	8.25 and 10.45	2
Not stated	9.42	5
Not stated	11.93	1

References: 1. Eason *et al*. (1990); 2. Hughes *et al*. (1998); 3. Kocher *et al.* (2000); 4. Perez-Maldonado (1997); 5. Ravindran *et al*. (2002); na = not available.

The low and variable energy utilisation in lupins is due largely to their soluble NSP content. The carbohydrate composition of lupins is presented in Table 2.12.

Table 2.12. Carbohydrate composition (g/kg) of whole seed, kernel and hulls of Australian sweet lupins cv. Gungurru (van Barneveld, 1999)

Variable	Whole	Kernel	Hull
Free sugars			
Arabinose	0.44	0.00	0.00
Xylose	0.00	0.00	0.00
Mannose	8.89	8.29	3.35
Galactose	34.1	37.0	11.2
Glucose	29.1	27.5	9.24
Insoluble NSP			
Rhamnose	2.34	0.93	2.25
Fucose	1.47	0.00	3.22
Ribose	0.00	0.00	0.00
Arabinose	42.1	40.6	63.3
Xylose	26.7	21.4	87.1
Mannose	4.45	2.97	10.45
Galactose	143	141	39.52
Glucose	8.58	19.2	14.1
Soluble NSP			
Rhamnose	0.49	0.29	0.42
Fucose	0.22	0.00	0.00
Ribose	0.19	0.14	0.00
Arabinose	3.23	3.31	5.59
Xylose	1.19	0.90	2.54
Mannose	2.63	1.53	5.61
Galactose	13.0	14.3	5.32
Glucose	0.95	0.73	0.85

Amino acid digestibility: Available data on the apparent ileal amino acid digestibility coefficient of lupins are presented in Table 2.13. The amino acids in sweet lupins are well digested. The average amino acid digestibility coefficient of amino acids in Australian sweet lupins is over 0.80.

Table 2.13. Apparent ileal amino acid digestibility coefficient of Australian sweet lupins

Amino acids	References	
Indispensable	1	2
Arginine	0.90	0.89
Histidine	0.84	0.84
Isoleucine	0.82	0.81
Leucine	0.84	0.83
Lysine	0.78	0.83
Methionine	0.83	0.82
Phenylalanine	0.83	0.83
Threonine	0.76	0.77
Tryptophan	0.79	na
Valine	0.80	0.70
Dispensable		
Alanine	0.80	0.80
Aspartic acid	0.82	0.82
Cystine	0.69	0.78
Glycine	0.82	0.82
Glutamic acid	0.88	0.86
Serine	0.81	0.80
Tyrosine	0.85	0.83

References: 1.Ravindran *et al.* (2002); 2.Ravindran *et al*. (2005); na = not available.

Feeding value: Perez-Maldonado (1997) reported that the egg production of layers fed mash diets based on field peas and sweet lupins was higher than those fed faba bean- and/or chickpea-based diets. In broiler experiments, it was found that birds fed lupin and chick pea-based diets had inferior weight gain and feed conversion ratio compared to those fed the other two grain legumes, and that digesta viscosity was higher in birds fed lupin-based diets. The inclusion level of lupins at 150-220 g/kg and 100-120 g/kg for starter and finisher diets, respectively, supported better gains and feed conversion ratio compared to field peas at 200-300 g/kg and chick peas at 150-220 g/kg.

Bekrić *et al*. (1990) showed that lupins can be included in broiler diets up to 250 g/kg with no adverse effects on performance when compared to commercial diets containing soybean meal. Hughes *et al*. (1998) suggested that broilers can tolerate up to a 400 g/kg inclusion level of lupins, but the birds will produce sticky wet droppings and therefore better

poultry health could be achieved by reducing the level of inclusion. There was no effect on excreta dry matter at an inclusion level of 200 g/kg (Van Barneveld, 1999).

Study conducted by Olkowski *et al*. (2001) examined the effects of sweet lupins (cv. Troll) in raw (400 g/kg), dehulled (350 g/kg) and autoclaved (400 g/kg) forms in broiler diets. The results indicated that birds fed lupin-based diets showed a poor growth and feed intake compared to those fed the control diet based on soybean meal. Acute signs of toxity (leg weakness, lack of coordination and torticollis) were observed in some chicks that were given the diet containing raw lupin during the first week of trial. During weeks two and three, some birds which received the raw lupin diets showed signs of muscle paralysis and skeletal deformity. The content of liver microsomal cytochrome P-450 was higher in birds fed the diet containing raw lupin which suggests a systemic effect. It was concluded that high levels of alkaloids in some varieties of sweet lupins could cause significant detrimental effects in broilers.

2.3.3.2. *Lupinus albus* (also called white lupin) is an annual plant. The flowers of white lupins can be blue or white (Huyghe, 1997). Seeds of white lupin are large, measuring 8–14 mm in diameter, and with a 1000-seed weight of 70 to 1000 g. They have a circular flattened shape and are cream in colour (Erbas *et al.,* 2005).

The alkaloid content of bitter cultivars ranges from 5 to 40 g/kg, while those of low-alkaloid cultivars range between 0.08 and 0.12 g/kg (Erbas *et al.,* 2005). Alkaloid-free cultivars of white lupins are now available and the development of these alkaloid-free mutants has allowed the exploitation of white lupins as a protein source for animals and humans.

Nutritional composition: White lupins have a high content of crude protein (306 to 400 g/kg) and crude fat (59 to 146 g/kg) and a high fibre content (96-161 g/kg) (Table 2.14). Considerable variation observed in the nutritional content of white lupins probably reflects genetic and environmental differences. Green and Oram (1983) reported that the principal components of white lupin seed oil are the unsaturated long chain fatty acids, oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3), which on average account for 50, 22 and 10%, respectively, of the total fatty acids. Brenes *et al*. (1993a) reported that the high portion of hull (16 % of the seed) was mainly responsible for the high fibre content of the whole seed. Thus the removal of the hull will markedly decrease the fibre content.

Table 2.14. The nutritional composition (g/kg) of white lupins

Nutrients	Average	Standard Deviation	Range	Sources
Dry matter	916	15	895-944	1, 2, 3, 7, 8, 11
Crude protein	356	32	306-410	1,2, 3, 4, 5, 6, 8, 10, 11
Crude fat	103	22	59-146	1, 2, 3, 5, 6, 7, 8, 11, 13
Crude fibre	139	20	96-161	1, 2, 3, 7, 8, 11
Neutral detergent fibre	181	21	161-202	1, 2
Acid detergent fibre	157	28	129-185	1, 2
Total fibre	360	24	342-394	5, 6
Soluble fibre	44	7.9	36-52	5
Insoluble fibre	325	17	308-342	5
Starch	156	128	15-327	1, 2, 5, 6
Ash	37	6.0	26-41	1, 2, 3, 5, 6, 11
Calcium	15	0.0	15	7
Phosphorus	20	0.0	20	7

References: 1. Brenes *et al.* (1993a); 2. Diaz *et al.* (2006); 3. Erbas *et al*. (2005); 4. Sgarbiery and Galeazzi (1978); 5. Martinez-Villaluenga *et al*. (2006); 6. Mohamed and Prayas-Duarte (1995); 7. Olver and Jonker (1997); 8. Olver (1997); 9. Saini (1989); 10. Gatel (1994); 11. Sujak *et al*. (2006); 12. Trugo *et al*. (1988); 13. Uzun *et al*. (2007).

As can be seen in Table 2.15, the carbohydrate fractions of the hulls and kernels in white lupin seeds are different. Higher amounts of free sugars are found in kernels than in the hulls. The hulls are high in soluble NSP (17.51 g/kg). The insoluble fractions of hulls and whole seeds are comparable.

Table 2.15. Carbohydrate composition (g/kg) of whole seed, kernel and hulls of white lupins cv. Kiev mutant (Van Barneveld, 1999)

	Whole	Kernel	Hull
Free sugars			
Arabinose	0.27	0.00	0.25
Mannose	9.64	6.11	3.89
Galactose	38.2	35.1	14.7
Glucose	38.3	23.1	14.2
Insoluble NSP			
Rhamnose	2.02	0.81	1.96
Fucose	0.00	0.00	1.83
Arabinose	41.5	35.8	51.2
Xylose	37.0	17.1	66.7
Mannose	3.15	1.77	3.16
Galactose	110	101	38.5
Glucose	50.7	12.4	4.95

Soluble NSP			
Rhamnose	0.34	0.14	0.50
Ribose	0.15	0.15	0.00
Arabinose	3.03	2.25	8.43
Xylose	0.57	0.48	1.77
Mannose	2.40	1.23	2.09
Galactose	6.64	6.75	4.72
Glucose	0.78	0.54	0.75

The amino acid composition (Table 2.16) indicates white lupins to be deficient in sulphur amino acids (methionine and cystine) and tryptophan, but they are rich source of arginine.

Table 2.16. Amino acid content (g/kg) of white lupins

Amino acid	References			
	1	2	3	4
Indispensable				
Arginine	35.0	10.4	38.6	43.1
Histidine	8.2	2.2	8.3	9.4
Isoleucine	16.3	4.9	14.3	18.0
Leucine	26.1	7.5	24.3	28.7
Lysine	14.9	4.7	16.4	19.3
Methionine	2.4	0.6	2.6	na
Phenylalanine	14.7	3.7	12.4	na
Threonine	13.0	3.7	11.6	14.8
Valine	13.8	4.5	14.5	17.2
Tryptophan	2.1	0.7	na	3.2
Dispensable				
Alanine	11.6	3.3	10.2	na
Aspartic acid	41.6	10.6	33.6	na
Cystine	5.0	1.3	5.1	na
Glycine	13.6	3.9	13.4	na
Glutamic acid	80.7	23.4	58.6	na
Proline	15.0	3.6	12.8	na
Serine	21.8	5.2	14.6	na
Tyrosine	16.0	4.6	13.4	na

References: 1. Diaz *et al*. (2006) (cv. Multitalia); 2. Mariscal-Landin *et al*. (2002) (cv. Lublanc); 3. Zrally *et al*. (2007)-cv Amiga; 4. Gatel (1994); na=not available.

Amino acid digestibility: Amino acids in white lupins are well digested (Table 2.17) with a minority of amino acids having digestibility coefficients of over 0.80.

Table 2.17. Apparent ileal amino acid digestibility coefficient of white lupins for broilers

Amino acid	
Indispensable	
Arginine	0.88
Histidine	0.81
Isoleucine	0.77
Leucine	0.79
Lysine	0.81
Methionine	0.84
Phenylalanine	0.79
Threonine	0.75
Valine	0.75
Dispensable	
Alanine	0.78
Aspartic acid	0.80
Cystine	0.83
Glycine	0.79
Glutamic acid	0.85
Serine	0.78
Tyrosine	0.81

Source: Ravindran *et al*. (2005).

Apparent metabolisable energy: The AME values of white lupins have been reported to range from 8.0 to 14.9 MJ/kg (Hughes *et al*., 1996; Hughes *et al*., 1998; Kocher *et al*., 2000). The higher AME content of white lupins compared to Australian sweet lupin is due to its higher oil content (Table 2.14*)*. Brenes *et al.* (1993a) reported that the AME value of the low alkaloid white lupins was 10.0 MJ/kg.

Feeding value: The nutritional value of lupins is determined, to a large extent, by the level of alkaloids in the seed. These bitter substances can inhibit feed intake and growth in poultry (Hill, 1977) and limit the utilisation of white lupins. However, with the development of new cultivars with low alkaloid content (<0.1 g/kg), this is no longer an issue.

Olver (1987) reported that feeding broilers up to eight weeks with 400 g/kg of white lupin (alkaloid content, 0.1 g/kg) produced no adverse effects on growth, feed efficiency or carcass characteristics. In another study, Olver and Jonker (1997) found that broiler chickens can tolerate up to 400 g/kg of white lupins (cv Hanti) without negatively influencing growth. A similar trend was also found in feeding ducklings to 3 or 6 weeks of age with diets containing up to 400 g/kg white lupin (cv Buttercup). It was concluded that these lupin varieties could replace all the soybean meal in broiler diets and that white lupins do not exert any anti-nutritive effect provided the concentration of alkaloids in the white lupin seed is less

than 0.1 g/kg. Olkowski *et al*. (2005), on the other hand, reported a significant decrease in feed intake and gains in broilers fed diet containing raw untreated white lupin seeds (400 g/kg). It is probable that a high-alkaloid cultivar was used in their study.

2.3.4. Peas (*Pisum sativum*)

Peas are an annual herbaceous plant. The seeds can be smooth or wrinkled, and green, white or brown in colour. The distinction between different peas is made by the colour of the tegument (translucent without tannins and coloured with tannins) and the colour of the cotyledons. Nevertheless, essential subdivisions are also based on the shape of the seed (round or wrinkled) and flower colour (white or coloured) (AEP, 2007).

Nutrient Composition: The variability in proximate composition, shown in Table 2.18, is probably reflective of differences in cultivar, growing condition and analytical methods. Compared to soybean meal, peas have moderate crude protein content, ranging from 215 to 307 g/kg. However, the crude fibre content of peas is higher (49 to 118 g/kg) than that of soybean meal (34 g/kg; Castell, 1990). Hickling (2003) reported that feed pea protein is highly digestible with an excellent amino acid balance. The starch content in peas is high, ranging between 334 and 511 g/kg DM (Table 2.18).

The value of pea protein is dictated by its composition and especially by the content of different storage protein fractions, which are genetically controlled (Tzitzikaz *et al*., 2006). Osborne and Campbell (1898) classified pea proteins into two major fractions, namely salt-soluble globulins and water-soluble albumins. These globulins were further subdivided into two main groups based on their sedimentation coefficients: the 11S fraction (legumin) and the 7S fraction (vicilin, convicilin). These two groups differ significantly in molecular weight, structure and amino acid composition.

Marquardt (1993) reported that high percentage of pea carbohydrates are less digestible by adult roosters, which could be attributed to the presence of oligosaccharides (raffinose, stachiyose, and verbascose) and NSP.

Table 2.18. The nutritional composition (g/kg) of peas

Nutrient	Average	Standard Deviation	Range	Sources
Dry matter	888	17	856-913	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
Crude Protein	259	23	215-307	3, 4, 5, 6, 7, 9, 10, 11,12
Crude fat	15	5.2	11-27	3, 4, 5, 6 , 8, 10, 11,12
Crude fibre	73	17	49-118	3, 4, 5, 6, 7, 8, 9, 11
Acid detergent fibre	96	28	33-145	1, 3, 4, 6, 7, 8, 9, 10,12

Neutral detergent fibre	180	37	84-230	7,12
Non starch polysaccharides	163	28	140-195	7,13
Starch	440	59	334-511	3, 5, 6, 7,13
Ash	32	3.3	25-37	3, 4, 5, 6, 7, 8, 9, 10, 11,12
Calcium	1.2	0.1	1.1-1.3	6, 8
Phosphorus	4.3	0.3	4.1-4.6	6,8

References: 1. Alonso *et al*. (2000b); 2. Canibe and Eggum (1997); 3. Diaz *et al.* (2006); 4. Eason *et al*. (1990); 5. AEP (2007); 6. Fleury (2004); 7. Hickling (2003); 8. Jaikaran *et al*. (1995 in Fleury, 2004); 9. Mariscal-Landin *et al*. (2002); 10. Perez-Maldonado *et al*. (1999); 11. Palander *et al*. (2006); 12. Wang and Daun (2004); 13. Nicolopoulou *et al*. (2007).

Similar to other legumes, peas are deficient in sulphur-containing amino acids (Table 2.19). Lysine concentration is relatively high in peas.

Table 2.19. Amino acid content (g/kg) of peas

Amino acid	References									
	1	2	3	4	5	6	7	8	9	10
Indispensable										
Arginine	14.2	9.2	10.2	8.6	10.2	22.2	19.8	23.0	8.5	10.1
Histidine	3.8	2.4	2.3	2.7	2.4	5.3	5.6	6.0	2.3	2.4
Isoleucine	8.7	4.3	4.3	4.7	4.5	8.6	9.2	9.3	3.3	3.9
Leucine	14.1	7.5	6.8	7.3	7.1	15.2	15.1	16.0	6.5	6.6
Lysine	13.0	7.7	7.1	7.6	6.7	13.0	15.3	15.6	6.3	6.9
Methionine	1.8	0.8	0.8	1.1	0.9	1.7	2.1	2.0	1.1	0.9
Phenylalanine	9.5	4. 9	4.3	4.6	4.3	10.1	9.9	10.5	4.4	4.2
Threonine	8.1	3.6	3.3	3.7	3.7	7.8	8.1	8.8	4.4	3.4
Tryptophan	1.8	0.8	0.8	0.9	0.9	na	na	na	0.8	0.8
Valine	9.1	4.8	4.7	5.0	4.8	9.9	11.1	10.9	4.0	4.3
Dispensable										
Alanine	8.9	4.4	4.2	4.5	4.3	9.2	9.4	10.3	4.1	4.0
Aspartic acid	26.5	11.3	10.2	11.2	11.2	23.2	24.1	26.1	12.5	10.3
Cystine	3.0	1.2	1.2	1.4	1.3	2.9	1.60	1.6	1.2	1.5
Glycine	8.5	4.2	3.9	4.3	4.0	9.2	9.3	9.9	4.3	4.1
Glutamic acid	34.6	20.0	19.2	19.7	19.2	35.1	35.8	37.7	15.6	16.1
Proline	8.5	3.9	3.7	4.1	4.4	8.9	11.5	na	4.8	4.1
Serine	11.3	4.7	4.4	4.7	4.6	10.1	10.6	11.8	5.6	4.3
Tyrosine	6.2	2.7	2.6	2.6	2.6	6.1	6.6	6.9	2.9	2.9

References: 1. Diaz *et al*. (2006); 2-5. Mariscal-Landin *et al*. (2002); 6. Perez-Maldonado *et al*. (1999); 7. Eason *et al*. (1990); 8. Ravindran *et al*. (2005); 9-10. Wang and Daun (2004).

Apparent metabolisable energy: The reported AME value of peas ranges between 10.86 and 13.40 MJ/kg (Table 2.20). The observed variation is reflective of differences in both pea cultivars and age of bird. Compared to faba beans (Table 2.7) and lupins (Table 2.11), the energy value of peas was high which may be attributed to their high starch content.

Table 2.20. The apparent metabolisable energy (MJ/kg) of peas

Cultivar	Bird class	AME	Reference
Finale	Young cockerels	11.56	Carré *et al*. (1991)
Finale	Adult cockerels	11.77	Carré *et al*. (1991)
Frisson	Young broiler chickens	10.86	Carré *et al*. (1991)
Frisson	Adult cockerels	11.28	Carré *et al*. (1991)
Glenroy	Adult hens	11.70	Perez-Maldonado *et al*. (1999)
Athos	Broiler chickens	13.40	Metayer *et al*. (2003)
Athos	Adult cockerels	12.93	Metayer *et al*. (2003)

Amino acid digestibility: The digestibility values in peas vary depending on factors such as age or the physiological stage of the animals and variety. Gatel (1994) reported that the digestibility coefficients of both winter and spring peas were considerably higher in young birds than in adults. Within pea varieties, it was found that coloured-flowered peas (*P. arvense*) were digested poorly (Gatel, 1994).

High trypsin inhibitor content is a major factor responsible for the low amino acid digestibility of raw peas. The amino acid digestibility of peas can be increased by heat processing such as extrusion cooking (Mariscal-Landín *et al*., 2002). The noticeable improvements in tryptophan and cystine digestibilities as a result of extrusion may be a direct response to the decrease in trypsin inhibitor contents. The reason for the former effect could be that trypsin inhibitor prevents protein hydrolysis at the carboxyl end of tryptophan. The latter effect may be associated with the abundance of cystine in pancreatic enzymes, the secretion of which is stimulated by trypsin inhibitor (Corring *et al*., 1986). As a result, an increase in the ileal endogenous losses of this amino acid could be observed.

Table 2.21. Apparent ileal amino acid digestibility coefficients of peas

Amino acid	Reference	
	1	2
Indispensable		
Arginine	0.83	0.90
Histidine	0.75	0.95
Isoleucine	0.71	0.74
Leucine	0.71	0.82
Lysine	0.83	0.79
Methionine	0.70	0.59
Phenylalanine	0.72	0.81
Threonine	0.69	0.72
Valine	0.71	0.38

Dispensable		
Alanine	0.73	0.75
Aspartic acid	0.78	0.86
Cystine	0.66	0.71
Glutamic acid	0.80	0.87
Glycine	0.71	0.78
Serine	0.71	0.74
Tyrosine	0.72	0.76

References: 1. Ravindran *et al*. (2005); 2. Perez *et al*. (1993).

Feeding value: Several studies have demonstrated the usefulness of peas as a protein source in poultry diets. Castell *et al*. (1996) reported that peas can be included into broiler diets up to 300 g/kg without any negative effects. Brenes *et al*. (1993b) found that the performance of broilers fed a diet with 475 g/kg of peas was similar to those fed maize-soy diets.

Igbasan and Guenter (1997) investigated the level of inclusion of peas (0, 200, 400 and 600 g/kg) on egg production, egg mass and feed conversion in layer diets. It was found that these performance indices were improved in birds fed a diet containing 200 g/kg of peas relative to those fed the wheat-soy basal diet and that performance was similar between those fed a diet containing 400 g/kg peas and basal diet. However, inclusion of 600 g/kg peas lowered egg production, egg mass and feed conversion. It was suggested that peas could be included at levels up to 400 g/kg without detrimental effects on the performance of laying hens. Bennet (2002) recommended that the maximum level of inclusion of peas in diets for young birds and layers was 200 and 300 g/kg, respectively. Similarly Perez-Maldonado (1997) found that the upper level of the practical inclusion of peas in broiler starter and finisher diets was 300g/kg.

2.4. Improving the feeding values of legumes

The adverse effects of feeding raw legumes to chickens have been demonstrated by several researchers (Liener and Kakade, 1980; Longstaff and McNab, 1991; Jansman, 1993a; Choct, 1997) and various feed processing technologies have been applied to reduce these effects.

Processing can produce both positive and negative effects on the feeding value of ingredients. On the positive side, processing can alter particle size, prevent spoilage, improve palatability and nutrient digestibility, remove potential allergens, improve functional behaviour for processing, inactivate or destroy ANF, remove specific parts of seed, and improve handling. The maximum destruction of ANF may require different processing treatments because of variations in ANF structure and their biological effects (Thorpe and Beal, 2001). The levels of ANF, localisation within the seeds and sensitivities to physical and

chemical factors are also essential in maximising the reduction or elimination of ANFs (Melcion and Van der Poel, 1993). On the other hand, processing can have a negative effect on the nutritional composition of feed ingredients or diets.

Processing can be applied by physical, chemical, thermal, or bacterial means.

2.4.1. Physical processing

Dehulling is the most commonly used method to reduce the deleterious effects of ANFs such as tannins and fibre, with the remaining kernel having higher energy and protein contents (Marquardt, 1993).

Traditionally, the removal of hulls from legume seeds is accomplished with attrition dehullers, roller mills or an abrasive-type dehuller (Melcion and Van der Poel, 1993). Attrition type dehullers and roller mills are particularly suitable for dehulling and splitting legume grains with loose seed coats (soybeans, peas, and faba bean), whereas abrasive type dehullers are suitable for dehulling grains with a more tightly adhering seed coats such as cowpea, mungbean and pigeon pea (Ehiwe and Reichert, 1987).

The beneficial effects of dehulling on the nutritional value of faba beans, lupins, and peas are well documented (Marquardt, 1993; Melcion and Van der Poel, 1993; Brufau *et al*., 1998; Alonso *et al*., 2000a; Brenes *et al*., 2003; Fleury, 2004; Breytenbach, 2005). Crude protein and fat contents have been reported to increase by approximately 10 to 23% and 16 to 22%, respectively, after dehulling (Table 2.22). Alonso *et al.* (2000b) reported that the fat content increased by about 39% in Australian sweet lupin and 32% in white lupins after dehulling. Crude fibre content was considerably decreased (by 76 to 83%) in dehulled faba bean and peas. Brenes *et al*. (2003) reported that dehulling reduced fibre content by approximately 70% in lupin seeds. Amino acid concentration of faba bean and Australian sweet lupin was also increased after dehulling.

Longstaff and McNab (1989) reported that available carbohydrate (the sum of free sugars, sucrose and starch content) of pea hulls was 31 g/kg DM, whilst hemicellulotic polysaccharides were the main component of pea hull NSPs. Igbasan and Guenter (1996) reported that the starch content of peas was increased following the removal of the pea hulls.

Table 2.22. Nutritional values (g/kg) of whole and dehulled faba beans, lupins and peas

Nutrient	Faba beans[1]		Australian sweet lupins[2]		White lupins[3]		Peas[4]	
	whole	Dehulled	whole	dehulled	whole	Dehulled	whole	dehulled
Dry matter	888	885	922	926	905	894	930	920
Crude protein	289	321	311	405	347	416	213	237
Crude fat	na	na	46	76	84	123	14	14
Crude fibre	153	26	172	51	134	49	63	15
Neutral detergent fibre	168	85	313	110	192	40	na	na
Acid detergent fibre	126	27	na	na	163	52	na	na
Ash	46	45	30	29	32	31	30	31
Tannins	95	2.6	na	na	na	Na	na	na
TI activity (TIU/mg)	4.4	6.5	na	na	na	Na	na	na
Indispensable amino acids								
Arginine	25.1	27.3	na	na	na	Na	na	na
Histidine	8.1	9.2	8.0	11.0	na	Na	na	na
Lysine	18.2	19.5	16.0	20.0	na	Na	na	na
Phenylalanine	11.8	13.5	13.0	17.0	na	Na	na	na
Leucine	21.4	24.7	22.0	29.0	na	Na	na	na
Isoleucine	12.4	14.3	14.0	19.0	na	Na	na	na
Valine	13.9	16.4	13.0	17.0	na	Na	na	na
Methionine	2.2	2.4	1.6	2.1	na	Na	na	na
Threonine	9.8	9.47	12.0	15.0	na	Na	na	na
Tryptophan	2.0	2.7	na	na	na	Na	na	na

[1]Mariscal-Landin *et al*. (2002); [2]Fernándes and Batterham (1995); [3]Brand (1996); [4]McCallum (2004)

Dehulling has been shown to significantly improve dry matter digestibility (Jansman and Mieczkowska, 1998), protein digestibility (Igbasan and Guenter, 1996; Brenes *et al*., 2003), starch digestibility (Longstaff and McNab, 1987) and AME (Brenes *et al*. 1993a; Annison *et al*., 1996; Breytenbach, 2005) of grain legumes. It has been demonstrated that dehulling of lupin seeds (low alkaloid, cv. Amiga) increased the AME values by 15 to 18%, whilst the digestibility of protein improved by 7% (Brenes *et al*., 1993a; Brenes *et al*., 2003). Breytenbach (2005) reported that dehulling increased the AMEn value of Australian sweet lupin from 8.61 to 8.81 MJ/kg.

In a study with laying hens, Igbasan and Guenter (1996) found that the improvement of AME of peas due to the removal of hulls was varied and depended on the cultivar. Brown seeded peas (cv Sirius) showed the highest improvement (24.1%), followed by green seeded

peas (cv Radley, 4.9%), and yellow seeded peas (cv Impala, 3.0%). The observed improvements were attributed to improvements in starch content as a result of the removal of indigestible fibre components and tannins in the hulls.

Brenes *et al*. (2003) showed that dehulling of lupins improved broiler performance. Olkowski *et al*. (2005) found that dehulling of white lupins markedly improved the weight gain of broilers. However, the weight gain was still lower than birds that were fed a diet based on soybean meal. Feed intake and feed conversion ratio were not affected by dehulling.

Farhoomand and Poure (2006) found that weight gain and the feed conversion ratio of broiler chicks fed a diet containing dehulled peas was higher than those fed whole raw peas. Igbasan and Guenter (1997) demonstrated that feeding birds with diets containing dehulled peas were found to significantly improve egg production, feed intake, egg weight, egg mass output, yolk colour, albumen height and shell thickness, but it had no beneficial effects on the feed conversion ratio and body weight of laying hens. The positive impact on laying performance was due to improvements in both the content and digestibility of nutrients in the dehulled meal.

2.4.2. Thermal processing

Summers (2006) classified processing into two main categories: thermal and non-thermal. Thermal processing is further divided into dry heat and wet heat treatments. In thermic processes with water, the main effects are to inactive heat-labile ANFs, such as protease inhibitors and lectins, and to increase nutrient digestibility, especially of starch. Dry thermic processes, on the other hand, improve palatability and the nutritional components of feed ingredients. Roasting, popping and micronising are examples of dry heat treatment, whilst pelleting, expansion, extrusion, compacting and steam flakes are included in wet heat treatment (Summers, 2006).

The use of appropriate processing temperatures is critical for the elimination of heat-labile ANFs found in legume seeds (D'Mello, 1991; Thorpe and Beal, 2001). Under-processing will fail to deliver full benefits on the digestibility of amino acids, since the ANFs will not be fully eliminated. Excessive heat treatment, or over processing, will also lower amino acid digestibility since amino acids may be destroyed or become unavailable due to the formation of indigestible complexes.

The amino acids which are most affected by over processing are lysine and cysteine. Cysteine is the most heat-labile amino acid, whilst the effect on lysine may be largely explained by the Maillard reaction in which free lysine binds with free carbonyl groups of

reducing sugars to form Maillard complexes. In advanced stages of the Maillard reaction, the amino acid becomes unavailable to the animal due to cross-linkages being formed between protein chains (Moughan, 2003).

Several studies have also shown that reduced protein digestibility due to thermal treatment is the result of protein aggregation (Deshpande and Damodaran, 1989; Dänicke *et al*., 1998; Wang, 2000; Carbonaro *et al*., 2005). Aggregation is a general term that encompasses several types of interactions or characteristics (Cromwell, 2006). The aggregation behavior of protein is affected by two main factors, namely, structural (internal factors) and environmental (external factors) (Wang, 2005). Primary and secondary structures of protein are included in structural factors, whilst factors such as temperature, pH, and protein concentration are included in external factors. Among all external factors, temperature is probably the most common and critical in affecting protein aggregation (Wang, 2005). Proteins unfold above certain temperatures and thermally-induced protein unfolding is often followed by immediate aggregation due to exposure of the hydrophobic residues.

Speed *et al*. (1997) reported that increasing temperature increases the rate of aggregation by increasing frequencies of both molecular collision and hydrophobic interaction. Increasing temperatures may also change the relative composition of secondary structures and alter the aggregation behaviour. Temperatures up to 70 ^{o}C usually affect most proteins reversibly or partially, while temperatures between 70 to 100 ^{o}C will break hydrogen bonds, disulphide bonds and the alpha helix secondary structure. Heating between 100 and 150 ^{o}C damages tertiary protein structures.

Between 105 and 150^{o}C, losses of lysine, serine and threonine become prominent while isopeptides such as lysinolysine and glutamyllysine are formed and cross-links between proteins are generated. The level of isopeptides formed in addition to the cross-links between proteins is proportional to the degree and temperature of heating. All this chemical alteration will decrease the digestibility of protein. Between 150 and 250^{o}C or higher, pyrolysis of amino acids occurs i.e. destruction of amino acids with a large number of potential end-products, some of which are carcinogenic. The more hydrophobic a protein is- the more probability that it form an aggregate.

Extrusion is a process where the feed is subjected to mixing, shearing, and heating under high pressure before the extrudate is finally forced through a die (Sørensen *et al*., 2002). Feed may undergo reactions during processing that could be beneficial if the

nutritional value is improved or detrimental if nutrient are destroyed or become resistant to digestion. Reactions that occur in the feed during extrusion are largely determined by shear forces, temperature, moisture, residence time and pH (Sørensen *et al*., 2002). In addition, the reactions depend on the type of reactant present, such as water, lipids, carbohydrate and proteins.

The functions performed by extrusion cooking include gelatinisation of starchy component, denaturation of proteins, stretching or restructuring of tactile components and the exothermic expansion of extruder and modification of liquids (Kearns, 1994; Sheriff and Sajeev, 2005). The principle aim of extrusion is to achieve a high level of starch gelatinisation and disruption of the grain structure. When the mass is cooked, the product is shaped by the die. The starch particles are expanded to form an open 'honeycomb' like structure, which is referred to as being 'gelatinised' (Gazia, 2003).

During extrusion, proteins start to denature and are converted from soluble to insoluble forms through bonding (Mitchell, 1992; Hubber, 2001). Some or all of these bonds are then broken by the increasing heat and shear to form a concentrated solution or melt phase which can produce a formation of covalent bonds at high temperatures. Upon cooling, non-covalent and disulfide bonds form, and finally, if the moisture content is low enough, amorphous regions form which becomes crystalline.

Extrusion may also affect the nutritional value of lipids as a result of oxidation, hydrogenation, isomerisation or polymerisation (Camire *et al*., 1990), and the composition of starch and dietary fibre (Korus *et al*., 2000; Vasanthan *et al*., 2002). According to Lue *et al*. (1991) the changes in the dietary fibre profile of grain flours after extrusion may be explained by three mechanisms. Firstly, the starch is degraded into fractions resistant to enzymatic attack, thus increasing the dietary fibre content. Secondly, degradation of fibre to low molecular weight fractions typically lowers the dietary fibre content. Thirdly, macromolecular degradation of fibre increases its solubility and changes its physiological effects.

The other benefit of the extrusion process includes decreased ANFs, increased digestibility of individual feed components, the destruction of pathogens, and the extension of feed storage time. Extrusion also lowers raw or bitter flavours commonly associated with many vegetable feed sources. Many of these undesirable flavours are volatile in nature and they are eliminated through the extrusion and decompression at the extruder die.

Van der Poel (1992) evaluated the effects of different extrusion conditions on the ANFs and protein dispersibility of two cultivars of peas. It was shown that the reduction in the levels of trypsin inhibitors and lectins was dependent on the processing variables. For the round-seeded pea variety (cv Finale), the moisture level in addition to the temperature were found to be important, although inactivation of trypin inhibitor activity was complete for all the processing conditions investigated. For the wrinkle-seeded peas (cv C306), the temperature used during extrusion cooking largely inactivated ANF. It was also shown that extrusion reduced the level of tannins by 30 – 40% and it improved the nutritional value in both varieties. It was suggested that the improvement of the nutritional value of legumes upon thermal treatment was associated with a decrease in the activity of proteinaceous ANF and it had positive effects on the digestibility of protein

Diaz *et al*. (2006) reported that trypsin inhibitor contents of faba beans, lupin and peas decreased after extrusion, but the tannin content of peas and lupin seeds increased after thermal processing. However, O'Doherty and Keady (2001) claimed that extrusion of peas had resulted in a remarkable decrease in tannin content (14 vs. 9 mg/g) and trypsin inhibitor activity content (2.0 mg/kg to 1.25 mg/kg). In a study by Van der Poel (1992), it was shown that extrusion cooking decreased the tannin content of faba beans decreased by 45 % (1.55% vs. 0.86%) using Folin Denis method of assay and by 10% (0.67% vs. 0.60%) using Vanillin assay.

The native starch granule, which consists predominantly of α-glucan in the form of amylose and amylopectin, is hydrolysed very slowly by α-amylase and amyloglucosidase compared with processed (gelatinised) starch whose crystalinity has been lost and where the accessibility of substrate to enzymes is greater and not restricted by α-glucan association such as double helices (especially in crystallites) (Tester *et al*., 2004).

When native starches are heated in excess water, the crystalline structure is disrupted and water molecules from hydrogen bonds to the exposed hydroxyl groups of amylose and amylopectin (Ratnayake *et al*., 2002; Tester *et al*., 2004). This causes an increase in granule swelling and solubility. Granule structure is completely lost and a thin paste or gel is formed. This process makes the starch completely digestible by hydrolysing enzymes. Thus, for starch to be readily digestible, it must be amorphous (especially physically damaged or gelatinised) not crystalline, freely accessible to digestive enzymes (not entrapped in food/feed particles) in small particles or preferably solubilised and not associated with other

molecules to form complexes (e.g. amylose-lipid complexes), not chemically modified in a form that prevents it from acting as a substrate for amylases.

Extrusion has been reported to have positive effects on *in vitro* protein digestibility (Alonso *et al*., 2000b; El-Hady and Habiba, 2003; Diaz *et al*., 2006), the fat digestibility (Dänicke *et al*., 1998; Lichovnikova *et al*., 2004), the digestibility of amino acids (except lysine and histidine) (Lichovnikova *et al*., 2004), and the starch digestibility (Alonso *et al*., 2000a; Diaz *et al*., 2006) of grain legumes. The improvement of protein digestibility after extrusion was probably due to the destruction of ANFs. In the case of starch digestibility, the improvement was probably due to changes in starch structure, such as fusion, gelatinisation, fragmentation and dextrinisation (Pérez-Navarrete *et al*., 2006).

Breytenbach (2005) reported that the AME value of Australian sweet lupin decreased after extrusion (8.61 MJ/kg vs. 7.52 MJ/kg). This decrease was attributed to the increased bulkiness (mash form) associated with expansion with a resultant decrease in energy intake.

2.4.3. Exogenous enzymes: During the past two decades, the use of exogenous enzymes has become a common practice in the feed industry due to their effectiveness and lower costs. According to Sheppy (2001) and McCleary (2001), the main objectives of enzyme supplementation in poultry diets are to (i) destroy or lower the content of anti-nutritional factors; (ii) increase the availability of nutrient components such as starch and proteins that are either enclosed within fibre-rich cell walls and, therefore, not as accessible to endogenous digestive enzymes; (iii) breakdown specific chemical bonds in raw materials which are not usually broken down by the animal's own enzymes; (iv) supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzymes production may be inadequate; (v) reduce the variability in nutritive value between samples of a feedstuff, (vi) improve gut health and (vii) decrease nutrient overload in the manure.

Five main types of enzymes are commonly used in poultry diets, which are NSP-degrading enzymes (i.e. xylanase and β-glucanase), protein-degrading enzymes (protease), starch-degrading enzymes (i.e. amylase), phytic acid-degrading enzymes (phytase) and lipid-degrading enzymes (lipase) (Sheppy, 2001; Mcleary, 2001). It is important to note that feed ingredients typically contain more than one anti-nutritive factor and, as a result, the addition of multienzymes may be more effective to improve nutrient digestibility.

Of these four enzyme groups, NSP-degrading enzymes are more relevant to grain legumes due to the presence of relatively high contents of NSP, especially in lupins and peas.

Currently, exogenous NSP enzymes are routinely used to mitigate the adverse effects of NSP and to minimise the variation in AME and also the performance of poultry fed diets based on viscous grains. The proposed mechanisms by which these enzymes improve energy and nutrient utilisation include degradation of NSP in the cell wall matrix and the release of encapsulated nutrients, lowering of digesta viscosity in the intestinal tract, increased accessibility of nutrients to endogenous digestive enzymes, stimulation of intestinal motility and improved feed passage rate.

Hughes *et al*. (2002) reported that the dry matter digestibility and AME of diets based on faba bean were increased by 9.6% and 22%, respectively, by adding an enzyme product with multi-carbohydrase activities including hemi-cellulase and pectinase.

For lupins, the responses seem to vary due to the type and quantity of the lupins in addition to the enzymes employed (Brenes *et al*., 2003). The addition of multi enzymes (carbohydrase, protease, and α-galactosidase) to a diet containing 70% raw lupins improved the weight gain and feed efficiency of broilers by 18 and 10%, respectively (Brenes *et al*., 1993a). Naveed *et al*. (1998) reported that the addition of xylanase or cellulase in lupin-based diets gave a beneficial effect on bird performance. Cowieson *et al*. (2003) studied the effect of amylase resistant starch in peas on the performance and nutrient digestibility of broilers, using an exogenous enzyme (carbohydrase). The results indicated that the supplementation pea-based diet with carbohydrase improved gain, feed conversion, and nutrient digestibility.

2.4.4. Plant breeding: Older cultivars are known to contain high concentrations of various anti-nutritional factors, especially protease inhibitors and tannins, which severely limited the inclusion levels of these ingredients in practical animal diets (Jansman, 2005). The levels of these constituents have been considerably reduced in current cultivars through plant breeding technologies and this has enhanced the usefulness of current cultivars of legumes in animal feeding.

2.5. Determination methods of amino acid digestibility

Protein quality of an ingredient is determined by its availability. The availability is a function of two processes which are digestion and absorption (Johnson, 1992). The availability of amino acid is commonly measured by the slope-ratio assay which involves measuring the performance with graded levels of pure amino acids such as L-lysine or DL-methionine. However, this method is subject to criticism because of the difficulty of assessing all amino acids in one time and the availability of amino acids is confounded with other, non-protein, dietary factors (Johnson, 1992). Due to these drawbacks, digestibility

assays have become the most common and favoured technique to measure availability largely because the values apply directly to the animal and all the amino acids can be measured in one assay (Ravindran *et al.*, 1999).

There are three different methods in determining the digestibility of amino acids in birds, namely, *in vivo* (growth and digestibility assays), indirect *in vivo* (plasma amino acid assays, nitrogen retention) and *in vitro* methods (enzymic digestion, chemical, or microbiological assays) (Sibbald, 1987; Ravindran and Bryden, 1999). Of these, the most commonly used method to determine amino acid digestibility is *in vivo* digestibility assays.

In vivo digestibility assays can be done by three different methods: excreta assay, ileal digesta assay or growth assay. Determination of excreta digestibility (by total collection or indicator method), was the most commonly used method during the early days of digestibility research, especially when the majority of published data on excreta amino acid digestibility was obtained by using the precision feeding assay developed by Sibbald (1979).

However, excreta digestibility measurements are considered to be an unreliable assessment based on several reasons. Firstly, the excreta contain not only amino acids from dietary origin but also microbial proteins from the caeca. Other considerations were that excreta samples can be contaminated by urine, feathers, scales and foreign materials (Ravindran *et al*., 1999) and microbial activity in the caeca, may influence amino acid digestibility by deaminating undigested amino acid residues (Johnson, 1992; Ravindran *et al*. 2005). The latter had been proven through the determination of digestibility using both intact and caecectomised birds, which indicated that the amino acid excretion in caecectomised birds was higher than in the intact birds (Green *et al*., 1987).

Ravindran and Bryden (1999) concluded that an ileal digestibility assay can be used as alternative method in order to overcome the limitations of the excreta assay. The ileal digestibility assay involves the collection of ileal content through either slaughter of the birds (cervical dislocation or euthanasia of the birds) or insertion of a canula into the distal ileum (Ravindran and Bryden, 1999). Ravindran and Bryden (1999) concluded that killing by cervical dislocation is not recommended because it increases the loss of endogenous protein due to increase shedding of mucosa cells into the gut lumen at the time of slaughter. In contrast, euthanasia of the birds by using substances such since pentobarbitone is currently preferred as this minimises both peristalsis and mucosal shedding.

The ileal amino acid digestibility values can be expressed as either as apparent or true digestibility. The apparent digestibility measures the digestibility of amino acids of both dietary and endogenous origin, whereas true digestibility takes into account the ileal endogenous loss of amino acids (Ravindran *et al*., 1999). Endogenous amino acid losses at the ileal level can be divided into a basal (or non-specific) and a specific fraction (Hoehler *et al*., 2006). The basal losses are related to the dry matter intake and they are independent of the raw material or diet composition. In contrast, the specific losses are influenced by the inherent characteristics of the raw material, such as the presence of ANFs that may stimulate endogenous secretions.

Reported amino acid digestibility values vary largely amongst samples of the same feedstuff. These differences are due to a number of factors such as methodological factors, including dietary amino acid levels, methods of determination and inherent factors (Sauer *et al*., 2000; Borin *et al*., 2002; Lemme *et al*., 2004; Rodehutscord *et al*., 2004; Ravindran *et al*., 2005).

There are three methods for the measurement of amino acid digestibility in raw materials, viz the direct, the difference and the regression methods (Lemme *et al*., 2004). All three methods are briefly outlined below:

2.5.1. The direct method. The direct method is the most common method used to measure amino acid digestibility of feed ingredients, largely because of the simplicity of the assay diet and calculations (Ravindran and Bryden, 1999). In this method**,** the feed ingredient under test usually represents the only amino acid source in the test diet, which is usually a semipurified diet (Lemme *et al*., 2004). The diet should be formulated to contain at least 180 g/kg crude protein in order to minimise the confounding effects of endogenous amino acids relative to unabsorbed dietary amino acids. Fan *et al*. (1994) reported that the ileal amino digestibility values of an ingredient determined with the direct method increased with the increasing dietary amino acid content.

When the direct method is employed to determine the digestibility of low-protein ingredients, one can expect that the dietary levels of some of the amino acids, which include the limiting ones, are considerably lower than their respective upper limit level. As a result, small differences in the dietary contents of these amino acids will elicit a relatively large change in their apparent ileal digestibilities. The assay diet is also fortified with minerals and vitamins. Energy is added in the form of purified forms of carbohydrates (starch, dextrose)

and fats. Calculation of the digestibility coefficient assumes that the amino acid digestibility of the diet is representative of that of the feed ingredient.

2.5.2. The difference method. The 'substitution method' or the 'difference method' is another assay that can be used to evaluate AIDC either in low or high protein ingredients when their inclusion levels in the assay diets are relatively high (Lewis and Southern, 2000). The drawbacks of the difference method are due to its complexity in developing the assay diets and final calculation. This method assumes that there was no interaction between the basal diet and the test ingredient, and the apparent ileal amino acid digestibility is an additive. The use of the difference method requires two diets (a basal diet and an assay diet) to be formulated (Lemme *et al*., 2004). The basal diet is typically a maize-soybean meal diet, whereas, the assay diet consists of a mixture (usually 50:50) of pre-determined ratios of the basal and the test feed ingredient. The digestibility of amino acids in the test ingredient is determined by using the difference in digestibility between the basal and assay diet, and the contribution level of the nutrient to the assay diet.

2.5.3. The regression method. The 'regression method' is a method where specific losses are claimed to be automatically included in the digestibility coefficient determination (Lemme *et al*., 2004). The digestibility of amino acids determined by this method was based on the assumption that with an increasing intake of amino acids from a certain feed ingredient, the amount of amino acid present at the terminal ileum is also affected by ingredient-specific factors (Rodehutscord *et al*., 2004). The assay diets (semi-purified diets) are formulated to contain graded inclusion levels of the test ingredient. Linear regression equations are then developed for the quantitative data of both amino acid intake and amino acid flow at the terminal ileum. According to Rodehutscord *et al*. (2004), the slope of the regression line represents only the ingredient-specific effects and this method does not require a correction for basal endogenous losses.

CHAPTER 3

Nutritional characterisation of grain legumes grown in New Zealand

3.1. Abstract

The effects of cultivars on the nutrient profile and protein quality of grain legumes were investigated. A total of 53 samples representing five cultivars of peas, four cultivars of chickpeas and two cultivars of each of white lupins, sweet lupins and soybeans were analysed for proximate, fibre and carbohydrate components, minerals and amino acids. No differences ($P > 0.05$) were found in the proximate and fibre composition between the cultivars of chickpeas, peas, white lupins and soybeans. Significant ($P < 0.05$) differences, however, were observed between the two cultivars of sweet lupins. Starch was the major carbohydrate component in chickpeas and peas, whereas non-starch polysaccharides were the major carbohydrates in lupins. The non-starch polysaccharide contents were markedly higher in the lupins, compared to peas and chickpeas. The legume proteins were deficient in lysine, methionine, cystine and threonine. The results from the protein quality assay showed that there were no differences ($P > 0.05$) in protein quality between cultivars of the different grain legume species. Raw soybeans had the lowest weight gain and protein efficiency ratio, and had the highest relative pancreatic weights and mortality rate. These data suggest that the raw soybeans contained high concentrations of anti-nutritional factors, possibly protease inhibitors. Mortality and relative pancreatic weights in birds fed raw forms of chickpeas, peas or lupins was low, suggesting that the cultivars evaluated did not contain significant levels of any ANFs. Overall, the present results demonstrate the nutritional potential of local cultivars of chickpea, pea and lupins as protein sources in poultry diets.

3.2. Introduction

Grain legumes, such as peas (*Pisum sativum*), lupins (*Lupinus* spp.) and chickpeas (*Cicer arietinum*), are excellent sources of protein and energy for poultry and pigs. The nutritional composition of these grain legumes are well documented and widely accepted by stock feed manufacturers in other parts of the world, especially in Europe where these legumes have long been used for animal feeding (Rubio *et al*., 1992; Annison *et al*., 1996; van Barneveld, 1999; Hickling, 2003; Rubio *et al*., 2003). However, whilst such overseas data could be useful, it is inadequate for accurate feed formulations under New Zealand conditions.

Interactions of cultivars, soil, climate and agronomic factors can cause appreciable differences in nutrient profiles between locally grown ingredients and those available elsewhere.

Another factor which limits the utilisation of grain legumes in poultry diets is the uncertainty about their nutritional quality. The variation reported in the nutritive value of grain legumes is related, partly, to variable amounts of anti-nutritional factors that depress nutrient digestion and bird performance. The anti-nutritional factors commonly found in grain legumes include protease inhibitors, lectins, tannins, amylase inhibitors and non-starch polysaccharides (Wiryawan, 1997; Alonso *et al*., 2000b; Choct, 2006). As a result, feeding raw legumes generally result in poor growth and feed efficiency in poultry (Kakade *et al*., 1974; Ortiz *et al*., 1993; Olkowski *et al*., 2005). However, each legume produces a different response (Viveros *et al*., 2001; Perez-Maldonado, 1997; Perez-Maldonado *et al*., 1999). Most current cultivars have also been bred for low levels of these anti-nutritional factors. To be cost effective, the grain legumes must be incorporated into diets in raw form without any processing, especially heat treatment. Some of the legume cultivars grown in New Zealand are of unspecified origin and may belong to 'older' cultivars with significant levels of anti-nutritional factors. For this reason, locally grown cultivars need to be screened for possible toxic factors in *in vivo* trials.

The present study was composed of two parts. The first characterised the nutrient profiles of cultivars of chickpea, pea, Australian sweet lupin (*L. angustifoilus*), white lupin (*L. albus*) and soybean grown in New Zealand. The second part followed on from the first and determined the protein quality and the possible presence of anti-growth factors in these cultivars. A modified protein quality assay was employed, where day-old broiler chicks were used, instead of growing rats, as the animal model to determine the protein efficiency ratio of grain legumes, relative to soybean meal.

3.3. Materials and Methods

3.3.1. Samples

A total of 57 samples representing five cultivars of peas, four cultivars of chickpeas and two cultivars each of white lupins, sweet lupins and soybeans (Table 3.1) were obtained from the field trial units of Crop and Food Research, which were located in Pukekohe (North Island), Marton (North Island), Ashburton (South Island) and Chertsey (South Island). The seed

samples were received at Massey, cleaned of any extraneous materials, and subsequently ground to pass through a 1-mm screen and then representative samples were taken for laboratory analysis.

Table 3.1. Details of cultivars evaluated within each legume

	No of samples
Peas	
Santana	5
Miami	5
Rex	3
Crusader	3
Courier	3
Chickpeas[1]	
Unknown cultivar # 1	4
Unknown cultivar # 2	4
Unknown cultivar # 3	4
Unknown cultivar # 4	4
White lupins	
Small	5
Promore	5
Australian sweet lupins	
Penny	5
Borre	3
Soybeans[2]	
Maturity V cultivar	2
Maturity VI cultivar	2

[1] All kabuli type; cultivars not known.
[2] New introduction.

3.3.2. Nutrient characterisation

The evaluation was carried out in two phases. In the first phase, all 53 samples (Table 3.1) were analysed for dry matter, crude protein, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash. During the second phase, samples within each legume were pooled within a cultivar, because location effects in the first phase were found to be small and inconsistent. This generated a total of 15 samples and included five pea samples, four chickpea samples, and two samples each of white lupins, Australian lupins and soybeans for the analyses of starch, non-starch polysaccharides, mineral contents and amino acid composition. All analyses were performed in duplicates.

3.3.3. Chemical analysis

3.3.3.1. Proximate and fibre composition: The dry matter (930.15), crude fat (920.35), ADF (973.18), NDF (2002.04) and ash (942.05) contents were determined according to the AOAC (2002) standard methods. Nitrogen content was determined by the combustion method (AOAC, 2002, method no. 968.06) using a CNS-2000 carbon, nitrogen and sulphur analyser (LECO® Corporation, St. Joseph, Michigan, USA). The crude protein content of the ingredients was calculated as N x 6.25.

3.3.3.2. Starch analysis: Starch content was measured using an assay kit (Megazyme, Boronia, Victoria) based on the use of thermostable α-amylase and amyloglucosidase (McCleary *et al*., 1997).

3.3.3.3. NSP analysis: Total, soluble and insoluble NSP were analysed using an assay kit (Englyst Fiberzym Kit GLC, Englyst Carbohydrate Services Limited, Cambridge, UK) based on the procedures described by Englyst *et al*. (1994).

3.3.3.4. Mineral analysis: The samples were wet acid digested with nitric and perchloric acid mixture, and concentrations of minerals were determined at specific wavelengths for each element (Ca, 393.3; P, 185.9; K, 766.4; Na, 589.5; Mg, 279.1; Fe, 259.9; Mn, 257.6; Zn, 213.9 and Cu, 324.8 nm) by an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA). The instrument was calibrated against standards (Junsei Chemical Co., Ltd., Tokyo, Japan) of known concentration.

3.3.3.5. Amino acid analysis: Amino acids were determined by performic acid oxidation with acid hydrolysis-sodium metabisulfite method (AOAC, 2002, method no. 994.12). In this method, sample was hydrolysed with 6N HCl (containing phenol) for 24 h at 110 ± 2^{o}C in glass tubes sealed under vacuum. Amino acids were then detected on a Waters ion-exchange HPLC system, and the chromatograms were integrated using dedicated software (Millenium, Version 3.05.01, Waters, Millipore, Milford, MA) with the amino acids identified and quantified using a standard amino acid mixture (Product no. A2908, Sigma, St. Louis, MO). The HPLC system consisted of an ion-exchange column, two 510 pumps, Waters 715 ultraWISP sample processor, a column heater, a post column reaction coil heater, a ninhydrin pump and a dual wavelength detector. Amino acids were eluted by a gradient of pH 3.3 sodium citrate eluent to pH 9.8 sodium borate eluent at a flow rate of 0.4 ml/ min and a column temperature of 60^{o}C. Cysteine and methionine were analysed as cysteic acid and

methionine sulphone, respectively, by oxidation with performic acid for 16h at 0^{o}C and neutralization with hydrobromic acid prior to hydrolysis.

3.3.4. Protein quality evaluation

A modified protein efficiency ratio assay, involving broiler (Ross 308) chicks, was conducted. The evaluation consisted of the 15 samples, analysed in the second analytical phase above, together with a commercial sample of soybean meal as the reference protein.

The legumes, in raw form (without any thermal processing) with hulls, were ground in a hammer mill to pass through a 3-mm sieve. These ground legumes were incorporated as the sole source of dietary protein in the assay diets. The assay diets were based on dextrose and the test ingredient (Table 3.2) and, the proportions of dextrose and the test legume were varied in each assay diet in order to obtain 180 g crude protein/kg.

Table 3.2. Composition of the assay diets

Ingredient	g/kg as fed basis
Grain legume/ soybean meal	to supply 180 g/kg protein
Soybean oil	20.0
Dicalcium phosphate	20.0
Limestone	18.0
Salt	3.0
Vitamin-trace mineral premix[1]	3.0
Dextrose	to 1000

[1] Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

Each of the 16 assay diets was offered to three replicate brooder cages (8 birds/ cage) of chicks from day 1 to day 12 post-hatching. The brooders were housed in an environmentally controlled room. The temperature was maintained at 31°C during the first week and then gradually reduced to 28°C during the second week. The birds received constant fluorescent illumination and, allowed free access to the diets and water. Body weights and feed intake were recorded on days 1 and 12. Mortality was recorded daily. On day 12, two birds were randomly selected from each cage, weighed, euthanized by cervical dislocation and pancreatic weights were recorded.

The protein efficiency ratio was calculated as follows:

PER = Weight gain (g/bird) / protein intake (g/bird)

3.3.5. Data analysis

Where appropriate, the nutrient composition data are presented as mean± standard deviation. By expressing the standard deviation as a percentage of the mean, coefficients of variation can be calculated.

The data for proximate and fibre composition, and protein quality assay (weight gain, PER and relative pancreatic weights) were analysed using one-way analysis of variance using the General Linear Models procedure of SAS (1997). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

3.4. Results

Peas

The proximate and fibre compositions of the five pea cultivars are summarised in Table 3.3. No cultivar differences ($P > 0.05$) were observed in these parameters, except in ADF contents. The ADF content of 'Courier' tended ($P = 0.06$) to be higher than those of other cultivars. The crude protein content of samples varied within the narrow range, from 230 to 283 g/kg. The crude fat content in peas was low, with an average value of 12.2 g/kg.

Table 3.3. Proximate and fibre compositions (g/kg dry matter basis; mean ± SD) of the five pea cultivars

	Cultivar					
	Santana	Miami	Rex	Crusader	Courier	All cultivars
Number of samples	5	5	3	3	3	19
Dry Matter	872± 3.7	873±4.3	871±2.0	875±2.1	868±8.6	872±4.7 (860-879)[1]
Crude protein	260±16.2	250±16.0	256±4.5	256±12.2	267±8.5	257±13.3 (230-283)
Crude fat	12.2±3.1	11.4±2.8	11.9±2.4	11.8±0.60	14.2±6.5	12.2±3.2 (8.1-21.7)
ADF[2]	62.4±6.6	64.4±3.1	68.9±4.2	65.1±0.32	93.0±7.1[*]	69.2±11.7 (56-101)
NDF	93.8±5.3	97.6±12.3	97.6±13.5	93.3±6.5	122±12.0	99.8±13.5 (83-136)
Ash	31.0±4.6	31.1±7.2	31.8±1.3	34.6±3.4	42.2±16.1	33.5±8.0 (30-60)

[1] Values in parentheses refer to range of values.
[2] P=0.06.
[*]Tended to differ from other cultivars.

The major component of pea carbohydrates was starch, comprising 415 g/kg of the seed (Table 3.4). Differences were observed between cultivars in the mineral concentrations (Table 3.5), with 'Courier' having higher levels of phosphorus, iron, manganese and zinc. In general, the concentrations of amino acids in different pea cultivars were similar (Table 3.6).

Table 3.4. Starch and non-starch polysaccharide contents (g/kg dry matter basis) of the five pea cultivars

	Cultivar					
	Santana	Miami	Rex	Crusader	Courier	Mean ± SD
Starch	423	397	402	418	435	415±15.5
Non-starch polysaccharides						
Soluble	17.4	15.0	18.4	16.6	17.0	16.9±1.25
Insoluble	154.0	145.2	144.0	166.4	176.0	157±13.8
Total	181.4	160.2	162.4	182.4	193.0	175.9±14.1

Table 3.5. Mineral composition (dry matter basis) of the five pea cultivars

	Cultivar					
	Santana	Miami	Rex	Crusader	Courier	Mean ± SD
Calcium, g/kg	1.30	1.10	0.90	0.90	0.80	1.00±0.20
Phosphorus, g/kg	3.80	4.20	4.30	4.90	5.30	4.50±0.60
Potassium, g/kg	12.0	11.0	12.0	12.0	12.0	11.8±0.40
Sodium, g/kg	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Magnesium, g/kg	1.40	1.30	1.30	1.40	1.60	1.40±0.10
Iron, mg/kg	79	200	104	206	512	220±172
Manganese, mg/kg	16	43	16	23	45	29±14.4
Zinc, mg/kg	35	36	43	48	60	44±10.2
Copper, mg/kg	6.0	8.0	8.0	8.0	9.0	7.80±1.10

Table 3.6. Amino acid concentration (g/kg dry matter) of pea cultivars

Amino acid	Santana	Miami	Rex	Crusader	Courier	Mean
Indispensable						
Arginine	23.4	22.5	23.3	22.7	23.8	23.1±0.53
Histidine	6.4	7.3	6.6	6.9	6.5	6.7±0.36
Isoleucine	10	10.8	9.8	10.2	10.2	10.2±0.37
Leucine	19.4	18.9	17.8	18.5	18.3	18.6±0.61
Lysine	18.2	17.4	17.7	17.6	18.5	17.9±0.45
Methionine	2.9	2.7	2.5	2.7	2.7	2.7±0.14
Phenylalanine	13.0	12.5	11.8	12.3	12.2	12.4±0.44
Threonine	8.8	7.8	9.7	9.4	9.6	9.1±0.79
Valine	12.3	12.3	11.4	12.3	12.2	12.1±0.39

Dispensable						
Alanine	12.5	11.6	11.1	12.3	12.8	12.1±0.69
Aspartic acid	31.7	32.3	32.2	32.4	33.6	32.4±0.70
Cystine	8.6	8.6	7.6	7.4	7.8	8.0±0.28
Glycine	10.4	11.1	9.9	11.1	11.2	10.7±0.57
Glutamic acid	40.4	39.1	41.9	40.8	43.7	41.2±1.73
Proline	11.1	10.8	9.9	11	10.7	10.7±0.47
Serine	11.4	10.2	10.3	10.1	10.7	10.5±0.53
Tyrosine	7.8	7.5	7.9	8.2	8.1	7.9±0.27

Chickpeas

The proximate and fibre composition of the four chickpea cultivars, summarised in Table 3.7, were found to be similar ($P > 0.05$). The crude fat content of chickpeas was low and the crude protein content was moderate, with values ranging between 170 and 276 g/kg. On average, chickpeas contained 345 g/kg starch and 151 g/kg total NSP (Table 3.8). The mineral contents in chickpea cultivars are presented in Table 3.9. The amino acid concentrations in the different chickpea cultivars were similar (Table 3.10).

Table 3.7. Proximate and fibre composition (g/kg dry matter basis; mean ± SD) of the four chickpea cultivars[1,2]

	Cultivar				
	Cultivar 1	Cultivar 2	Cultivar 3	Cultivar 4	All cultivars
No of samples	4	4	4	4	16
Dry Matter	877±6.6	877±5.6	878±5.0	881±6.4	878±5.5 (869-885)[3]
Crude protein	219±35.3	215±31.4	212±34.3	208±49.0	214±34.3 (170-276)
Crude fat	77±8.1	78±6.8	79±8.2	80±11.2	79±7.9 (70-91)
ADF	51±8.8	51±9.5	49±9.4	47±11.2	50±8.9 (37-59)
NDF	65±6.7	63±8.3	64±9.5	63±9.2	64±7.7 (50-74)
Ash	35±5.7	34±6.4	35±8.7	34±5.2	34±6.0 (24-44)

[1] All are 'Kabuli'; cultivars not known.
[2] Cultivar effects were not significant ($P > 0.05$).
[3] Values in parentheses refer to range of values.

Table 3.8. Starch and non-starch polysaccharide contents (g/kg dry matter basis) of the four chickpea cultivars

	Cultivar				
	1	2	3	4	Mean ± SD
Starch	356	341	332	348	345±10.2
Non-starch polysaccharides					
Soluble	10.1	8.2	10.8	9.5	9.65±1.1
Insoluble	141.1	150.0	138.9	134.2	141±6.6
Total	151.2	158.2	149.7	143.7	150.7±6.0

Table 3.9. Mineral composition (dry matter basis) of the four chickpea cultivars

	Cultivar				
	1	2	3	4	Mean ±SD
Calcium, g/kg	1.70	1.60	1.90	1.80	1.75±0.13
Phosphorus, g/kg	4.0	4.4	4.3	4.1	4.20±0.18
Potassium, g/kg	13	13	13	13	13±0.00
Sodium, g/kg	0.20	0.20	0.20	0.20	0.20±0.00
Magnesium, g/kg	1.5	1.5	1.5	1.4	1.48±0.15
Iron, mg/kg	109	108	154	95	117±25.8
Manganese, mg/kg	42	42	43	39	42±1.73
Zinc, mg/kg	48	48	50	47	48±1.26
Copper, mg/kg	8	9	9	8	8.50±0.58

Table 3.10. Amino acid concentrations (g/kg dry matter) of chickpea cultivars

Amino acid	Cultivar 1	Cultivar 2	Cultivar 3	Cultivar 4	Mean ± SD
Indispensable					
Arginine	18.3	18.9	18.1	17.3	18.2±0.66
Histidine	6.4	6.2	6.6	5.9	6.3±0.30
Isoleucine	9.6	9.4	9.2	8.8	9.3±0.34
Leucine	17.8	17.1	16.2	16.6	16.9±0.69
Lysine	15.3	15.3	15.2	14.4	15.1±0.44
Methionine	3.7	3.4	3.3	3.5	3.5±0.17
Phenylalanine	13.4	13.1	13.0	12.4	13.0±0.42
Threonine	8.2	8.1	8.7	8.2	8.3±0.27
Valine	10.2	10.0	10.5	9.5	10.1±0.42
Dispensable					
Alanine	11.1	11.0	10.8	10.5	10.9±0.26
Aspartic acid	29.0	28.1	27.8	26.1	27.8±1.21
Cystine	8.2	7.4	7.2	7.2	7.6±0.24
Glycine	8.8	8.6	8.5	8.5	8.6±0.14
Glutamic acid	35.1	35.6	34.4	32.4	34.4±1.41
Proline	9.0	8.7	9.0	8.4	8.8±0.29
Serine	10.5	10.0	10.8	9.9	10.3±0.4
Tyrosine	6.4	6.1	7.0	6.0	6.4±0.45

Australian sweet lupins

Significant ($P < 0.05$) differences were observed in the proximate and fibre compositions of the two sweet lupin cultivars (Table 3.11). The contents of protein and fat were higher ($P < 0.05$) and those of ADF and NDF were lower ($P < 0.05$) in cultivar 'Penny' compared to cultivar 'Borre'. Sweet lupin carbohydrates were characterised by negligible levels of starch and high concentrations of NSP (Table 3.12). The mineral data showed that sweet lupins are

excellent sources of calcium and phosphorus (Table 3.13). The amino acid concentrations in the two cultivars were similar (Table 3.14).

Table 3.11. Proximate and fibre composition (g/kg dry matter basis; mean ± SD) of the two sweet lupin cultivars

	Cultivar		
	Penny	Borre	Both cultivars
No of samples	5	3	8
Dry Matter	882±8.7	888±1.5	885±7.3 (873-890)[1]
Crude protein	445±32.2[a]	381±6.2[b]	421±41.1 (374-483)
Crude fat	60±8.8[a]	43±5.8[b]	53±11.5 (36-75)
ADF	176±14.2[b]	220±17.6[a]	192±27.0 (159-240)
NDF	221±21.0[b]	266±23.5[b]	238±30.7 (189-258)
Ash	45±5.9	42±1.8	44±4.8 (35-42)

[a, b] Means in a row bearing different superscripts are significantly different ($P < 0.05$)
[1] Values in parentheses refer to range of values.

Table 3.12. Starch and non-starch polysaccharide contents (g/kg dry matter basis) of the two sweet lupin cultivars

	Cultivar		
	Penny	Borre	Mean ± SD
Starch	4.40	4.00	4.20±0.28
Non-starch polysaccharides			
Soluble	48.2	51.2	49.7±2.1
Insoluble	278.6	322.0	315±51.9
Total	326.8	373.2	350±32.8

Table 3.13. Mineral composition (dry matter basis) of the two sweet lupin cultivars

	Cultivar		
	Penny	Borre	Mean ± SD
Calcium, g/kg	2.4	3.9	3.15±1.06
Phosphorus, g/kg	6.7	5.2	5.95±1.06
Potassium, g/kg	13.0	12.0	12.5±0.71
Sodium, g/kg	0.20	0.40	0.30±0.14
Magnesium, g/kg	3.2	2.0	2.60±0.85
Iron, mg/kg	94	61	78±23.3
Manganese, mg/kg	70	45	58±17.8
Zinc, mg/kg	72	48	60±17.0
Copper, mg/kg	13	7	10±4.2

Table 3.14. Amino acid concentrations (g/kg dry matter) of sweet lupin cultivars

	Cultivar		
	Penny	Borre	Mean ± SD
Indispensable amino acids			
Arginine	45.1	38.9	42.0±4.38
Histidine	13.9	11.6	12.8±1.63
Isoleucine	18.3	15.6	17.0±1.91
Leucine	26.5	20.6	23.6±4.17
Lysine	20.8	18.8	19.8±1.41
Methionine	3.1	2.6	2.9±0.35
Phenylalanine	18.5	15.6	17.1±2.05
Threonine	15.1	14.0	14.6±0.78
Valine	16.7	14.5	15.6±1.56
Dispensable amino acids			
Alanine	16.0	13.6	14.8±1.70
Aspartic acid	50.3	40.2	45.3±7.14
Cystine	19.8	13.8	16.8±2.12
Glycine	18.3	15.6	17.0±1.91
Glutamic acid	98.2	81.2	89.7±12.0
Proline	18.3	16.6	17.5±1.20
Serine	20.8	17.8	19.3±2.12
Tyrosine	12.9	10.5	11.7±1.70

White lupins

No significant ($P > 0.05$) differences were observed in the proximate and fibre compositions of the two white lupin cultivars (Table 3.15). The notable feature was the high fat content of white lupins. White lupins contained negligible levels of starch and high concentrations of NSP (Table 3.16). The mineral data showed that sweet lupins are excellent sources of calcium and phosphorus (Table 3.17). An interesting finding was the very high concentrations of manganese in white lupins. The amino acid concentrations in the two cultivars were similar (Table 3.18).

Table 3.15. Proximate and fibre composition (g/kg dry matter basis; mean ± SD) of the two white lupin cultivars[1]

	Cultivar		
	Small	Promore	Both cultivars
No of samples	4	4	8
Dry matter	891±6.5	891±7.4	891±6.5 (884-901)[2]
Crude protein	368±10.9	356±11.4	362±11.9 (341-378)
Crude fat	111±11.0	114±15.0	113±12.3 (100-135)
ADF	157±16.4	159±13.9	158±14.2 (135-177)

NDF	196±25.8	199±22.8	197±22.6 (161-223)
Ash	37.3±5.4	38±4.1	37.6±4.4 (31-43)

[1] Cultivar effects were not significant (P>0.05).
[2] Values in parentheses refer to range of values.

Table 3.16. Starch and non-starch polysaccharide contents (g/kg dry matter basis) of the two white lupin cultivars

	Cultivar		
	Small	Promore	Mean ± SD
Starch	2.0	2.9	2.5±0.63
Non-starch polysaccharides			
Soluble	31.1	34.5	32.8±2.4
Insoluble	255.2	248.0	252±5.1
Total	286.3	282.5	284±2.7

Table 3.17. Mineral composition (dry matter basis) of white lupin cultivars

	Cultivar		
	Small	Promore	Mean ± SD
Calcium, g/kg	2.7	3.0	2.9±0.21
Phosphorus, g/kg	4.8	4.6	4.7±0.14
Potassium, g/kg	12	13	12.5±0.71
Sodium, g/kg	0.40	0.50	0.45±0.07
Magnesium, g/kg	1.8	1.8	1.8±0.00
Iron, mg/kg	50	63	57±9.2
Manganese, mg/kg	630	690	660±42.4
Zinc, mg/kg	40	38	39±1.4
Copper, mg/kg	7	7	7±0.00

Table 3.18. Amino acid concentrations (g/kg dry matter) of white lupin cultivars

	Cultivar		
Amino acid	Small	Promore	Mean ± SD
Indispensable			
Arginine	41.0	38.0	39.5±2.1
Histidine	9.7	7.9	8.8±1.3
Isoleucine	15.0	13.1	14.1±1.3
Leucine	27.9	27.1	27.5±0.6
Lysine	17.6	16.5	17.1±0.8
Methionine	2.9	2.9	2.9±0.0
Phenylalanine	13.5	12.4	13.0±0.8
Threonine	14.3	12.7	13.5±1.1
Valine	14.6	13.4	14.0±0.8

Dispensable			
Alanine	15.6	16.5	16.1±0.6
Aspartic acid	39.5	40.1	39.8±0.4
Cysteine	6.4	5.6	6.0±0.6
Glycine	14.9	13.1	14.0±1.3
Glutamic acid	66.6	68.3	67.5±1.2
Proline	15.1	13.5	14.3±1.1
Serine	18.7	16.1	17.4±1.8
Tyrosine	15.5	14.4	15.0±0.8

Soybeans

The proximate and fibre compositions of the two soybean cultivars are presented in Table 3.19. Cultivar effects were not significant (P > 0.05) for these parameters. Soybean carbohydrate composition showed negligible levels of starch (Table 3.20). The mineral (Table 3.21) and amino acid (Table 3.22) concentrations in the two soybean cultivars were comparable. The seeds contained high concentrations of potassium, relative to the other grain legumes species.

Table 3.19. Proximate and fibre composition (g/kg dry matter basis; mean ± SD) of the two soybean cultivars[1]

	Cultivar		
	Maturity V	Maturity VI	Both cultivars
No of samples	2	2	4
Dry matter	895±12.7	892±14.8	893±11.5 (881-904)[2]
Crude protein	404±7.7	405±27.4	405±16.4 (386-425)
Crude fat	178±17.2	185±1.7	182±10.8 (166-190)
ADF	71±6.5	102±36.6	87±28.1 (66-128)
NDF	102±15.7	94±16.7	98±14.1 (91-113)
Ash	58±1.6	55±2.3	56±2.2 (53-59)

[1] Cultivar effects were not significant (P > 0.05).
[2] Values in parentheses refer to range of values.

Table 3.20. Starch and non-starch polysaccharide contents (g/kg dry matter basis) of the two soybean cultivars

	Cultivar		
	Maturity V	Maturity VI	Mean ± SD
Starch	7.5	8.8	8.2±0.9
Non-starch polysaccharides			
Soluble	22.4	28.8	25.6±4.5
Insoluble	135	157	146±16.1
Total	158	187	172.±20.6

Table 3.21. Mineral composition (dry matter basis) of soybean cultivars

	Cultivar		
	Maturity V	Maturity VI	Mean ± SD
Calcium, g/kg	2.0	1.8	1.9±0.14
Phosphorus, g/kg	8.6	7.7	8.2±0.64
Potassium, g/kg	22	22	22.0±0.00
Sodium, g/kg	0.10	0.10	0.10±0.00
Magnesium, g/kg	2.4	2.5	2.5±0.07
Iron, mg/kg	99	98	99±0.71
Manganese, mg/kg	24	28	26±2.82
Zinc, mg/kg	55	58	57±2.12
Copper, mg/kg	19	22	21±2.12

Table 3.22. Amino acid concentrations (g/kg dry matter) of soybean cultivars

	Cultivar		
Amino acid	Maturity V	Maturity VI	Mean ± SD
Indispensable			
Arginine	29.7	28.4	29.1±0.92
Histidine	12.1	10.9	11.5±0.85
Isoleucine	18.7	17.7	18.2±0.71
Leucine	32.7	31.3	32.0±0.99
Lysine	26.7	25.4	26.1±0.92
Methionine	5.5	6.5	6.0±0.71
Phenylalanine	21.4	19.9	20.7±1.06
Threonine	17.5	16.6	17.1±0.64
Valine	20.9	19.4	20.2±1.06
Dispensable			
Alanine	22.6	21.6	22.1±0.71
Aspartic acid	38.6	38.1	38.4±0.35
Cystine	13.2	14.4	13.8±0.42
Glycine	18.1	17.5	17.8±0.42
Glutamic acid	68.2	69.1	68.7±0.71
Proline	20.8	22.2	21.5±0.99
Serine	21.2	20.4	20.8±0.57
Tyrosine	15.0	14.5	14.8±0.35

Comparison of the five legume species

A summary of comparison of nutritional profiles of the five legume species is shown in Tables 3.23 to 3.27. The data were not subjected to statistical analysis since the aim was to present an overview of relative nutritive values rather than to provide a statistical comparison. As could be expected, there were marked differences between the legumes in terms of protein, fat and fibre contents (Table 3.23). Lupins had protein contents that were comparable to soybeans, but also had high fibre contents which were more than double those

found in other species. Peas had intermediate protein levels, whilst chickpeas had the lowest. The fat contents were highest in soybeans, intermediate in white lupins and lowest in peas.

Table 3.23. Comparison of average proximate and fibre composition (g/kg dry matter basis) of the five legumes

	Peas	Chickpeas	Sweet lupins	White lupins	Soybeans
No of samples	19	16	8	8	4
Dry matter	872	878	885	891	893
Crude protein	257	214	421	362	405
Crude fat	12	79	53	113	182
ADF	69	50	192	158	87
NDF	100	64	238	197	98
Ash	34	34	44	38	56

Amongst the five species, peas and chickpeas were good sources of starch (Table 3.24), whilst the lupins and soybeans were almost devoid of starch. All legumes were excellent sources of dietary fibre. The total NSP contents of lupins were almost double that of the other three legumes.

Table 3.24. Comparison of average carbohydrate composition (g/kg dry matter basis) of the five legumes

	Peas	Chickpeas	Sweet lupins	White lupins	Soybeans
No of samples	5	4	2	2	2
Starch	415	345	4.20	2.50	8.20
Soluble NSP	17	10	50	33	256
Insoluble NSP	157	141	318	252	141
Total NSP	17	151	368	285	167

The mineral data showed marked differences in some minerals between the species (Table 3.25). In particular, high concentrations of potassium in soybeans, manganese in white lupins and iron in peas are noteworthy.

Table 3.25. Summary - Comparison of average mineral composition of the five legumes

	Peas	Chickpeas	Sweet lupins	White lupins	Soybeans
No of samples	5	4	2	2	2
Calcium, g/kg	1.00	1.75	3.15	2.90	1.90
Phosphorus, g/kg	4.50	4.20	5.95	4.70	8.20
Potassium, g/kg	11.8	13.0	12.5	12.5	22.0
Sodium, g/kg	<0.01	0.20	0.30	0.45	0.10
Magnesium, g/kg	1.40	1.48	2.60	1.80	2.50
Iron, mg/kg	220	117	78	57	99
Manganese, mg/kg	29	42	58	660	26
Zinc, mg/kg	44	48	60	39	57
Copper, mg/kg	7.8	8.5	10.0	7.0	21

A summary of the amino acid concentrations (g/kg dry matter) of the five legumes is shown in Table 3.26. Amino acid concentration in a commercial soybean sample is also included for comparison purposes. There were differences in total amino acid concentrations, largely reflecting the differences in protein contents between legume species.

Table 3.26. Comparison of average amino acid concentrations (g/kg dry matter) of the five legumes and a commercial sample of soybean meal

	Peas	Chickpeas	Sweet lupins	White lupins	Soybeans	Commercial Soybean meal[1]
No of samples	5	4	2	2	2	1
Indispensable amino acids						
Arginine	23.1	18.2	42.0	39.5	29.1	35.9
Histidine	6.7	6.3	12.8	8.8	11.5	15.6
Isoleucine	10.2	9.3	17.0	14.1	18.2	23.9
Leucine	18.6	16.9	23.6	27.5	32.0	35.5
Lysine	17.9	15.1	19.8	17.1	26.1	31.2
Methionine	2.7	3.5	2.9	2.9	6.0	6.8
Phenylalanine	12.4	13.0	17.1	13.0	20.7	25.5
Threonine	9.1	8.3	14.6	13.5	17.1	21.3
Valine	12.1	10.1	15.6	14.0	20.2	25.4
Dispensable amino acids						
Alanine	12.1	10.9	14.8	16.1	22.1	23.8
Aspartic acid	32.4	27.8	45.3	39.8	38.4	55.4
Cystine	8.0	7.6	16.8	12.0	13.8	13.8
Glycine	10.7	8.6	17.0	14.0	17.8	23.5

Glutamic acid	41.2	34.4	89.7	67.5	68.7	88.9
Proline	10.7	8.8	17.5	14.3	21.5	23.2
Serine	10.5	10.9	19.3	17.4	20.8	26.6
Tyrosine	7.9	6.4	11.7	15.0	14.8	18.9

[1] Crude protein content of the soybean meal was 487 g/kg (dry matter basis).

A comparison of the amino acid profile (g/16 g nitrogen) of the five legumes and soybean meal is shown in Table 3.27. This table enables comparison of the ingredients on a protein basis and it gives an indication of the limiting amino acids. It can be seen that the lysine concentrations in chickpea and pea proteins were comparable to that in soy protein, but the lysine concentration in lupin protein was lower. Compared to soybean meal, methionine concentrations in chickpeas were higher and those in peas and lupins were lower. Threonine concentrations in all five legumes were lower than that in soybean meal.

Table 3.27. Comparison of amino acid profile (g/16 g nitrogen) of the five legumes and a commercial sample of soybean meal

	Peas	Chickpeas	Sweet lupins	White lupins	Soybeans	Commercial Soybean meal
No of samples	5	4	2	2	2	1
Indispensable amino acids						
Arginine	9.00	8.48	9.98	10.91	7.17	7.99
Histidine	2.62	2.93	3.03	2.43	2.84	3.20
Isoleucine	3.97	4.32	4.03	3.88	4.49	4.91
Leucine	7.23	7.91	5.59	7.60	7.90	8.23
Lysine	6.96	7.03	4.70	4.71	6.43	6.57
Methionine	1.05	1.62	0.68	0.80	1.48	1.40
Phenylalanine	4.81	6.06	4.05	3.58	5.10	5.63
Threonine	3.53	3.88	3.46	3.73	4.21	4.37
Valine	4.71	4.70	3.71	3.87	4.98	5.22
Dispensable amino acids						
Alanine	4.69	5.07	3.52	4.43	5.46	4.89
Aspartic acid	12.62	12.97	10.75	10.99	9.47	11.38
Cystine	3.12	3.50	4.00	3.32	3.40	3.36
Glycine	4.18	4.02	4.03	3.87	4.40	4.83
Glutamic acid	16.02	16.06	21.31	18.63	16.96	18.25
Proline	4.16	4.10	4.14	3.95	5.31	4.76
Serine	4.10	5.07	4.58	4.81	5.14	5.46
Tyrosine	3.07	2.98	2.78	4.13	3.64	4.23

Protein quality of legumes

A summary of results from the *in vivo* evaluation of protein quality of the five legumes, in relation to soybean meal, is presented in Table 3.28. Within each legume, no differences ($P > 0.05$) were seen in the protein quality (measured as weight gain and protein efficiency ratio) of the different cultivars. However, there were significant ($P < 0.05$) differences in the protein quality of different legumes. None of the legumes were comparable ($P < 0.05$) to soybean meal. Amongst the five species, birds fed the chickpea diets had the highest ($P < 0.05$) weight gain and PER and those fed the soybean diets had the lowest ($P < 0.05$) values. No differences ($P > 0.05$) were noted between the protein quality measures of peas, sweet lupins and white lupins.

Table 3.28. Protein quality of legumes, relative to soybean meal, on the basis of protein efficiency ratio (PER), 1-12 days post-hatching[1]

	Weight gain, g/bird	PER[2]	Relative PER (SBM=100)	Pancreas, g/kg body weight[3]	Mortality, per 24 birds
Soybean meal	120	2.18	100	4.21	0
Peas					
Santana	61	1.30	60	3.56	1
Miami	61	1.35	62	3.87	1
Rex	63	1.39	64	3.04	1
Crusader	58	1.27	58	3.76	0
Courier	67	1.34	61	4.37	0
Chickpea					
Cultivar 1	94	1.87	86	4.36	0
Cultivar 2	80	1.85	85	4.05	0
Cultivar 3	83	1.74	80	3.84	0
Cultivar 4	97	1.76	81	4.07	1
Sweet lupins					
Penny	63	1.24	56	3.55	0
Borre	62	1.18	54	3.49	0
White lupins					
Small	61	1.21	55	3.76	1
Pramore	61	1.07	49	3.14	0
Soybean					
Maturity V	9.7	0.38	17	7.27	5
Maturity VI	9.3	0.42	19	6.56	4

Pooled SEM	3.22	0.052	2.20	0.147	-
Significance, P<	0.001	0.001	0.001	0.001	-
LSD, P<0.05	9.24	0.151	6.31	0.421	

[1] Each mean is an average of three pens of 8 birds each.
[2] PER was calculated as body weight gain (g) divided by protein intake (g).
[3] Each mean is an average of six birds.

The birds fed a diet containing raw soybeans had a higher mortality and higher ($P < 0.05$) relative weights of pancreas compared to those fed other dietary treatments. Relative pancreas weights and the mortality of birds fed chickpeas, peas and lupins were similar ($P > 0.05$) to those fed the soybean meal diets.

3.5. Discussion

Nutrient profiles

Cultivar differences in the nutrient composition of grain legumes are well documented (Green and Oram, 1983; Castell *et al.*, 1996; Duc *et al*., 1991; Nicolopoulo *et al*., 2007; Sujak *et al*., 2006) and one of the aims of the present evaluation was to investigate the effects of cultivars on the nutrient profile of legumes grown in New Zealand. However, there were no significant differences in the proximate and fibre composition between cultivars of chickpeas, peas, white lupins and soybeans. Significant differences were observed only between the two cultivars of sweet lupins.

The proximate composition of chickpeas, peas, sweet lupins and white lupins is within the range reported in the literature (Jood *et al.*, 1998; Perez-Maldonado *et al*., 1999). As anticipated, the protein and fat contents of the different species differed considerably. All legumes were found to be good protein sources (> 200 g/kg DM), but the protein contents of the two lupin species (421 g/kg DM for Australian sweet lupins and 362 g/kg DM for white lupins) were much higher than those of chickpeas (214 g/kg DM) and peas (257 g/kg DM). The average fat contents of chickpeas (79 g/kg DM) and white lupins (113 g/kg) were higher than peas (12.2 g/kg) and sweet lupins (53 g/kg).

It is evident from the present study that starch is the major carbohydrate component in chickpeas (345 g/kg DM) and peas (415 g/kg DM). The moderate fat and starch contents of chickpeas and the high starch content of peas make these legume seeds as excellent sources of available energy. The starch content of chickpeas and peas was within the range reported

by Ribeiro (1990), Castell *et al*. (1996) and Viveros *et al*. (2001). However, higher starch values of peas (437 to 460 g/kg as is basis) have been reported in some studies (Hickling, 2003; Diaz *et al*., 2006).

In agreement with published data (Mohamed and Prayas-Duarte, 1995; Steenfeldt *et al*., 2003), lupins (< 5 g/kg DM) and soybeans (8.2 g/kg DM) were almost devoid of starch. However, the starch content observed for soybeans was much lower than the starch content of 109 to 117 g/kg DM for soybeans as reported by Stevenson *et al*. (2006). This variability may be a result of differences in cultivar and development stages. Maturity V and maturity VI cultivars were used in the present study, whereas high protein, lypoxygenase free, and low-linoleic acid cultivars were used in the study of Stevenson *et al*. (2006). Also the development stages of soybean seeds analysed by these researchers was 20 days prior to harvest and not at commercial maturity.

Non-starch polysaccharides were the major carbohydrates in lupins. In particular, the soluble NSP concentrations were markedly higher in the lupins (49.7 g/kg DM in sweet lupins and 32.8 g/kg DM in white lupins), compared to soybeans (25.6 g/kg DM), peas (16.9 g/kg DM) and chickpeas (9.65 g/kg DM). The soluble NSP content of Australian sweet lupins obtained in the present study was higher than the values (22 to 40 g/kg DM) reported by the previous researchers (Smits and Annison, 1996; Gdala *et al*., 1997; van Barneveld, 1999). In white lupins, the value is within the range reported in the literature (Smits and Annison, 1996; Van Barneveld, 1999; Knudsen, 2001). The soluble NSP of peas was lower than the values (25 to 59 g/kg DM) reported in the literature (Englyst and Hudson, 1996; Smits and Annison, 1996; Gdala *et al*., 1997; Knudsen, 1997; Periago *et al*., 1997; Knudsen, 2001)

In the review by Wang *et al*. (2003), it was reported that micro and macro mineral concentrations in legume seeds were influenced by genetic diversity. Compared to cereal grains, the legumes were found to be excellent sources of minerals, both major and trace minerals. Of interest is the tendency of legume species to accumulate specific minerals as indicated by very high concentrations in the seeds. It was found in the present study that manganese, potassium and iron were preferentially accumulated in the seeds of white lupins, soybeans and peas, respectively.

The high manganese level in white lupins was in agreement with Brand *et al*. (2001) but the value obtained in the present study was higher than that of a previous study conducted by Brand *et al*. (2001). The iron level of peas obtained in the present study was higher than

those published by Wang and Daun (2004). These authors reported that potassium was the mineral found in high concentrations in peas. These discrepancies may be due to the differences in cultivar and growing conditions.

The amino acid composition of the legume species evaluated were comparable to those reported previously (Perez-Maldonado *et al*., 1999; Ravindran *et al*., 2005). The amino acid composition data indicate that these legumes are excellent sources of supplementary protein. The amino acid profiles suggest that the legume proteins are deficient in lysine, sulphur-containing amino acids and threonine, which is a characteristic of legumes in general. However, cereal proteins generally have higher concentrations of these amino acids and will counter these deficiencies either partially or completely, depending on the diet formulation. Furthermore, the low cost of synthetic forms of methionine, lysine and threonine will make it possible to balance practical diets with these amino acids.

Protein quality

The data indicate that there were no differences in protein quality between cultivars of the different grain legume species.

All grain legume species evaluated in this study are known to contain a range of heat-labile and heat-stable anti-nutritive factors including protease inhibitors, tannins, lectins, phytate and NSP, which limit their utilisation in raw form in animal diets (Liener and Kakade, 1980; Ortiz *et al*., 1993; Choct, 1997; Wiryawan, 1997). In the current study, except for NSP, none of the other anti-nutrients were determined. However, the data on relative pancreatic weights and mortality from the PER assay gives an indirect indication of the level of anti-nutrients.

The significant difference in weight gain was probably due to the difference in either protein quality or anti nutritional factors. Raw soybeans supported the lowest weight gain and, had the lowest PER and higher relative pancreatic weights. Heavy mortality was also observed in this group, with a fifth of the birds dying during the 12-day assay period. These data are suggestive of the presence of high concentrations of anti-nutrients, possibly protease inhibitors, in raw soybeans. It is known that both lectins and the protease inhibitors in raw soybeans induce pancreatic hyperplasia and hypertrophy, causing pancreatic enlargement (Grant, 1989).

The mortality of birds fed raw forms of chickpeas, peas or lupins was similar to the control soybean meal group, suggesting that these did not have significant levels of any anti-nutritive factors. The relative pancreatic weights of birds fed these legumes confirm that the level of protease inhibitors in these legumes were probably low. Overall, the present results suggest that the levels of anti-nutrients found in the chickpea, pea and lupin cultivars grown in New Zealand are not of nutritional significance.

3.6. Conclusions

It was evident from the present study that, except in sweet lupins, cultivar type had no effect on the proximate and fibre composition of grain legumes. The primary carbohydrate component of chickpeas and peas was starch, whilst NSPs formed the major carbohydrates in lupins. No differences were found in protein quality between cultivars of the different grain legume species. Results from the *in vivo* assay suggest that the levels of ANFs found in the chickpea, pea and lupin cultivars grown in New Zealand are not of nutritional significance. These data also indicate that the lower PER values of the legumes evaluated, compared to that of soybean meal, are probably due to deficiencies in the limiting amino acids, namely, lysine and sulphur-containing amino acids. This poor protein utilisation may be overcome, to a large extent, by supplementation of crystalline forms of these amino acids.

CHAPTER 4

Determination of apparent metabolisable energy and ileal amino acid digestibility of grain legumes for growing broilers

4.1. Abstract

The apparent metabolisable energy and apparent ileal digestibility coefficient of amino acids of faba beans, Australian sweet lupins, white lupins and peas for growing broiler chickens were determined. The assayed samples included four cultivars of faba beans (PGG Tic, Spec Tic, South Tic and Broad), three cultivars of Australian sweet lupins (Wallan, Tanjil and Borre), three cultivars of white lupins (Promore, Kiev mutant and Ultra) and four cultivars of peas (Santana, Miami, Courier and Rex). A sample of soybean meal was included in the assay for comparison purposes. The assay diets were developed by substituting soybean meal and legumes for 50 and 25% (w/w), respectively, of a maize-soy basal diet. All diets contained 3 g/kg titanium dioxide as an indigestible marker. The diets were offered *ad libitum* in mash form to four replicate cages of broilers (4 birds/cage) from day 28 to d 35 post-hatching. Total excreta collection was made during the last four days for AME determination. The birds were killed on day 35 and the contents of the terminal ileum were collected for amino acid digestibility determination. The AME values and apparent ileal digestibility coefficient of amino acids varied considerably ($P < 0.05$) between legume species. Cultivar effects ($P < 0.05$) on the AME values were observed for faba beans and white lupins. In faba bean, the AME value of South Tic cultivar was found to be higher ($P < 0.05$) than those of Spec Tic and Broad cultivars, but similar ($P > 0.05$) to that of PGG Tic. In white lupins, ultra cultivar had a lower ($P < 0.05$) AME value than those of Promore and Kiev mutant. No differences in the AME were observed between the cultivars of Australian sweet lupins and peas. The AME of faba beans, white lupins and peas were similar ($P > 0.05$), but were higher ($P < 0.05$) than that of Australian sweet lupins. The AME values of all legume species were lower ($P < 0.05$) than that of soybean meal. In all legume species, the apparent ileal digestibility coefficient of amino acids was found to be similar ($P > 0.05$) between cultivars. White lupins had the highest apparent ileal digestibility coefficient of amino acids, but this was not different ($P > 0.05$) from those for Australian sweet lupins and peas. The apparent ileal digestibility coefficient of amino acids of both lupin species was comparable ($P > 0.05$) to that of soybean meal.

4.2 Introduction

The search for new plant protein sources has attracted considerable attention in recent years as a result of the ban on inclusion animal protein meals in diet formulations and the skyrocketing price of soybean meal. In addition, continued growth in the poultry industry is driving the demand for raw materials.

Leguminous seeds such as faba beans, lupins and peas represent potential ingredients of good protein quality in non-ruminant diets and it offers the prospects to increase the protein self-reliance of poultry feeding. However, the use of these ingredients in poultry diets remains limited because of the uncertainty of their nutritional value and the presence of anti-nutritional factors. Most legume species contain one or more anti-nutritional factors, which decrease their nutritional value by increasing endogenous nitrogen losses and impairing nutrient utilisation (Mansoori and Acamovic, 2007; Gabriel *et al*., 2007a). However, the level of anti-nutritional factors in grain legumes varies depending on the species and cultivar (Gatel, 1994; Smits and Annison, 1996).

There have been a number of studies reporting the feeding value of faba beans, lupins and peas in broiler diets (Olkowkski *et al*., 2005; Brenes *et al*, 2003; Perez-Maldonado, 1997; Olver and Jonker, 1997; Igbasan and Guenter, 1996), but published data on energy availability and ileal amino acid digestibility of grain legumes are limited (Perez-Maldonado *et al*., 1999; Hughes *et al*., 2002; Ravindran *et al*., 2002). Moreover, available data on the energy value and amino acid digestibility of grain legumes have often been obtained in studies which have evaluated only one sample of legume. Knowledge of the variability of these parameters is essential for more precise feed formulations. The aim of the present study was to determine and compare the AME, nitrogen-corrected AME (AMEn) and ileal amino acid digestibility values of locally-grown cultivars of faba bean, Australian sweet lupin, white lupin and peas for growing broilers.

4.3. Materials and Methods

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC 05/20 and 05/21) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

4.3.1. Ingredients: A total of 15 samples, representing 14 grain legumes (Figures 1 to 4) and a sample of commercial soybean meal, were assayed. The legume samples included four cultivars of faba beans (PGG Tic, Spec Tic, South Tic and Broad), three cultivars of

Australian sweet lupin (Wallan, Tanjil, and Borre), three cultivars of white lupin (Promore, Kiev mutant and Ultra) and four cultivars of pea (Santana, Miami, Courier and Rex). The legume seeds, with hulls, were ground to pass through a 3-mm sieve in a hammer mill prior to inclusion into the diets. Because of low grain yield, chickpeas were excluded from further evaluations in this thesis research and were replaced by faba beans.

Figure 4.1. Faba beans

Figure 4.2. Australian sweet lupins

Figure 4.3. White lupins

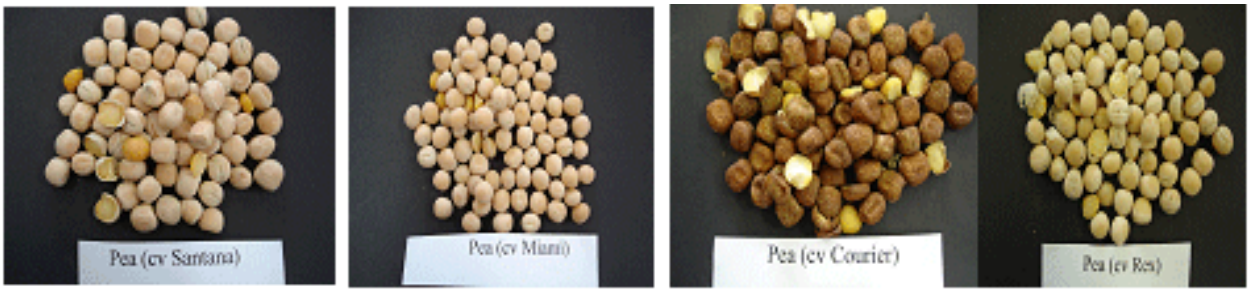


Figure 4.4. Peas

4.3.2. Birds and housing

Day-old male broilers (Ross 308), obtained from a commercial hatchery, were raised in floor pens and fed a commercial broiler starter diets (230 g/kg crude protein) till day 21. Feed and water were available at all times. The temperature was maintained at 32^{o}C during the first week and gradually decreased to approximately 23^{o}C by the end of the third week. Ventilation was controlled by a central ceiling extraction fan and wall inlet ducts. On day 21, 256 birds of uniform body weight were selected and randomly assigned to 64 cages (4 birds per cage). The birds were offered a commercial broiler finisher diet (180 g/kg crude protein) until the introduction of assay diets on day 28. On day 28, four replicate cages were randomly assigned to each assay diet.

4.3.3. Diets

A basal diet based on the maize and soybean meal was formulated (Table 4.1). Fifteen assay diets were then developed by substituting the soybean meal and the 14 legume for 50 and 25% (w/w), respectively, of the basal diet. All diets contained titanium dioxide (3 g/kg) as an indigestible marker to calculate the apparent ileal amino acid digestibility of amino acids.

Table 4.1. Composition (g/kg air dry basis) of the basal diet

Ingredient	
Maize	594.6
Soybean meal	351.8
Soybean oil	17.8
Dicalcium phosphate	21.7
Limestone	7.8
Salt	2.0
Sodium bicarbonate	2.3
Trace mineral-vitamin premix[1]	3.0

[1]Provided per kg diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; trans-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamin, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium panthothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg.

4.3.4. Excreta collection

The AME assay was conducted using the classical total excreta collection method. The diets, in mash form, were fed to birds from day 28. Feed intake and excreta output were measured quantitatively per cage from day 32 for four consecutive days. The excreta from each cage were pooled, mixed, sub-sampled and freeze-dried. The dried excreta samples, together with samples of the diets, were subsequently ground to pass through a 0.5-mm sieve and then

stored in airtight plastic containers for analysis of dry matter, gross energy and nitrogen content.

4.3.5. Collection of ileal digesta

On day 35 post-hatching, all birds were euthanised by an intracardial injection of sodium pentobarbitone solution (1 ml per 2 kg live weight) and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. Digesta samples were pooled within a cage. The ileum was defined as the portion of the small intestine extending from vitelline diverticulum to a point 40 mm proximal to the ileo-caecal junction. The digesta samples were frozen at -20^{o}C in airtight containers immediately after collection and subsequently freeze-dried. The digesta samples in addition to samples of ingredients and diets, were subsequently ground to pass through a 0.5-mm sieve and then stored in airtight plastic containers. The diet and digesta samples were then analysed for dry matter, titanium dioxide and amino acids, whilst ingredient samples were analysed for dry matter and amino acids.

4.3.6. Chemical Analysis

4.3.6.1. Proximate composition: The dry matter content of ingredients, diets and excreta was determined in a convection oven at 105^{o}C (AOAC 930.15, AOAC 925.10 AOAC, 2005). Ash was determined as the organic residue present after incineration at 550^{o}C until loss of organic matter (Method 923.03). Ether extract was determined using the Mojonnier method (AOAC 989.05, 2005). Nitrogen content was determined by the Dumas method (Sweeney, 1989) using a CNS-2000 carbon, nitrogen and sulphur analyser (AOAC 968.06-LECO Corporation, St Joseph, MI, USA). A conversion factor of 6.25 was used to convert N into the crude protein content.

4.3.6.2. Starch: Total starch was determined by using the amyloglucosidase/α-amylase method (Megazyme total starch assay kit, Megazyme International Ireland Ltd, Wicklow, Ireland).

4.3.6.3. Soluble, insoluble and total NSP: Soluble, insoluble and total NSP concentrations were determined using Megazyme total dietary fibre assay kit address based on the methods of Lee *et al*. (1992) and Prosky *et al*. (1992) (AOAC 991.43).

4.3.6.4. Gross energy: Gross energy was determined using an adiabatic oxygen calorimeter (Gallenkamp Autobomb, London, UK) standardised with benzoic acid.

4.3.6.5. Amino acid analysis: Amino acid concentrations of ingredient, digesta and diet samples were determined as described in Chapter 3 section 3.3.3.5.

4.3.6.6. Titanium dioxide: The samples were ignited at 500°C in order to burn all organic material and the remaining minerals were digested (using 66% sulphuric acid) in order to release titanium which was then determined using a colorimetric assay (Short *et al*., 1996)

4.3.6.7. Trypsin inhibitor: The procedure to determine trypsin inhibitor was that of Kakade *et al*. (1974) as modified by Valdebouze *et al*. (1980). One gram of finely ground sample was suspended by constant stirring for 30 min in 100 ml of water adjusted to pH 2.9 (2.8-3.0) with 0.1 M hydrochloric acid. Portions of 0, 0.6, 1.0, 1.4, 1.8 ml of sample suspension were pipetted into graduated test tubes and adjusted to 2 ml with distilled water. To each tube, 2 ml of trypsin solution (trypsin from porcine pancreas) and 5 ml Nα-benzoyl-DL-arginine-ρ-nitroanilide hydrochloride (BAPNA) solution (previously warmed at 37^{o}C) were added. The tubes were incubated in a water bath at 37^{o}C. The reaction was terminated 10 minutes later by adding 1 ml 30% acetic acid, mixed and then filtered (Whatman No. 3). The absorbance of the filtrate was measured at 410 nm against a reagent blank prepared by adding 1 ml of 30% acetic acid to a test tube containing 2 ml water and 2 ml trypsin solution. The differential of absorbance in the presence of inhibitor is a measure of trypsin inhibitor activity. Trypsin inhibitor activity (TIA) was expressed in units of trypsin inhibited (TIU) per milligram sample. All analyses were conducted in duplicate

4.3.7. Calculations

4.3.7.1. AME calculations: The AME values were calculated using the following formulas:

$$AME_{diet}\ (MJ/kg) = \frac{(\text{feed intake} \times GE_{diet}) - (\text{excreta output} \times GE_{excreta})}{\text{Total feed intake}}$$

$$AME_{legume}\ (MJ/kg) = \frac{\text{AME of legume diet} - (\text{AME basal diet} \times 0.75)}{0.25}$$

$$AME_{soybean\ meal}\ (MJ/kg) = \frac{\text{AME of soybean meal diet} - (\text{AME basal diet} \times 0.50)}{0.50}$$

Correction for zero nitrogen retention was made using a factor of 36.54 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958).

4.3.7.2. Digestibility calculations: The apparent ileal digestibility coefficients of amino acids were calculated, using titanium oxide as the indigestible marker, as shown below

$$\text{AIDC of diet} = \frac{(AA / Ti)\ \text{diet} - (AA / Ti)\ \text{ileal}}{(AA/Ti)\ \text{diet}}$$

$$\text{AIDC of legume} = \frac{(\text{AIDC of legume diet} \times \text{AA in legume diet}) - (\text{AIDC of basal diet} \times 0.75 \times \text{AA in basal diet})}{0.25 \times \text{AA in test ingredient}}$$

$$\text{AIDC of soybean meal (SBM)} = \frac{(\text{AIDC soybean meal diet} \times \text{AA in SBM diet}) - (\text{AIDC of basal diet} \times 0.50 \times \text{AA in basal diet})}{0.50 \times \text{AA in SBM}}$$

Where, (AA / Ti) diet = ratio of amino acid to titanium in diet, and
(AA / Ti) ileal = ratio of amino acid to titanium in ileal digesta.

4.3.8. Statistical Analysis

All data were calculated by one-way analysis of variance (ANOVA) using the General Linear Model procedure of SAS (1997). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Fisher's Least Significant Difference Test (LSD).

4.4. Results

Chemical composition

The chemical composition of the grain legumes assayed is presented in Table 4.2. Considerable differences were observed between legume species in terms of chemical composition. Amongst the four legumes species, white lupins had the highest crude protein (349 to 363 g/kg DM) and ether extract (113.2 to 133.8 g/kg DM) contents. Australian sweet lupins, faba beans and peas had moderate levels of crude protein content.

Starch contents were highest in peas (454 to 559 g/kg DM), followed by faba beans (341 to 405 g/kg DM). The total NSP contents was higher in Australian sweet lupins (431.5 to 495.9 g/kg DM), followed by white lupins (355.1 to 370.1 g/kg DM). Trypsin inhibitor activity was found to be negligible in all legume species (< 1 TIU/mg).

Table 4.2. Chemical composition (g/kg DM) of faba beans, Australian sweet lupins, white lupins and peas

	Dry Matter	Crude Protein	Ether extract	Ash	Starch	Non-starch polysaccharides			TI (TIU/mg)
						Soluble	Insoluble	Total	
Faba beans									
PGG Tic	873	306	20.7	33.7	405	19.9	185	205	0.45
Spec Tic	863	300	19.5	32.7	341	21.6	218	240	0.40
South Tic	872	305	21.3	33.0	389	17.4	182	199	0.42
Broad	876	229	23.7	43.3	367	16.1	227	243	0.55
Australian sweet lupins									
Walan	910	288	63.2	39.5	4.43	31.6	463	495	0.23
Tanjil	906	265	73.3	40.1	4.04	31.9	464	496	0.15
Borre	893	320	61.5	38.9	3.17	29.5	402	432	0.25
White lupins									
Promore	883	351	131.7	39.4	6.14	28.8	339	368	0.25
Kiev Mutant	887	349	133.8	36.9	1.70	31.1	324	355	0.24
Ultra	888	363	113.2	40.9	6.54	50.1	320	370	0.20
Peas									
Santana	887	234	21.5	31.0	470	19.1	140	159	0.23
Miami	880	220	20.6	33.0	559	7.9	138	146	0.28
Courier	871	253	21.4	35.0	454	3.6	183	187	0.25
Rex	869	219	24.7	33.3	469	22.8	130	153	0.22

The amino acid contents of the grain legumes and soybean meal are presented in Tables 4.3 to 4.5. Overall, arginine was the most abundant indispensable amino acid, whereas glutamic acid was found to be the abundant dispensable amino acid in all legumes. All legumes were moderate sources of lysine, but deficient in methionine and cystine.

Table 4.3. Amino acid concentration (g/kg DM) for the faba bean cultivars and soybean meal

Amino acid	Faba beans				Soybean meal
	PGG Tic	Spec Tic	South Tic	Broad	
Indispensable					
Arginine	25.0	23.8	25.0	21.2	35.1
Histidine	7.01	6.43	6.84	5.96	19.7
Isoleucine	9.55	8.91	9.72	8.49	19.9
Leucine	17.6	16.7	18.1	14.5	35.3
Lysine	14.4	13.7	15.0	13.0	28.6
Methionine	2.26	2.11	2.21	2.18	6.68
Phenylalanine	9.61	9.22	9.71	8.54	22.2
Threonine	7.51	7.38	8.13	7.08	18.7
Valine	10.9	10.2	10.8	9.87	21.0
Dispensable					
Alanine	10.5	9.93	10.7	9.39	19.8
Aspartic acid	26.2	28.0	27.9	22.1	51.1
Cystine	3.86	3.57	3.71	3.22	7.00
Glycine	10.2	9.56	10.2	8.82	18.0
Glutamic acid	40.0	39.6	40.3	32.9	85.1
Proline	8.79	8.82	8.68	7.00	23.5
Serine	9.16	8.83	9.40	8.15	19.6
Tyrosine	7.78	7.39	7.98	6.74	16.5

Table 4.4. Amino acid concentration (g/kg DM) for Australian sweet and white lupins cultivars and soybean meal

Amino Acid	Australian sweet lupins			White lupins			Soybean meal
	Wallan	Tanjil	Borre	Promore	Kiev mutant	Ultra	
Indispensable							
Arginine	31.8	26.0	32.4	37.7	34.6	36.6	35.1
Histidine	8.51	7.99	8.99	8.92	8.78	8.79	19.7
Isoleucine	11.0	9.88	11.7	14.2	13.2	12.8	19.9
Leucine	19.2	17.5	21.3	26.5	25.3	26.3	35.3
Lysine	15.4	14.9	15.9	16.9	16.4	16.9	28.6
Methionine	2.55	2.53	2.44	2.96	2.77	2.73	6.68
Phenylalanine	10.4	9.79	11.1	13.3	12.6	13.4	22.2
Threonine	12.5	12.0	13.4	14.2	13.1	13.9	18.7
Valine	11.5	10.3	12.0	14.2	13.2	13.8	21.0
Dispensable							
Alanine	11.1	10.5	11.5	12.4	12.0	11.7	19.8
Aspartic acid	27.2	25.3	29.7	35.4	33.9	33.8	51.1
Cystine	5.48	4.90	5.24	5.27	5.53	5.02	7.00
Glycine	11.8	10.3	12.7	13.4	12.1	12.7	18.0
Glutamic acid	59.3	47.0	65.3	68.1	62.6	63.3	85.1
Proline	9.88	8.56	11.1	11.7	11.0	12.9	23.5
Serine	10.9	9.93	12.7	16.3	14.4	14.4	19.6
Tyrosine	9.66	8.86	10.7	15.6	11.5	14.4	16.5

Table 4.5. Amino acid concentration (g/kg DM) for pea cultivars and soybean meal

Amino acid	Peas				Soybean meal
	Santana	Miami	Courier	Rex	
Indispensable					
Arginine	22.0	19.4	22.6	20.3	35.1
Histidine	6.46	5.90	6.30	6.56	19.7
Isoleucine	9.68	9.21	9.66	9.13	19.9
Leucine	17.5	16.3	17.2	16.5	35.3
Lysine	17.3	17.4	17.9	16.6	28.6
Methionine	2.59	2.63	2.56	2.32	6.68
Phenylalanine	10.9	11.0	11.9	10.7	22.2
Threonine	8.97	8.27	9.18	8.04	18.7
Valine	10.5	10.2	10.6	9.88	21.0
Dispensable					
Alanine	10.0	9.25	10.3	9.43	19.8
Aspartic acid	28.6	22.1	29.8	25.7	51.1
Cystine	3.10	3.63	3.14	3.13	7.00
Glycine	10.4	9.12	11.0	9.22	18.0
Glutamic acid	39.6	33.1	39.9	36.5	85.1
Proline	9.61	8.76	9.70	9.19	23.5
Serine	10.3	9.21	10.6	9.86	19.6
Tyrosine	8.30	7.27	8.44	6.99	16.5

Apparent metabolisable energy and amino acid digestibility values

The AME values of different cultivars of faba beans varied, from 8.80 to 11.97 MJ/kg (Table 4.6). The AME and AMEn values of South Tic cultivar were significantly ($P < 0.05$) higher than those values of Spec Tic and Broad cultivars, but similar ($P > 0.05$) to that of PGG Tic. No significant cultivar differences ($P > 0.05$) were observed in the apparent ileal amino acid digestibility coefficients, except for proline ($P < 0.05$). The digestibility coefficient of proline in PGG Tic, South Tic and Spec Tic was higher ($P < 0.05$) than in Broad cultivar. Proline and cystine were the least digestible dispensable amino acids, whilst arginine was the most digestible indispensable amino acid in faba beans.

Table 4.6. Apparent metabolisable energy (AME), nitrogen–corrected apparent metabolisable energy (AMEn), and apparent ileal digestibility coefficient of amino acids in four cultivars of faba beans for broilers[1]

	PGG Tic	Spec Tic	South Tic	Broad	Pooled SEM
AME, MJ/kg DM	10.79[ab]	9.15[b]	11.97[a]	8.80[b]	0.650
AMEn, MJ/kg DM	9.78[ab]	8.25[b]	10.60[a]	8.46[b]	0.590
Ileal digestibility coefficients					
Indispensable amino acids					
Arginine	0.881	0.900	0.898	0.930	0.013
Histidine	0.739	0.738	0.739	0.648	0.046
Isoleucine	0.825	0.833	0.842	0.816	0.031
Leucine	0.831	0.846	0.854	0.813	0.039
Lysine	0.880	0.896	0.896	0.905	0.021
Methionine	0.829	0.825	0.834	0.758	0.034
Phenylalanine	0.851	0.882	0.882	0.919	0.035
Threonine	0.777	0.782	0.811	0.710	0.032
Valine	0.807	0.816	0.822	0.813	0.024
Mean	0.824	0.835	0.842	0.812	0.027
Dispensable amino acids					
Alanine	0.854	0.891	0.887	0.802	0.042
Aspartic acid	0.844	0.885	0.868	0.878	0.027
Cystine	0.597	0.544	0.552	0.558	0.038
Glycine	0.782	0.784	0.789	0.673	0.037
Glutamic acid	0.876	0.891	0.890	0.869	0.022
Proline	0.569[a]	0.583[a]	0.642[a]	0.369[b]	0.048
Serine	0.837	0.808	0.812	0.716	0.030
Tyrosine	0.774	0.788	0.812	0.845	0.028
Mean	0.767	0.772	0.782	0.714	0.026
Overall mean[2]	0.797	0.805	0.814	0.766	0.026

[a,b,c]Means in a row with different superscripts differ ($P < 0.05$).
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

No cultivar differences ($P > 0.05$) were observed in the AME, AMEn, and apparent ileal digestibility coefficient of amino acids of Australian sweet lupins (Table 4.7). The AME values were determined to range between 6.38 and 7.12 MJ/kg DM. In general, the average digestibility coefficients of indispensable and dispensable amino acids were found to be above 0.83. Arginine

had the highest digestibility coefficient (0.92-0.95), whilst the lowest was methionine (0.74-0.83).

Table 4.7. Apparent metabolisable energy (AME), nitrogen–corrected apparent metabolisable energy (AMEn), and apparent ileal digestibility coefficient of amino acids in three cultivars of Australian sweet lupins for broilers[1]

	Walan	Tanjil	Borre	Pooled SEM
AME, MJ/kg DM	6.38	6.73	7.12	0.885
AMEn, MJ/kg DM	5.35	6.18	5.52	0.745
Ileal digestibility coefficients				
Indispensable amino acids				
Arginine	0.949	0.936	0.923	0.015
Histidine	0.797	0.766	0.802	0.029
Isoleucine	0.863	0.835	0.845	0.013
Leucine	0.905	0.861	0.848	0.037
Lysine	0.857	0.874	0.870	0.019
Methionine	0.741	0.796	0.830	0.039
Phenylalanine	0.919	0.895	0.838	0.046
Threonine	0.819	0.802	0.842	0.025
Valine	0.840	0.827	0.818	0.038
Mean	0.854	0.844	0.846	0.028
Dispensable amino acids				
Alanine	0.847	0.826	0.814	0.035
Aspartic acid	0.834	0.837	0.846	0.033
Cystine	0.844	0.826	0.816	0.029
Glycine	0.834	0.811	0.825	0.030
Glutamic acid	0.923	0.907	0.901	0.024
Proline	0.873	0.772	0.814	0.059
Serine	0.862	0.769	0.826	0.043
Tyrosine	0.863	0.834	0.819	0.036
Mean	0.860	0.823	0.833	0.032
Overall mean[2]	0.857	0.834	0.840	0.030

[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

The AME and AMEn values of white lupins were determined to range between 8.05 and 9.68 MJ/kg. Ultra had a lower ($P < 0.05$) AME and AMEn values than Promore and Kiev mutant

cultivars (Table 4.8). The AME and AMEn values of Promore and Kiev mutant were found to be similar (P > 0.05). Overall, the digestibility coefficients of all amino acids were high, ranging between 0.80 for cystine to 0.95 for arginine.

Table 4.8. Apparent metabolisable energy (AME), nitrogen–corrected apparent metabolisable energy (AMEn), and apparent ileal digestibility coefficient of amino acids in three cultivars of white lupin for broilers

	Promore	Kiev mutant	Ultra	Pooled SEM
AME, MJ/kg DM	9.68[a]	9.58[a]	8.05[b]	0.430
AMEn, MJ/kg DM	7.67[a]	8.38[a]	6.34[b]	0.380
Ileal digestibility coefficients				
Indispensable amino acids				
Arginine	0.945	0.944	0.949	0.009
Histidine	0.808	0.805	0.816	0.031
Isoleucine	0.864	0.895	0.874	0.014
Leucine	0.877	0.911	0.894	0.019
Lysine	0.891	0.905	0.908	0.017
Methionine	0.803	0.866	0.822	0.036
Phenylalanine	0.908	0.933	0.929	0.021
Threonine	0.829	0.828	0.850	0.017
Valine	0.841	0.821	0.879	0.048
Mean	0.863	0.879	0.880	0.018
Dispensable amino acids				
Alanine	0.814	0.875	0.850	0.029
Aspartic acid	0.849	0.886	0.863	0.019
Cystine	0.808	0.811	0.797	0.044
Glycine	0.851	0.862	0.868	0.019
Glutamic acid	0.904	0.944	0.927	0.011
Proline	0.831	0.855	0.870	0.028
Serine	0.852	0.843	0.842	0.023
Tyrosine	0.882	0.862	0.892	0.019
Mean	0.849	0.867	0.864	0.018
Overall mean[2]	0.856	0.873	0.872	0.018

[a,b,c]Means in a row with different superscripts differ (P < 0.05).
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

It can be seen from Table 4.9 that the AME and AMEn values of the four pea cultivars were similar (P > 0.05). The range of AME and AMEn values were from 9.82 to 10.78 MJ/kg DM and 9.11 to 10.16 MJ/kg DM, respectively. No cultivar differences (P > 0.05) were observed in the digestibility coefficients of amino acids, except for arginine. The courier cultivar had a lower (P < 0.05) arginine digestibility compared to the three other cultivars. The average digestibility coefficient of amino acids was above 0.82. Arginine was the best digested amino acid (0.88 to 0.93) and cystine was the least digestible (0.60 to 0.69) amino acid in field peas.

Table 4.9 Apparent metabolisable energy (AME), nitrogen-corrected apparent metabolisable energy (AMEn) and apparent ileal digestibility coefficient of amino acids in three cultivars of field peas for broilers[1,2]

	Santana	Miami	Courier	Rex	Pooled SEM
AME, MJ/kg DM	10.78	10.15	10.39	9.82	0.550
AMEn, MJ/kg DM	10.16	9.81	9.71	9.11	0.490
Ileal digestibility coefficients					
Indispensable amino acids					
Arginine	0.920[a]	0.925[a]	0.885[b]	0.929[a]	0.010
Histidine	0.774	0.829	0.806	0.865	0.036
Isoleucine	0.846	0.840	0.850	0.825	0.018
Leucine	0.844	0.831	0.843	0.856	0.025
Lysine	0.891	0.905	0.868	0.889	0.013
Methionine	0.826	0.750	0.852	0.840	0.072
Phenylalanine	0.865	0.887	0.857	0.886	0.023
Threonine	0.782	0.795	0.798	0.740	0.026
Valine	0.837	0.830	0.831	0.846	0.015
Mean	0.843	0.844	0.843	0.853	0.021
Dispensable amino acids					
Alanine	0.840	0.806	0.856	0.847	0.025
Aspartic acid	0.846	0.845	0.845	0.840	0.026
Cystine	0.612	0.683	0.689	0.596	0.041
Glycine	0.812	0.793	0.811	0.799	0.019
Glutamic acid	0.911	0.902	0.896	0.892	0.017
Proline	0.847	0.710	0.632	0.878	0.076
Serine	0.813	0.791	0.821	0.802	0.028
Tyrosine	0.827	0.791	0.829	0.791	0.021

Mean	0.813	0.790	0.797	0.806	0.025
Overall mean[3]	0.829	0.818	0.822	0.831	0.022

[a,b]Means in a row with different superscripts differ (P < 0.05).
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

A comparison of the AME, AMEn and apparent ileal digestibility coefficient of amino acids of the four legumes and soybean meal is presented in Table 4.10. No differences (P > 0.05) were observed in the AME values of faba beans, white lupin and peas. The AME and AMEn values was found to be high in field peas and faba beans, intermediate in white lupins, and low in Australian sweet lupins. The AME values of faba beans, Australian sweet and white lupins, and peas were lower (P < 0.05) than that of soybean meal.

The AMEn values of faba beans and peas were similar (P > 0.05) to that of soybean meal, whilst those of both lupin species were lower (P < 0.05) than that of soybean meal. The AMEn values of faba beans and peas were higher (P < 0.05) than those of both lupins.

Significant differences (P < 0.05) were found between ingredients for the digestibility coefficients of arginine, histidine, isoleucine, threonine, cystine, glycine, proline, and tyrosine. Both lupin species had the highest arginine digestibility and did not differ (P > 0.05) from each other. The digestibility coefficient of arginine in field peas was comparable (P > 0.05) to the Australian sweet lupins, but lower (P < 0.05) than the white lupins. Histidine digestibility in soybean meal was higher (P < 0.05) than those in grain legumes. The lowest digestibility coefficient of histidine was found in faba beans.

Soybean meal had the highest isoleucine digestibility, but it did not differ (P > 0.05) from values obtained from Australian sweet and white lupins. Threonine digestibility coefficients in Australian sweet and white lupins were similar (P > 0.05) to that of soybean meal. The digestibility of threonine in peas did not differ (P > 0.05) from faba beans and Australian sweet lupins. Faba beans had a lower (P < 0.05) cystine digestibility than the rest of grain legumes. No differences (P > 0.05) in cystine digestibility were observed between Australian sweet and white lupins and soybean meal.

Glycine digestibility in faba beans was lower (P < 0.05) than lupins but similar (P > 0.05) to those in peas and soybean meal. Glutamic acid digestibility coefficient of both lupin species was higher (P < 0.05) than soybean meal, but similar (P > 0.05) to that of peas. Faba beans had a lower (P < 0.05) proline digestibility coefficient than the rest of grain legumes and soybean meal.

Table 4.10. Apparent metabolisable energy (AME), nitrogen-corrected apparent metabolisable energy (AMEn), and apparent ileal amino acid digestibility coefficients in four legumes and soybean meal for broilers

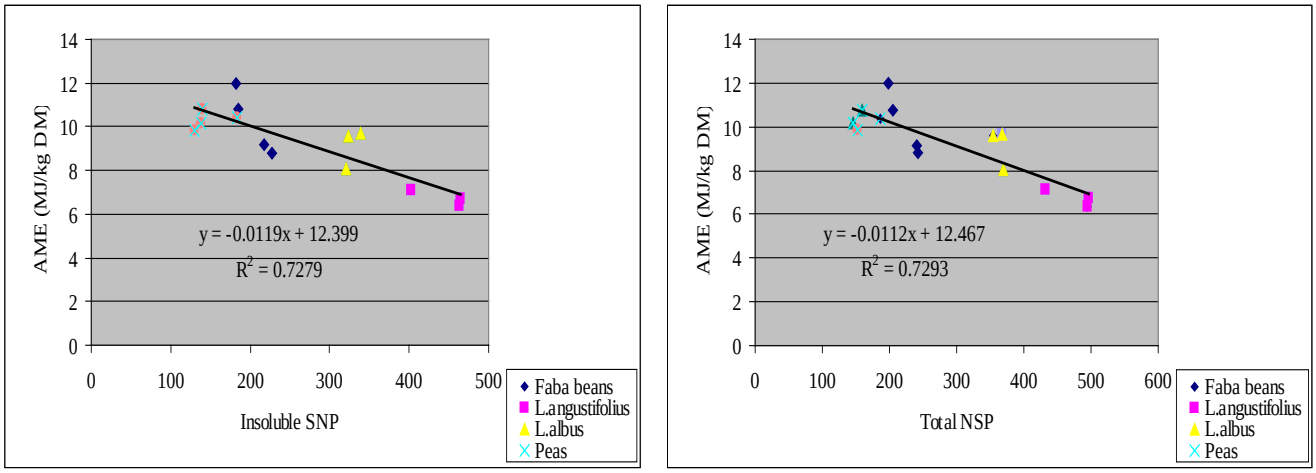
	Faba beans[1]	Australian sweet lupins[2]	White lupins[3]	Peas[4]	SBM[5]	Pooled SEM
AME, MJ/kg DM	10.18[b]	6.75[c]	9.12[b]	10.33[b]	12.51[a]	0.430
AMEn, MJ/kg DM	9.27[a]	5.68[c]	7.46[b]	9.74[a]	9.61[a]	0.398
Ileal digestibility coefficients						
Indispensable amino acids						
Arginine	0.902[c]	0.936[ab]	0.946[a]	0.914[bc]	0.918[bc]	0.012
Histidine	0.715[c]	0.789[b]	0.810[b]	0.815[b]	0.910[a]	0.032
Isoleucine	0.829[c]	0.848[abc]	0.877[ab]	0.841b[c]	0.891[a]	0.014
Leucine	0.835	0.871	0.894	0.843	0.876	0.018
Lysine	0.894	0.867	0.901	0.888	0.915	0.010
Methionine	0.811	0.720	0.830	0.816	0.898	0.030
Phenylalanine	0.884	0.884	0.923	0.873	0.888	0.020
Threonine	0.769[b]	0.821[ab]	0.836[a]	0.781[ab]	0.855[a]	0.016
Valine	0.815	0.828	0.847	0.835	0.879	0.020
Mean	0.828	0.847	0.874	0.845	0.892	0.012
Dispensable amino acids:						
Alanine	0.857	0.829	0.846	0.837	0.869	0.022
Aspartic acid	0.868	0.839	0.866	0.844	0.865	0.015
Cystine	0.564[c]	0.761[a]	0.806[a]	0.648[b]	0.809[a]	0.023
Glycine	0.755[b]	0.823[a]	0.860[a]	0.804[ab]	0.853[ab]	0.018
Glutamic acid	0.881	0.910	0.925	0.901	0.901	0.028
Proline	0.538[b]	0.820[a]	0.852[a]	0.759 [a]	0.887 [a]	0.041
Serine	0.792	0.819	0.846	0.807	0.869	0.031
Tyrosine	0.806[c]	0.839[bc]	0.878 [ab]	0.811[c]	0.895 [a]	0.016
Mean	0.758[c]	0.837[ab]	0.860[a]	0.801[bc]	0.869[a]	0.016
Overall mean[6]	0.795b[c]	0.843[ab]	0.867[ab]	0.825[bc]	0.881[a]	0.014

[a,b,c,d]Means in a row with different superscripts differ ($P < 0.05$).
[1]Mean of 4 cultivars (4 replicates per cultivar and 4 birds per replicate)
[2]Mean of 3 cultivars (4 replicates per cultivar and 4 birds per replicate)
[3]Mean of 3 cultivars (4 replicates per cultivar and 4 birds per replicate)
[4]Mean of 4 cultivars (4 replicates per cultivar and 4 birds per replicate)
[5]Mean of 4 replicates (4 birds per replicates)
[6]Average digestibility of 17 amino acids.

Relationship between soluble NSP content and the AME values of grain legumes

The linear regression between insoluble and total NSP content and the apparent metabolisable energy value of grain legumes was presented in Figure 4.5. The AME of grain legumes was significantly correlated (R^2 = 0.73) with their insoluble and total NSP contents. As the insoluble and total NSP increased, the AME value decreased.

Figure 4.5. Linear regression between soluble, insoluble and total NSP content and AME value of grain legumes



4.5. Discussion

Chemical composition: The considerable cultivar differences observed in the chemical composition within the legume species are consistent with published data (Brand *et al*., 2004; French, 2005; Glencross, 2005). The results showed that the grain legumes tested in the present study represent valuable sources of protein (> 200 g/kg DM) and starch (> 300 g/kg DM, except for lupin species). The highest protein content was found in the two lupin species followed by faba beans and peas. Grain legumes were moderately excellent sources of lysine, but deficient in sulphur-containing amino acids (methionine and cystine). The deficiency of sulphur-containing amino acids may be overcome by combining them with cereals which are excellent sources of methionine and cystine. Another practical option is to supplement legume-based diets with crystalline forms of methionine.

Both lupin species had higher non-starch polysaccharide contents than faba beans and peas. In particular, the high contents of soluble non-starch polysaccharides in lupins have implications for digesta viscosity and nutrient utilisation in birds. The level of trypsin inhibitor activity found in all legume species tested was very low (< 1 TIU/mg) suggesting that these legumes could be incorporated in raw form in poultry diets.

Apparent metabolisable energy and apparent ileal amino acid digestibility coeffcient of faba beans: In the present study, the apparent metabolisable energy of faba beans ranged from 8.80 to 11.97 MJ/kg DM. In general, these values were lower than the values (10.30 - 13.70 MJ/kg DM) reported by earlier researchers (Perez-Maldonado, 1997; Brufau *et al*., 1998; Hughes *et al*., 2002; Metayer *et al*., 2003). This discrepancy probably reflects cultivar differences in terms of their physical and chemical characteristics in addition to differences in the assay methodology. In addition, factors such as seasonal effects, growth sites, year of harvest, crop treatment and grain fumigant, post harvest storage condition, and period of storage may also cause variations in the apparent metabolisable energy values of legume grains (Brufau *et al*., 1998; Hughes and Choct, 1999). The lower apparent metabolisable energy value in Spec Tic may be attributed to its low starch and the high soluble non-starch polysaccharide contents.

The average amino acid digestibility of faba beans was high, with arginine being the most digestible amino acid, while cystine and proline were the poorest. On average, the apparent ileal digestibility coefficient values were found to be were higher than that of Ravindran *et al*. (2005). This variability was probably due to the differences in the assay methodology.

Apparent metabolisable energy and apparent ileal amino acid digestibility coefficient of lupin species: The apparent metabolisable energy values of white lupins (8.05 - 9.68 MJ/kg DM) were higher than that of Australian sweet lupins (6.38 - 7.12 MJ/kg DM). The superior apparent metabolisable energy values of white lupins may be attributed to their higher fat content (Table 4.2). The apparent metabolisable energy values of white lupins have been reported to range from 8.0 to 14.9 MJ/kg (Hughes *et al*., 1996, 1998; Kocher *et al*., 2000) and the values obtained in the present study for white lupins were within the range of the values reported in the literature. The apparent metabolisable energy values determined for Australian sweet lupins in this study were also within the range of the previous published data (Eason *et al*, 1990; Hughes *et al*., 1998; Perez-Maldonado *et al*., 1999; Kocher *et al*., 2000).

No cultivar effects were observed in the apparent ileal digestibility coefficient of amino acids in either lupin species. The average apparent ileal digestibility coefficient of Australian sweet lupins determined in the present study was comparable to those published by Ravindran *et al*. (2002), but slightly higher than that of Ravindran *et al*. (2005). For white lupins, the average apparent ileal digestibility coefficient of amino acids was slightly higher than those published by R4avindran *et al*. (2002). These differences may be due to the differences in cultivar in terms of chemical composition and the methodology used.

Apparent metabolisable energy and apparent ileal amino acid digestibility coefficient of peas: The apparent metabolisable energy values of peas (9.82 - 10.78 MJ/kg) determined in the present study was lower than those reported by Carré *et al* (1991) and Perez-Maldonado *et al*. (1999). These differences may be related to difference in cultivars, the methodology used, and other possible factors such as seasonal effects, growth sites, year of harvest, crop treatment and grain fumigant, post harvest storage condition, and period of storage which could have a bearing on energy availability of grain legumes (Brufau *et al*., 1998; Hughes and Choct, 1999). The average apparent digestibility of amino acids in peas varied between 0.82 and 0.83, with the highest values for arginine (0.88 to 0.93) and glutamic acid (0.89 to 0.91), and the lowest values for cystine (0.60 to 0.69). The apparent ileal digestibility coefficient of amino acids obtained in this study was higher than those published by Ravindran *et al*. (2002) and Pérez *et al*. (1993). These differences might be due to the differences in the methodology used.

Comparison of apparent metabolisable energy and apparent ileal amino acid digestibility coefficient of faba beans, lupins, peas and soybean meal: In general, the apparent metabolisable energy of different grain legumes varied considerably. The superiority of peas and the inferiority of lupins in terms of their apparent metabolisable energy values are in agreement with previous studies (Perez-Maldonado *et al*., 1999; Metayer *et al*., 2003; Palander *et al*., 2006).

Although the lupin species were rich in fat, their apparent metabolisable energy values were lower than those of faba beans and peas. This observation could be attributed to the high levels of non-starch polysaccaharide (Table 4.2; Figure 4.5), particularly the soluble non-starch polysaccaharide fraction. It is known that the soluble fibre fraction increases gut viscosity (Smits and Annison, 1996), leading to reduced mixing of digestive enzyme and substrates in the intestinal lumen (Choct, 1997), and lowering overall nutrient digestibility. The absence of starch in lupins is another reason for the low apparent metabolisable energy values.

In comparison to soybean meal, the apparent metabolisable energy values of all grain legumes were lower. The lower apparent metabolisable energy values of these legume seeds could be improved by applying processing technology (dehulling and/or thermal processing) or supplementing the diets with appropriate exogenous feed-enzymes. A study conducted by Moran *et al*. (1968) showed that pelleting was effective in improving the apparent metabolisable energy values of pea-based diets and reducing the variability in apparent metabolisable energy values. According to Hickling (2003), the improvements in the apparent metabolisable energy of heat-treated peas were due to gelatinisation of starch and the rupture of the cell wall matrix.

Hughes *et al*. (2002) reported that the dry matter digestibility and apparent metabolisable energy of diets based on faba bean were increased by 9.6% and 22%, respectively, by adding an enzyme product with multi-carbohydrase activities, including hemi-cellulase and pectinase.

The apparent ileal digestibility coefficient of amino acids of both lupin species and peas were comparable to those of soybean meal. The apparent ileal digestibility coefficient of arginine and lysine were high in all legumes tested. The relatively high apparent ileal digestibility coefficient values of arginine compared to other amino acids was probably due to the high content of this amino acid in all legumes.

A number of reports have shown that the content and digestibility of cystine were low in grain legumes (Longstaff and McNab, 1991; Brufau *et. al*., 1998; Palander *et. al*., 2006). In the present study, it was observed that the apparent ileal digestibility coefficient of cystine was lower than other amino acids in faba beans and peas (0.564 - 0.648). The apparent ileal digestibility coefficient of cystine in lupins was higher (0.761 - 0.806) and these values were comparable to that in soybean meal (0.748). The apparent ileal digestibility coefficient of methionine in all legumes was high and comparable to that in soybean meal.

4.6. Conclusions

It is evident from this study that considerably differences occurred in the chemical composition, apparent metabolisable energy values and apparent ileal digestibility coefficient of amino acids between legume species. Cultivar effects on apparent metabolisable energy values were found only for faba beans and white lupins. The apparent metabolisable energy value of South Tic cultivar of faba bean was higher than those of Spec Tic and Broad cultivars, but similar to that of PGG Tic. Ultra cultivar of white lupins had lower apparent metabolisable energy value than that of Promore and Kiev mutant cultivars. Overall, faba beans, white lupins, and peas had comparable apparent metabolisable energy values although these values were higher than those of Australian sweet lupins and lower than that of soybean meal.

In all legume species, there were no cultivar effects on the apparent ileal digestibility coefficient of amino acids. White lupins had the highest apparent ileal digestibility coefficient of amino acids, but did not differ from values obtained for Australian sweet lupins and peas. The apparent ileal digestibility coefficient of amino acids of both lupin species was comparable to that of soybean meal.

On the basis of these results, future studies are warranted to evaluate the effects of feed processing technology (i.e. dehulling or thermal processing) and enzyme supplementation) to improve the available energy values and nutrient digestibility of grain legumes.

CHAPTER 5

The influence of diets containing different grain legumes on the performance and digestive tract development of growing broiler chickens

5.1 Abstract

The effects of feeding diets containing 200 g/kg of faba beans, Australian sweet lupins, white lupins, and peas on the performance and the digestive tract development of broiler starters were investigated. The legumes included four cultivars of faba beans (PGG Tic, Spec Tic, South Tic and Broad), three cultivars of Australian sweet lupins (Wallan, Tanjil and Borre), three cultivars of white lupins (Promore, Kiev mutant and Ultra), and four cultivars of peas (Santana, Miami, Courier and Rex). The experiment was conducted as a complete randomised design consisting of 15 treatments, involving a maize-soy control diet and 14 experimental diets. The diets, in pellet form, were fed *ad libitum* to four pens of eight male broilers each from day 1 to 21 post-hatch. The results showed that the weight gain of birds fed diets containing different cultivars of grain legumes was similar ($P > 0.05$) to that of control diet. Feed intake and feed per gain of birds fed diets containing the majority of grain legume cultivars did not differ ($P > 0.05$) from those fed a maize-soy diet. The relative pancreas weight of birds fed the majority of legume cultivars was similar ($P > 0.05$) to that of control diet. Birds fed diets containing the majority of both lupin cultivars had higher ($P < 0.05$) relative empty weight of duodenum, jejunum, ileum and small intestine. Excluding peas, birds fed diets containing the majority of faba beans and lupin cultivars had higher ($P < 0.05$) relative digesta content of proventriculus and gizzard The relative length of duodenum, jejunum, ileum and small intestine and caeca of birds fed diets containing the majority of grain legume cultivars was found to be similar ($P > 0.05$) to those fed a maize-soy diet. Between legume species, feed per gain of broilers fed diets containing white lupins was similar ($P > 0.05$) to those fed the maize-soy diet and faba bean- and pea- based diets. The relative empty weight of duodenum, jejunum, ileum, small intestine and caeca of birds fed a diet containing white lupins was higher ($P < 0.05$) than that of control diet. The relative digesta content of birds fed faba bean- and sweet lupin- based diets was higher ($P < 0.05$) than that of control diet. Excluding sweet lupins, birds fed diets containing grain legumes had similar ($P > 0.05$) relative length of jejunum, ileum and small intestine to that of control diet. Overall, these results suggest that faba beans, Australian sweet lupins, white lupins, and peas could be included at 200 g/kg level of inclusion as a partial replacement for soybean meal, in diets for broiler starters.

5.2. Introduction

Commercial poultry feed industry is mainly dependent on the conventional protein sources, soybean meal and meat and bone meal, for practical diet formulations. Soybean meal is imported and comprise up to 30% of poultry diets, which results in a high production cost. The recent ban on the use of ingredients of animal origin in livestock diets makes the situation has become more critical for the poultry industry. Therefore, evaluation of alternative protein ingredients, which are locally available and economical, and can be used as substitutes for conventional protein meals, is required.

The interest of using grain legumes such as faba beans, lupins and peas as protein sources for poultry as an alternative to conventional protein sources has been increasing (Olver, 1987; Brufau *et al.*, 1998; Farrel *et al*., 1999; Brand *et al*., 2004; Li *et al*., 2006). These legume seeds not only offer a valuable source of protein, but also provide the energy due to their starch (in faba beans and peas) and oil (lupin in particular) contents (Hickling, 2003; Palander *et al*., 2006; Diaz *et al*, 2006). However, these legume seeds also contain variable amounts of anti-nutritional factors such as non-starch polysaccharides, tannins and protease inhibitors which can reduce nutrient digestibility, performance and adversely affect digestive tract development (ie. pancreatic and small intestinal enlargements) in birds. Thus, for the maximal use of these legume meals, it is important to know how much of these ingredients can be included in practical broiler diets whilst still maintaining performance.

Athough considerable research has been carried out on the suitability of faba beans, lupins and peas either partly or fully replacing soybean meal in broiler diets, the data on their inclusion levels are contradictory (Perez-Maldonado, 1997; Olver and Jonker, 1997; Metayer *et al*., 2003; McNeill *et al*., 2004; Olkowski *et al*., 2005). In addition, the majority of available data on the nutritional value of grain legumes for poultry have been generated in studies which focused only on one sample of legume. Thus, the present experiment was carried out in order to examine the effects of feeding diets containing 200 g/kg of different cultivars of Australian sweet lupins, white lupins, and peas on the performance and the digestive tract development of broiler starters. This inclusion level was chosen on the basis of available published data.

5.3. Materials and methods

All experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC 05/20 and 05/21) and were in accordance with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

5.3.1. Ingredients: The ingredients included four cultivars of faba beans (PGG Tic, Spec Tic, South Tic and Broad), three cultivars of Australian sweet lupins (Wallan, Tanjil, an Borre), three cultivars of white lupins (Promore, Kiev mutant and Ultra) and four cultivars of peas (Santana, Miami, Courier and Rex). Before incorporation into test diets, legume seeds with hulls were ground in a hammer mill to pass through a 3 mm sieve.

5.3.2. Birds and housing: Day-old male broilers (Ross 308) obtained from a commercial hatchery were individually weighed and assigned to 60 cages (8 birds per cage) in electrically heated battery brooders, so that the average initial weight per cage was similar. Details of the housing were as described in Chapter 4, Section 4.3.2.

5.3.3. Diets: Fifteen diets (Table 5.1), including a maize-soybean meal control diet and 14 experimental diets containing 200 g/kg of legumes, were formulated using the determined values of AME and digestible amino acids (reported in Chapter 4) to contain similar levels of metabolisable energy and digestible amino acids (lysine, methionine and cystine, and threonine). After mixing, the experimental diets were cold pelleted (70°C). Each of the 15 dietary treatments was then randomly assigned to four pens which contain eight chicks each. The diets were offered *ad libitum* from day 1 to 21. Water was freely available throughout the trial.

5.3.4. Measurements: Body weights and feed intake were recorded at weekly intervals throughout the trial. Mortality was recorded daily. Feed per gain was corrected for mortality and represent grams of feed consumed by all birds in a pen divided by grams of body weight gain per pen, plus the body weight of the birds that died. Excreta was scored on a scale of 1 to 5 (1 = normal, dry, friable litter and 5 = representing wet and cakey litter). On day 21, three birds, closest to the mean pen weight, were selected per cage, weighed and sacrificed by cervical dislocation. The length of each intestinal segment was determined with a non-rigid tape on a wet glass surface to prevent inadvertent stretching. The length (± 0.1 mm) of the duodenum (from the pyloric junction to the distal most point of insertion of the duodenal mesentery), the length of the jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), the length of ileum (from the junction with Meckel's diverticulum to ileal-caecal junction) and the sum of the lengths from the ostium to the tip of each caeca were determined. Following division and freeing of each of these components from any adherent mesentery, their full and empty weights (± 0.1 g) were determined along together with those of the crop, proventriculus and gizzard.

5.3.5. Statistical analysis: Cage means were used to derive performance data. For digestive tract measurements, individual birds were considered as the experimental units. All data were calculated by one-way analysis of variance using the General Linear Model procedure of SAS (1997). For clarity and simplicity, data from each legume species were analysed separately along together with those from the maize-soy control diet. Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Fisher's Least Significant Difference test.

Table 5.1. Ingredient composition and calculated analysis (g/kg as is) of experimental diets

Ingredients	Maize-soy control diet	Faba beans			Australian sweet lupins				White lupins			Peas			
		PGG Tic	Spec Tic	South Tic	Broad	Wallan	Tanjil	Borre	Prom ore	Kiev mutant	Ultra	Santana	Miami	Courier	Rex
Maize	567	448	424	462	448	397	390	404	430	429	414	412	398	415	393
Soybean meal	317	226	235	223	226	239	247	238	235	235	237	258	266	253	268
Grain legume	-	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Meat meal	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
Tallow	10.0	10.0	10.0	9.8	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Soybean oil	6.0	15.2	30.4	5.0	15.2	53.0	51.8	47.1	25.1	26.0	38.8	19.5	25.5	22.2	28.3
L-lysine HCl	1.1	1.2	1.1	1.1	1.2	0.8	0.5	0.6	0.3	0.4	0.3				
DL-methionine	2.9	3.5	3.6	3.6	3.5	3.1	3.1	3.1	3.0	3.0	3.1	3.3	3.1	3.3	3.3
L-threonine	0.1	0.7	0.6	0.6	0.7	-	-	-				0.1	0.0	0.1	0.1
Dicalcium phosphate	10.3	9.9	9.9	9.9	9.9	11.8	11.7	11.8	11.7	11.7	11.8	11.4	11.3	11.4	11.3
Salt	1.6	1.6	1.6	1.6	1.6	1.7	1.8	1.8	1.8	1.8	1.9	2.0	2.0	2.0	2.0
Sodium bicarbonate	1.0	0.7	0.7	0.6	0.7	0.8	0.7	0.7	0.6	0.6	0.5	0.6	0.6	0.6	0.6
Trace-mineral-vitamin premix[1]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Calculated analysis															
AME, MJ/kg	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2
Crude protein	245	247	247	246	247	246	245	250	256	256	258	245	245	245	245
Digestible lysine	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.9	13.1	12.8	13.0
Digestible methionine	6.1	6.4	6.4	6.4	6.3	5.9	6.0	6.0	6.0	5.9	6.0	6.3	6.1	6.3	6.3
Digestible met + cys	8.8	8.9	8.9	9.0	8.8	8.9	8.9	8.9	8.9	9.0	8.9	8.9	8.9	8.9	8.9
Digestible threonine	7.2	7.2	7.2	7.2	7.1	7.5	7.5	7.7	7.8	7.6	7.8	7.2	7.2	7.2	7.2
Calcium	9.6	9.6	9.6	9.6	9.6	10.2	10.2	10.2	10.2	10.2	10.2	9.9	9.9	9.9	9.9
Available phosphorus	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
Sodium	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Potassium	8.2	8.2	8.2	8.2	8.4	8.0	8.1	8.0	8.0	8.0	8.0	8.2	8.3	8.1	8.3
Chloride	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6

[1] For composition, please refer to Table 4.1.

5.4. Results

The effects of dietary treatment on the performance and digestive tract development of birds

The effects of feeding diets containing 200 g/kg of each cultivar of faba beans, Australian sweet lupins, white lupins and peas on the performance, excreta score and digestive tract development of broilers are presented in Table 5.2. Excluding weight gain, significant differences ($P < 0.0009$ to 0.0001) were found in feed intake, feed per gain and excreta score between birds fed diets which contained all cultivars of faba beans, Australian sweet lupins, white lupins and peas and those birds fed a maize-soy basal diet. Feed intake and feed per gain of birds fed diets which contained the majority of grain legume cultivars were found to be similar ($P > 0.05$) to those fed a maize-soy diet. Birds fed diets containing faba bean cultivars had lower ($P < 0.05$) excreta score than those birds fed a basal diet and diets which contained the other legume cultivars. The mortality of birds fed diets containing the majority cultivar of grain legumes had lower ($P < 0.05$) mortality than those birds fed a maize-soy basal diet.

The relative liver weight of birds fed all cultivars of both lupin species was higher ($P < 0.05$) than those birds fed basal diets and faba bean- and pea-based diets. No significant difference ($P > 0.05$) was found in spleen weight between all treatment diets. The relative pancreas weight of birds fed grain legume based diets, except for Kiev mutant cultivar, was similar to those fed a maize-soy basal diet.

Excluding Courier cultivar of peas, the relative empty weights of crops and proventriculus of birds fed grain legume diets did not differ ($P > 0.05$) from those fed the maize-soy control diet. The relative empty gizzard weight of faba beans (except for PGG Tic) and Borre cultivar of Australian sweet lupin was higher ($P < 0.05$) than that of maize-soy diet. Birds fed diets which contained the majority of both lupin cultivars had higher ($P < 0.05$) relative empty weight of duodenum, jejunum, ileum and small intestine. The birds fed diets which contained all three cultivars of white lupins had higher ($P < 0.05$) relative empty weight of caeca.

Excluding peas, the majority of faba beans and both lupin cultivars had higher ($P < 0.05$) relative digesta content of proventriculus and gizzard. No differences ($P > 0.05$) were found in relative digesta content of ileum and caeca between birds fed all grain legume cultivars and those fed a maize-soy basal diet. The birds fed diets containing the majority of

both lupin cultivars had higher ($P < 0.05$) relative digesta weight of duodenum, jejunum and small intestine.

The relative length of duodenum, jejunum, ileum, small intestine and caeca of birds fed diets containing the majority of grain legume culvars was found to be similar ($P > 0.05$) to those fed a maize-soy basal diet.

Comparison of the performance and digestive tract development of birds fed grain legume diets and maize-soybean meal diet

As can be seen in Table 5.3, the birds fed diets which contained faba beans, sweet lupins, white lupins and peas had similar ($P > 0.05$) weight gain to those birds fed a maize-soy diet. Feed per gain of birds fed white lupin-based diet was higher ($P < 0.05$) than those of control diet, faba bean- and pea- based diets, but the observed value was similar ($P > 0.05$) to that of sweet lupins. It is interesting to note that birds fed diets which contained faba beans, sweet lupin, white lupin and peas had lower ($P < 0.05$) mortality rate compared to those fed a maize-soy control diet.

No significant difference ($P > 0.05$) was found in relative spleen weight between birds fed grain legume diets and those fed a maize-soy basal diet. The birds fed diets which contained white lupins had higher ($P < 0.05$) relative liver weight than those fed a maize-soy diet and diets contained faba beans, sweet lupin and peas. The relative empty weight of duodenum, jejunum, ileum, small intestine and caeca of birds fed a diet which contained white lupins was higher than those fed a maize-soy basal diet and diets which contained faba beans and peas, but the observed values were similar to that of sweet lupins.

The relative gizzard digesta content of birds fed faba bean- and sweet and white lupin-based diets was found to be higher ($P < 0.05$) than that of control diet. Birds fed diets which contained sweet (except for Wallan) and white lupins had higher ($P < 0.05$) the relative digesta content of jejunum than those fed a maize-soy basal diet.

Birds fed diets which contained the majority of faba bean, sweet lupin, white lupin and pea cultivars had similar ($P > 0.05$) relative length of jejunum, ileum and small intestine to that of maize-soy diet. The relative length of duodenum of birds fed faba bean- based diet did not differ ($P > 0.05$) from those fed a maize-soy diet, but the observed value was lower ($P < 0.05$) than those fed diets which contained both lupin species and peas.

Table 5.2. The effect of dietary legumes on the performance and digestive tract development of broilers

	Maize-soy control diet	Faba beans			*Australian sweet lupins*		*White lupins*			Peas					Pooled SEM
		PGG Tic	Spec Tic	South Tic	Broad	Wallan	Tanjil	Borre	Promore	Kiev mutant	Ultra	Santana	Miami	Courier	Rex	
Performance[1]																
Weight gain (g/bird)	933	973	978	967	948	940	961	948	963	973	911	952	962	888	952	21.56
Feed intake (g/bird)	1240^{bc}	1270^{abc}	1247^{abc}	1248^{abc}	1254^{abc}	1308^{ab}	1287^{ab}	1284^{ab}	1327^{a}	1320^{ab}	1265^{abc}	1290^{ab}	1261^{bbc}	1185^{c}	1252^{abc}	30.11
Feed per gain (g/g)	1.328^{cde}	1.306^{def}	1.275^{f}	1.291^{ef}	1.325^{def}	1.392^{a}	1.341^{abcde}	1.354^{abcd}	1.379^{abc}	1.358^{abcd}	1.388^{ab}	1.356^{abcd}	1.312^{def}	1.336^{bcde}	1.315^{def}	0.019
Excreta score	2.94^{ab}	1.63^{ef}	1.56^{f}	1.75^{ef}	1.81^{ef}	2.94^{cde}	2.25^{bcd}	2.50^{abc}	2.75^{abc}	2.94^{ab}	2.75^{abc}	3.19^{a}	3.13^{ab}	1.86^{def}	2.69^{abc}	0.23
Mortality (%)	9.4^{a}	3.1^{ab}	3.1^{ab}	0.0^{b}	0.0^{b}	0.0^{b}	3.1^{ab}	3.1^{ab}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	3.1b^{ab}	2.38
Relative organ weights (g/kg BW)[2]																
Liver	26.8^{fg}	27.2^{efg}	26.6^{fg}	27.5^{defg}	27.3^{efg}	28.9^{bcde}	29.6^{bc}	29.3^{bcd}	30.2^{b}	29.4^{bc}	32.2^{a}	26.7f^{g}	26.1^{g}	28.1^{cdef}	27.1^{efg}	0.66
Spleen	0.967	0.942	1.129	0.942	0.892	0.817	0.917	0.908	0.850	0.958	0.883	0.990	0.983	0.900	0.817	0.07
Pancreas	3.10^{abc}	3.37^{a}	3.23^{ab}	3.24^{ab}	3.34^{a}	2.80^{cd}	2.94^{cd}	3.96^{bcd}	2.90^{cd}	2.67^{d}	2.86^{cd}	2.90^{cd}	3.00^{bc}	2.99^{bc}	2.80^{cd}	0.11
Relative empty weights (g/kg BW)[2]																
Crop	2.66^{bc}	2.81^{bc}	2.82^{bc}	2.65^{bc}	2.60^{bc}	2.96^{ab}	2.87^{bc}	2.91^{abc}	2.62^{bc}	2.76^{bc}	2.94^{abc}	2.56^{bc}	2.52^{c}	3.42^{a}	2.78^{bc}	0.15
Proventriculus	4.53^{bcde}	4.35^{cde}	5.13^{abc}	4.33^{cde}	4.77^{abcd}	5.12^{abc}	4.90^{abcd}	5.49^{ab}	4.22^{cde}	3.59^{e}	4.96^{abc}	3.90^{de}	3.70^{e}	5.92^{a}	5.07^{abc}	0.37
Gizzard	10.3^{de}	11.5^{cd}	12.2^{bc}	12.8^{ab}	14.0^{a}	11.26^{cd}	11.50^{bcd}	12.21^{bc}	11.00^{cd}	10.32^{de}	11.53^{bcd}	9.11^{e}	9.03^{e}	10.74^{d}	10.70^{d}	0.50
Duodenum	6.57^{cde}	6.88^{abcd}	6.18^{de}	6.04^{e}	6.15^{de}	7.40^{a}	7.25^{ab}	6.44^{cde}	6.93^{abcd}	6.99^{abc}	7.54^{a}	6.30^{cde}	6.63^{bcde}	6.55^{bcde}	6.26^{cde}	0.28
Jejunum	10.3^{def}	11.1^{abcde}	9.6^{f}	10.1^{ef}	10.2^{ef}	11.8^{a}	11.5^{abc}	10.9^{abcde}	11.7^{ab}	11.3^{abcd}	11.9^{a}	10.7^{bcde}	11.2^{abcd}	10.9^{abcde}	10.5^{cdef}	0.34
Ileum	7.51^{bc}	8.18^{ab}	7.02^{c}	7.22^{c}	7.21^{c}	7.62^{abc}	8.02^{ab}	8.06^{ab}	8.38^{a}	8.04^{ab}	8.15^{ab}	7.73^{abc}	8.04^{ab}	8.24^{ab}	7.64^{abc}	0.28
Small intestine[3]	24.4^{defg}	26.1^{abcd}	22.7^{g}	23.4^{fg}	23.6^{fg}	26.8^{abc}	26.8^{abc}	25.5^{abcdef}	27.0^{ab}	26.3^{abcd}	27.6^{a}	24.7^{bcdef}	25.9^{abcde}	25.7^{abcdef}	24.4^{cdefg}	0.84
Caeca	1.28^{d}	1.31^{d}	1.31^{d}	1.35^{d}	1.27^{d}	1.42^{abcd}	1.37^{abcd}	1.41^{abcd}	1.53^{a}	1.52^{ab}	1.52^{ab}	1.39^{abcd}	1.36^{bcd}	1.50^{abc}	1.29^{d}	0.07

Relative digesta contents (g/kg BW)[2]																
Crop	2.59^{def}	2.52^{ef}	3.26^{cde}	3.61^{bc}	5.24^{a}	3.24^{cde}	4.50^{ab}	1.89^{f}	1.95^{f}	3.58^{bcd}	3.97^{bc}	2.40^{ef}	3.95^{bc}	3.94^{bc}	5.45^{a}	0.61
Proventriculus	1.99^{fg}	3.96^{abc}	4.11^{ab}	4.23^{ab}	3.78^{bcd}	4.39^{ab}	2.70^{a}	2.69^{ef}	2.99^{de}	2.41^{efg}	2.42^{efg}	1.68^{g}	1.61^{g}	2.72^{ef}	2.89^{cde}	0.44
Gizzard	3.56^{ef}	7.21^{b}	8.61^{b}	8.16^{b}	10.86^{a}	7.56^{b}	7.17^{a}	5.76^{a}	5.86^{c}	4.94^{cd}	5.60^{c}	4.36^{de}	2.78^{f}	3.76^{ef}	2.85^{ef}	0.77
Duodenum	6.51^{d}	7.29^{bcd}	6.82^{cd}	6.84^{cd}	6.52^{d}	6.93^{abcd}	8.03^{ab}	7.12^{bcd}	7.77^{abc}	8.46^{a}	7.07^{bcd}	7.49^{abcd}	7.49^{abcd}	6.79^{bcd}	6.75^{bcd}	0.41
Jejunum	20.4^{e}	22.8^{bcde}	22.4^{cde}	22.8^{bcde}	22.5^{cde}	23.4^{bcde}	25.5^{abc}	26.9^{a}	25.3^{abc}	26.7^{a}	25.9^{ab}	24.1^{abcd}	22.6^{bcde}	21.5^{de}	24.2^{abcd}	1.17
Ileum	18.4	18.6	18.3	18.2	20.5	20.7	22.2	19.8	21.6	20.5	21.5	18.5	19.5	19.2	19.1	1.29
Small intestine[3]	45.3^{d}	48.7^{cd}	47.5^{d}	47. 8^{d}	49.5^{bcd}	51.0^{abcd}	55.7^{a}	53.8^{abc}	54.7^{ab}	55.7^{a}	54.5^{abc}	50.1^{abcd}	49.6^{bcd}	47.5^{d}	50.1^{abcd}	2.11
Caeca	1.69	1.58	1.70	1.93	1.89	1.87	1.76	1.79	2.38	2.04	2.05	1.95	1.85	2.28	1.83	0.200
Relative length (cm/kg BW)[2]																
Duodenum	27.0^{de}	28.0^{abcde}	27.4^{cde}	27.6^{cde}	24.5^{f}	28.7^{abc}	27.1^{de}	27.1^{de}	27.8^{abcde}	28.2^{abcd}	29.2^{a}	26.6^{e}	28.7^{abc}	29.1^{ab}	27.6^{bcde}	0.53
Jejunum	61.6^{cdefg}	59.7^{fg}	58.5^{g}	62.6^{bcdefg}	59.8^{efg}	67.1^{ab}	65.1^{abcd}	64.4^{abcde}	63.4^{abcdef}	64.3^{abcdef}	66.1^{abc}	61.1^{defg}	63.0^{abcdefg}	67.3^{a}	64.0^{abcdef}	1.67
Ileum	68.4^{bcd}	67.0^{bcd}	65.6^{d}	67.6^{bcd}	67.0^{bcd}	71.2^{abc}	69.6^{bcd}	69.9^{bcd}	68.0^{bcd}	69.1^{bcd}	72.1^{ab}	66.6^{cd}	68.1^{bcd}	75.2^{a}	69.8^{bcd}	1.85
Small intestine[3]	157^{cdef}	155^{def}	152^{ef}	158^{bcdef}	151^{f}	167^{abc}	162^{bcd}	161^{bcdef}	159^{bcdef}	162^{bcde}	167^{ab}	154^{def}	160^{bcdef}	172^{a}	161^{bcde}	3.57
Caeca	15.0^{cd}	14.5^{d}	15.1^{cd}	15.2^{cd}	15.1^{cd}	17.0^{a}	15.9^{abc}	15.3^{cd}	15.9^{abc}	16.8^{ab}	17.2^{ab}	16.0^{abc}	15.3^{cd}	17.2^{a}	15.6^{bcd}	0.48

^{a,b,c,d,e,f,g} Means in a row with different superscripts differ ($P < 0.05$).

[1]Each value represents the mean of 4 replicates (8 birds/replicate).

[2]Each value represents the mean of 12 birds.

[3] Small intestine = duodenum + jejunum + ileum.

Table 5.3. Comparison of the performance and digestive tract development of birds fed grain legume diets and maize-soybean meal diet

	Maize-soy control diet[1]	Faba beans[2]	Australian sweet lupin[3]	White lupins[4]	Peas[5]	Pooled SEM
Performance[6]						
Weight gain (g/bird)	933	973	950	949	939	14.06
Feed intake (g/bird) (P=0.053)	1240^{b}	1255^{ab}	1293^{ab}	1304^{a}	1247^{b}	18.96
Feed per gain (g/g)	1.328^{bc}	1.299^{c}	1.362^{ab}	1.375^{a}	1.330^{bc}	0.012
Excreta score	2.94^{a}	1.69^{b}	2.54^{a}	2.81^{a}	2.72^{a}	0.163
Mortality (%)	9.4^{a}	1.6^{b}	2.1^{b}	0.0^{b}	0.8^{b}	1.42
Relative organ weights (g/kg BW)[7]						
Liver	26.8^{c}	27.2^{c}	26.6^{b}	27.5^{a}	27.3^{c}	0.43
Spleen	0.967	0.977	0.880	0.894	0.927	0.04
Pancreas	3.10^{ab}	3.37^{a}	3.23^{c}	3.24^{c}	3.34^{bc}	0.07
Relative empty weights (g/kg BW)[7]						
Crop	2.66	2.71	2.90	2.77	2.79	0.09
Proventriculus	4.53	4.65	5.17	4.26	4.58	0.24
Gizzard	10.3^{cd}	12.6^{a}	11.7^{b}	10.9^{bc}	9.9^{d}	0.33
Duodenum	6.57^{b}	6.31^{b}	7.04^{a}	7.16^{a}	6.43^{b}	0.18
Jejunum	10.3^{c}	10.2^{c}	11.4^{ab}	11.6^{a}	10.9^{bc}	0.22
Ileum	7.51^{b}	7.41^{b}	7.89^{ab}	8.19^{a}	7.91^{bc}	0.18
Small intestine[8]	24.4^{c}	23.9^{c}	26.3^{ab}	27.0^{a}	25.2^{bc}	0.54
Caeca	1.28^{c}	1.32^{bc}	1.40^{b}	1.52^{a}	1.39^{b}	0.04
Relative digesta contents (g/kg BW)[7]						
Crop	2.59^{b}	3.66^{a}	2.67^{b}	3.16^{ab}	3.89^{a}	0.27
Proventriculus	1.99^{b}	4.05^{a}	3.99^{a}	2.61^{b}	2.29^{b}	0.23
Gizzard	3.56^{c}	8.60^{a}	8.27^{a}	5.47^{b}	3.53^{c}	0.35
Duodenum	6.51^{c}	6.87^{bc}	7.52^{ab}	7.77^{a}	7.13^{abc}	0.26
Jejunum	20.4^{c}	22.7^{b}	25.3^{a}	26.0^{a}	23.1^{b}	0.74
Ileum	18.4^{b}	18.9^{ab}	20.9^{a}	21.2^{a}	19.1^{ab}	0.80
Small intestine[8]	45.3^{c}	40.4^{bc}	53.5^{d}	55.0^{a}	49.3^{b}	1.31
Caeca (P=0.0564)	1.69^{b}	1.76^{b}	1.81^{ab}	2.16^{a}	1.98^{ab}	0.13
Relative length (cm/kg BW)[7]						
Duodenum	27.0^{c}	26.9^{c}	27.6^{abc}	28.4^{a}	27.9^{ab}	0.37
Jejunum	61.6^{bc}	60.1^{c}	65.5^{a}	64.6^{ab}	63.8^{ab}	1.07
Ileum	68.4	66.8	70.2	69.7	69.9	1.90
Small intestine[8]	157^{ab}	154^{b}	163^{a}	162^{a}	162^{a}	2.31
Caeca	15.0^{b}	14.9^{b}	16.1^{a}	16.6^{a}	16.1^{a}	0.31

^{a,b,c,d}Means in a row with different superscripts differ (P < 0.05).

[1]Mean of 4 replicates (8 birds per replicate)
[2]Mean of 4 cultivars (4 replicates per cultivar)
[3]Mean of 3 cultivars (4 replicates per cultivar)
[4]Mean of 3 cultivars (4 replicates cultivar)
5Mean of 4 cultivars (4 replicates per cultivar)
[6] Each value represents 8 birds per replicate
[7]Each value represents 12 birds per replicate
[8] Small intestine = duodenum + jejunum + ileum.

5.5. Discussion

In general, the results showed that feeding diets containing 200 g/kg faba beans, Australian sweet lupins and peas had no deleterious effects on the performance of broiler starters. These findings are consistent with published data reported by the earlier researchers (Brenes *et al*., 1989; Bekrić *et al*., 1990; Farrell *et al*., 1999; Castell *et al*., 1996; Olver and Jonker, 1997; Perez-Maldonado, 1997; Bennet, 2002). Farrell *et al*. (1999) who showed that feeding 200 g/kg faba bean to broilers grown to 21 d of age gave good growth response and feed efficiency. In their study, the weight gain and feed per gain were found to improve with increasing inclusion levels of faba beans. In a study by Metayer *et al*. (2003), it was reported that diets which contained 200 and 250 g/kg of faba beans (cv Gloria and Divine) supported good live weight and feed per gain of broilers over trial periods of 14, 35 and 56 days. During the first 14 days, the live weight of birds fed faba bean based-diets were higher than those fed the control diet with soybean meal. Over 35 days, the feed efficiency of birds fed diets which contained faba beans surpassed that of the control diet.

In contrast, other studies (Farrell *et al*., 1999; Steenfeldt *et al*., 2003; Viveros *et al*., 2007) have shown that the use of 200 g/kg Australian sweet and white lupins reduced the growth rate and feed efficiency of broiler starters. This discrepancy may be explained by differences in diet formulation. In the present study, the experimental diets were formulated to contain similar levels of energy and digestible amino acids, whilst in the study by other researchers (Farrell *et al*., 1999; Viveros *et al*., 2007), the diets were formulated on the basis of total amino acids. In the study of Steenfeldt *et al*. (2003), the experimental diets were not balanced in regards to energy or amino acids.

Poor growth rate and feed efficiency of broilers fed diets with 200 g/kg peas was reported by McNeill *et al*. (2004). In the study by Li *et al*. (2006), good body weight and feed efficiency of broiler starters was observed at an inclusion level of 100 g/kg peas, but performance was adversely affected at the inclusion level of 250 g/kg peas. In their study, the diets were not balanced for metabolisable energy and digestible amino acids. The basis of

the diet formulation appears to be a major reason for the inconsistency in optimum inclusion levels reported in the literature.

The improvements in excreta quality in birds fed diets containing faba beans and Courier cultivar of peas were noteworthy. This may be, in part, related to the low NSP content in these legume seeds (Table 4.2). However, it is interesting to note that even though peas had lower NSP content than faba beans (130-183 g/kg DM vs. 199-263 g/kg DM, Table 4.2), the excreta quality of faba beans was more dry and friable than peas, except for the courier cultivar. Poor excreta quality would be a major concern when lupins and peas are used at 200 g/kg level. In this context, the exogenous NSP enzymes may have a potential role.

The higher relative empty weight of gizzard, duodenum, jejunum, ileum and small intestine in birds fed diets containing of faba bean and both lupin cultivars may be related, at least in part, to the effects of dietary NSP. A similar reason may be responsible for the increase in the relative caeca empty weight of birds fed diets containing white lupin cultivars. Jørgensen *et al.* (1996) showed that feeding high fibre diets increased visceral organ mass and intestinal length relative to empty body weight compared with those birds fed low fibre diets.

Choct (1997) reported that the negative effects of non-starch polysaccharides were associated with the physiological and morphological effects on the digestive tract due to their viscous nature and the interaction with gut microflora. In the review by Gabriel *et al.* (2006), it was reported that the interaction of bacteria with the intestinal mucosa and the production of various metabolites such as short- chain fatty acids (SCFA) and polyamines results in anatomical and physiological changes in the digestive tracts. It was also stated that, in the caeca, the presence of microorganisms induces a higher relative weight and a thicker wall.

It is noteworthy that birds fed diets which contained the majority of faba bean, sweet lupin, white lupin and pea cultivars had similar relative length of duodenum, jejunum, ileum and small intestine to those fed the maize-soy diet. This implied that the increase in the relative empty weight of these digestive organs was likely due to the enlargement in the size of intestinal organ mass (a thicker wall).

The higher relative digesta content in some segments of digestive tract of birds fed grain legumes may be explained by the increase in gut viscosity caused by dietary soluble NSP which could lead to lower gastric emptying rate of solids and liquids, and transit time in the small intestine. However, this effect had no negative effect on the weight gain of birds.

5.6. Conclusions

In conclusion, the present data shows that when diets are properly balanced in terms of available energy and digestible amino acids, faba beans, lupins and peas can be included at a level of 200 g/kg in broiler starter diets, with no detrimental effect on performance. However, excreta quality was poorer in diets containing lupins and peas and this may limit the use of these legumes in practical diets. The changes observed in some segments of the digestive tract of birds fed grain legumes may be related, at least in part, to the effects of dietary NSP on digesta viscosity and the interaction with gut microflora. It is likely that excreta quality may be improved by supplementation of diets containing lupins and peas with NSP degrading enzymes. On the other hand, the dry and friable excreta quality observed in birds fed faba bean diets is an added advantage and this should encourage the commercial use of this legume in broiler diets.

CHAPTER 6

Influence of feeding diets with grain legumes on the performance and digestive tract development of broilers housed in floor pens

6.1 Abstract

The aim of the present study was to evaluate the effect of the inclusion of faba beans, white lupins, and peas in two different basal diets on the performances and gross morphology of the gastrointestinal tract of broilers housed in floor pens over a 35 d grow-out period. The experimental design was a 2 x 4 factorial arrangement of treatments which evaluated two basal wheat-soy diets (with or without meat meal) and legume grains (no legume grains, or faba beans, white lupins and peas at 200 g/kg inclusion). All diets were formulated to contain similar levels of metabolisable energy and digestible amino acids. Each of the eight diets was fed *ad libitum* to six pens of 30 male broilers from day 1 to 35 posthatch. A 3-phase feeding programme (starter, grower and finisher) was employed. The starter, grower and finisher diets were offered from day 1 to 7, 8 to 21 and 22 to 35, respectively. During the starter period, legume x meat meal interaction was significant ($P < 0.05$) for weight gain and feed intake. Birds fed faba bean and white lupin diets containing meat meal had a higher ($P < 0.05$) weight gain and feed intake than those without meat meal. During day 1 to 21, an interaction ($P < 0.05$) between legumes and meat meal was observed for weight gain, with the gain of birds fed the pea diet without meat meal being higher ($P < 0.05$) that than those fed pea diet with meat meal. Over the 35 d trial period, with the exception of feed intake, legumes had no effect ($P > 0.05$) on performance, carcass recovery and litter score. Weight gain and feed per gain of birds fed diets without meat meal were better ($P < 0.05$) than those with meat meal. Legume x meat meal interaction was not significant ($P > 0.05$) in any performance traits. The main effects of legumes were significant ($P < 0.01$ to 0.05) for the relative weights of liver and gizzard, and the relative digesta weight of the crop and proventriculus. Birds fed meat meal diets had lower ($P < 0.05$) relative weights of liver, pancreas and small intestine and relative digesta weight of small intestine than those fed diets with no meat meal diets. No interaction ($P > 0.05$) was found for any digestive tract traits, except for the relative digesta weight in the crop. It was concluded that the dietary inclusion of grain legumes at 200 g/kg either in wheat-soybean meal or wheat-soybean meal-meat meal basal diets could support a good performance of birds over the 35-day grow out period.

6.2. Introduction

In the study reported in Chapter 5, the effects of diets containing different cultivars of faba bean, peas and white lupins on the performance, development of digestive tract, and excreta score of broiler starters housed in cages were examined in a 21-day feeding trial. The major finding of this experiment is that grain legumes can be included at a level of 200 g/kg in broiler starter diets without deleterious effect on performance when diets are properly balanced in terms of available energy and digestible amino acids. Nevertheles, excreta quality was poorer in diets containing lupins and peas.

The aim of the present study was to examine the effect of feeding diets which contained 200 g/kg faba beans, white lupins and peas on the performance, digestive tract traits and carcass characteristics of broilers housed in floor pens over a 35 day grow-out period. The legumes were incorporated either in a wheat-soybean meal or a wheat-soybean meal-meat meal diet.

6.3. Materials and methods

All experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC 05/20 and 05/21) and was in accordance with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

6.3.1. Ingredients: The feed ingredients included faba beans, white lupins and peas. Before incorporation into test diets, legume seeds with hulls were ground in a hammer mill to pass through 3 mm sieve.

6.3.2. Birds and housing: A total of 1440 day-old male broilers (Ross 308), obtained from commercial hatchery were assigned on the basis of body weight to the 48 floor pens (30 birds/pen) on sawdust litter in an environmentally controlled room. The temperature was maintained at 31°C during the first week and then it was gradually reduced to 22°C at 35 days of age. The birds received constant fluorescent illumination and, were allowed free access to the diets and water.

6.3.3. Diets: The study was conducted as a 2 x 4 factorial arrangement of treatments consisting of .two basal diets (with or without meat meal) and legumes (no legume, or faba beans, white lupins and peas at 200 g/kg inclusion. A total of eight diets were formulated. All diets were formulated to contain similar levels of metabolisable energy and digestible lysine, methionine and threonine (Tables 6.1 to 6.3). The determined values of AME and digestible amino acid for faba beans used to formulate the diet were taken from the values of PGG Tic

cultivar, whilst for white lupin and peas, the values were taken from the average value of three cultivars of white lupin and four cultivars of peas (see chapter 4), respectively. The zinc bacitracin and enzymes were used in this experiment in order to eliminate the viscosity effect of soluble NSP of wheat. The diets were cold pelleted (70°C) and each of the eight dietary treatments was randomly assigned to six pens. A 3-phase feeding programme (starter, grower and finisher) was employed. The starter, grower and finisher diets were offered from day 1 to 7, 8 to 21 and 22 to 35, respectively.

Table 6.1. Composition (g/kg as is) of treatment diets for starter (day 1 to 7)

	Wheat-soy				Wheat-soy-meat meal			
	Control	Faba beans	White lupins	Peas	Control	Faba beans	White lupins	Peas
Wheat	546	453	460	416	550	504	523	492
Soybean meal	342	281	265	312	271	168	144	179
Meat meal	-	-	-	-	100	100	100	100
Legume	-	150	150	150	-	150	150	150
Fishmeal	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Soybean oil	22.4	26.9	35.4	33.1	12.6	12.6	18.9	15.4
L-lysine	0.9	0.9	0.9	-	-	1.1	1.4	0.3
DL-methionine	2.5	3.0	2.8	2.8	2.1	2.9	2.8	2.8
L-threonine	0.3	0.7	0.1	0.2	-	0.8	0.3	0.5
Limestone	11.4	11.3	9.9	10.4	3.0	2.2	-	-
Dicalcium phosphate	15.7	15.7	17.3	16.9	4.0	1.5	3.2	2.8
Salt	0.6	0.6	0.6	1.0	0.8	0.4	0.2	0.6
Sodium bicarbonate	2.9	2.5	2.7	2.3	0.9	1.2	1.6	1.1
Trace mineral-vitamin premix[1]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Zinc bacitracin	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Xylanase[2]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Calculated analysis								
AME, MJ/kg	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9
Crude protein	265	265	265	265	284	272	269	265
Digestible lysine	13.5	13.5	13.5	14.0	13.6	13.5	13.5	13.5
Digestible methionine	3.7	3.5	3.5	3.6	4.0	3.7	3.7	3.8
Digestible met + cys	7.1	6.7	6.9	6.9	7.3	6.7	6.9	6.8
Digestible threonine	7.1	7.1	6.6	7.1	6.8	6.8	6.2	6.8
Calcium	10.0	10.0	10.0	10.0	11.4	10.6	10.3	10.1
Available phosphorus	4.8	4.8	4.8	4.8	5.2	4.8	4.8	4.8
Sodium	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Potassium	9.2	9.4	8.7	9.3	8.2	4.8	6.9	7.4
Chloride	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6

[1] Please refer to Table 4.1.
[2]Kemzyme, Kemin (Asia) Pte Ltd, Singapore.

Table 6.2. Composition (g/kg as is) of treatment diets for grower (day 8 to 21)

	Wheat-soy				Wheat-soy-meat meal			
	Control	Faba beans	White lupins	Peas	Control	Faba beans	White lupins	Peas
Wheat	611	487	496	437	684	521	545	503
Soybean meal	298	216	196	260	184	136	97.3	146
Meat meal	-	-	-	-	85.0	85.0	85.0	85.0
Legume	-	200	200	200	-	200	200	200
Tallow	35.0	35.0	35.0	35.0	13.4	26.4	35.0	34.3
Soybean oil	10.3	16.4	27.7	24.8	10.0	10.0	13.9	10.0
L-lysine	1.7	1.7	1.7	-	2.5	1.5	2.0	0.5
DL-methionine	2.2	2.9	2.6	2.6	2.2	2.6	2.5	2.6
L-threonine	0.6	1.1	0.2	0.3	0.9	1.0	0.4	0.7
Limestone	11.9	11.7	9.9	10.5	2.9	2.7	0.8	1.5
Dicalcium phosphate	19.8	19.8	22.0	21.4	7.7	7.6	9.9	9.4
Salt	1.0	1.1	1.1	1.7	0.6	1.1	0.8	1.4
Sodium bicarbonate	3.7	3.2	3.5	2.7	3.0	1.8	2.5	1.7
Trace mineral-vitamin premix[1]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Zinc bacitracin	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Xylanase[2]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Calculated analysis								
AME, MJ/kg	12.1	12.1	12.1	12.1	12.1	12.1	12.1	12.1
Crude protein	220	220	220	220	221	231	225	220
Digestible lysine	11.0	11.0	11.0	11.2	11.0	11.0	11.0	11.0
Digestible methionine	2.9	2.7	2.7	2.8	3.0	2.9	2.8	3.0
Digestible met + cys	6.0	5.5	5.8	5.8	6.0	5.6	5.8	5.7
Digestible threonine	6.7	6.8	6.0	6.8	6.4	6.5	5.7	6.5
Calcium	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Available phosphorus	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Sodium	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Potassium	8.1	8.4	7.4	8.4	6.4	7.3	6.2	6.6
Chloride	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6

[1]Please refer to Table 4.1.
[2]Kemzyme, Kemin (Asia) Pte Ltd, Singapore.

Table 6.3. Composition (g/kg as is) of treatment diets for finisher (day 22 to 35)

	Wheat-soy				Wheat-soy-meat meal			
	Control	Faba bean	Lupin	Peas	Control	Faba bean	Lupin	Peas
Wheat	618	493	503	445	687	550	569	510
Soybean meal	289	207	187	249	174	102	72.8	136
Meat meal	-	-	-	-	85.0	85.0	85.0	85.0
Legume	-	200	200	200	-	200	200	200
Tallow	35.0	35.0	35.0	35	27.7	35.0	35.0	35.0
Soybean oil	15.3	21.4	32.7	29.5	5.0	6.8	17.3	14.4
L-lysine	0.7	0.7	0.7	-	1.5	1.2	1.5	-
DL-methionine	1.7	2.4	2.1	2.1	1.7	2.3	2.1	2.1
L-threonine	0.3	0.8	-	0.05	0.6	1.0	0.2	0.3
Limestone	12.4	12.2	10.4	11.0	3.3	3.1	1.3	1.9
Dicalcium phosphate	19.1	19.2	21.3	20.8	7.1	7.1	9.3	8.8
Salt	2.3	2.4	2.3	2.6	1.9	2.1	1.9	2.3
Sodium bicarbonate	1.5	0.9	1.3	0.9	0.7	0.01	0.5	-
Trace mineral-vitamin premix[1]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Zinc bacitracin	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Xylanase[2]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Calculated analysis								
AME, MJ/kg	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Crude protein	215	215	215	215	215	218	215	215
Digestible lysine	10.0	10.0	10.0	10.9	10.0	10.0	10.0	10.4
Digestible methionine	3.1	2.9	2.8	3.0	2.9	2.8	2.7	2.9
Digestible met + cys	6.3	5.8	6.0	6.0	5.8	5.4	5.6	5.6
Digestible threonine	6.7	6.8	6.1	6.7	6.0	6.1	5.3	6.1
Calcium	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Available phosphorus	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Sodium	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Potassium	8.0	8.3	7.3	8.1	6.2	6.7	5.6	6.5
Chloride	2.1	2.1	2.1	2.0	2.1	2.1	2.1	2.0

[1]Provided per kg diet: Co, 0.3 mg; Cu, 15 mg; Fe, 60 mg; I, 1 mg; Mn, 120 mg; Zn, 100 mg; choline chloride, 320 mg; trans-retinol, 0.009 mIU; cholecalciferol, 0.004 mIU; dl-α-tocopheryl acetate, 50 mg; menadione, 4 mg; thiamin, 1.5 mg; riboflavin, 6 mg; niacin, 25 mg; calcium panthothenate, 8.6 mg; pyridoxine, 6 mg; cyanocobalalamin, 16 mg; folic acid 3 mg; biotin, 0.15 mg; molybdenum, 0.5 mg; selenium, 0.4 mg.
[2]Kemzyme, Kemin (Asia) Pte Ltd, Singapore.

6.3.4. Measurements: Body weights and feed intake were recorded on a pen basis on days 7, 21 and 35, and feed per gain values were calculated. Litter was scored for quality on days 21 and 35 for quality on a scale of 1 to 5 (1 = normal, dry, friable litter and 5 = representing wet

and cakey litter) based on visual observation. On day 35, the two birds closest to the mean pen weight were selected from each replicate, weighed and sacrificed by cervical dislocation in order to determine relative digestive tract size and carcass recovery. Digestive tract measurements were made as described in Chapter 5 section 5.3.4. Carcass recovery was determined using the carcass weight as a proportion of body weight. Carcass weight measurements were undertaken after defeathering and removal of feet, head and viscera. Dressing percentage was calculated by dividing the carcass weight by the live body weight.

6.3.5. Statistical analysis: The pen means were used to derive performance data. For digestive tract and carcass measurements, individual birds were considered as the experimental units. The data were analysed by a two-way analysis of variance using the General Linear Model procedure of SAS (1997). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Fisher's Least Significant Difference Test.

6.4. Results

During the starter period, the main effects of meat meals and legumes were not significant ($P > 0.05$) for performance parameters. However, a legume x meat meal interaction ($P < 0.001$) was observed for the weight gain and feed intake. The weight gain and feed intake of birds fed faba bean and white lupin-based diets containing meat meal were higher ($P < 0.05$) than those of faba bean- and lupin- based diets without meat meal. On the other hand, the weight gain and feed intake of birds fed pea diets supplemented with meat meal was comparable ($P > 0.05$) to that of pea diet without meat meal. The basal diet without meat meal resulted in a better ($P < 0.05$) performance compared to that with meat meal. Feed per gain of birds during the starter phase was unaffected ($P > 0.05$) by dietary treatments.

Table 6.4. Performance of broilers as influenced by legume grains and meat meal inclusion, 1-7 days post-hatching[1]

Legumes	Meat meal	Weight gain (g/bird)	Feed intake (g/bird)	Feed per gain (g/g)
No legume	-	185[a]	174[a]	0.945
	+	178[bc]	170[abc]	0.960
Faba beans	-	178[bc]	166[b]	0.935
	+	186[a]	177[a]	0.949
White lupins	-	174[b]	165[b]	0.948
	+	181[ac]	172[ac]	0.953
Peas	-	183[ac]	172[ac]	0.941
	+	177[bc]	168[bc]	0.948
SEM[2]		2.38	2.10	0.007
Main effects				
Legume				
No legume		182	173	0.953
Faba beans		182	171	0.942
White lupins		178	169	0.951
Peas		181	170	0.944
Meat meal				
-		180	169	0.942
+		181	172	0.952
Probabilities, P <				
Legume		NS	NS	NS
Meat meal		NS	NS	NS
Legume x Meat meal		***	***	NS

[a,b,c]Means in a column with different superscripts differ (P < 0.05).
NS, not significant; ***, P < 0.001.
[1]Each value represents the mean of six replicates (30 birds/replicate).
[2]Pooled standard error of mean.

During 1-21 day post-hatch, the main effect of legumes was found to be significant (P < 0.001) for weight gain, but there was an interaction (P < 0.001) between legumes and meat meal (Table 6.5). Feeding birds with the pea-based diet without meat meal produced better (P < 0.05) weight gain than those fed the pea-based diet containing meat meal. Birds fed legume diets, with the exception for the white lupin, had similar (P > 0.05) weight gain to those fed diets without legumes. Inclusion of meat meal had no effect (P > 0.05) on weight gains and

feed intake, but it influenced (P < 0.05) feed per gain. Diets without meat meal produced lower (P < 0.05) feed per gain than those with meat meal. The litter score of birds fed white lupin diet was lower (P < 0.05) than those fed diets with no legume diet and faba bean, but comparable (P > 0.05) to those fed the pea diets. Meat meal had no effect (P > 0.05) on the litter score. Legume x meat meal interaction was not significant (P > 0.05) for litter scores.

Table 6.5. Performance of broilers as influenced by legume grains and meat meal inclusion, 1-21 days post-hatching[1]

Legumes	Meat meal	Weight gain (g/bird)	Feed intake (g/bird)	Feed per gain (g/g)	Litter score
No legume	-	1095[b]	1443	1.322	1.58
	+	1094[b]	1450	1.328	1.42
Faba beans	-	1100[bc]	1430	1.304	1.75
	+	1133[ac]	1478	1.311	1.50
White lupins	-	1150[a]	1469	1.291	1.25
	+	1155[a]	1506	1.315	1.25
Peas	-	1131[a]	1464	1.296	1.58
	+	1091[b]	1456	1.338	1.33
SEM[2]		11.6	17.4	0.010	0.113
Main effects					
Legume					
No legume		1094[b]	1447	1.325	1.50[a]
Faba beans		1117[b]	1455	1.317	1.62[a]
White lupins		1153[a]	1488	1.302	1.25[b]
Peas		1111[b]	1460	1.317	1.46[ab]
Meat meal					
-		1119	1452	1.303[b]	1.54
+		1118	1473	1.323[a]	1.42
Probabilities, P <					
Legume		***	NS	NS	**
Meat meal		NS	NS	*	NS
Legumes x Meat meal		*	NS	NS	NS

[a,b,c] Means in a column with different superscripts differ (P < 0.05).
NS, not significant; **, P < 0.01; *, P < 0.05.
[1]Each value represents the mean of six replicates (30 birds/replicate).
[2]Pooled standard error of mean.

Over the 35-day trial period, legumes had no effect (P > 0.05) on the weight gain and feed per gain of broilers (Table 6.6). The feed intake of birds fed the white lupin diet was higher (P < 0.05) than those fed diets with no legumes, faba beans and peas. Weight gain and feed per gain of birds fed diets without meat meal were better (P < 0.05) than those broilers fed diets with no meat meal. Dietary treatments had no effect (P > 0.05) on litter scores and carcass recovery. Legume x meat meal interaction was not significant (P > 0.05) in any performance traits, litter score and on carcass recovery.

Table 6.6. Performance of broilers as influenced by legume grains and meat meal inclusion, 35 d post-hatching[1]

Legumes	Meat meal	Weight gain (g)	Feed intake (g)	Feed per gain (g/g)	Litter score	Carcass recovery (%)
No legume	-	2459	3718	1.516	1.25	72.1
	+	2438	3738	1.538	1.67	73.3
Faba beans	-	2500	3687	1.497	1.83	72.5
	+	2431	3709	1.542	1.58	72.0
White lupins	-	2576	3861	1.523	1.50	71.9
	+	2495	3803	1.560	1.75	71.7
Peas	-	2548	3772	1.491	1.33	72.6
	+	2369	3694	1.582	1.33	73.0
SEM[2]		39.0	39.3	0.028	0.148	0.332
Main effects						
Legume						
No legume		2449	3728[b]	1.527	1.46	72.6
Faba beans		2466	3698[b]	1.520	1.71	72.2
White lupins		2536	3832[a]	1.542	1.62	71.8
Peas		2458	3733[b]	1.537	1.33	72.8
Meat meal						
-		2521[a]	3760	1.507[b]	1.48	72.2
+		2433[b]	3736	1.555[a]	1.58	72.5
Probabilities, P <						
Legumes		NS	*	NS	NS	NS
Meat meal		**	NS	*	NS	NS
Legumes x Meat meal		NS	NS	NS	NS	NS

[a,b,c] Means in a column with different superscripts differ (P < 0.05).
NS, not significant; **, P < 0.01; *, P < 0.05.
[1]Each value represents the mean of six replicates (30 birds/replicate).
[2]Pooled standard error of mean.

The effects of dietary treatments on the gross morphology of the digestive tract of broilers are presented in Table 6.7. In general, it was found that the dietary treatments had no any effects on the majority of gross morphology parameters. The main effects of legumes were found to be significant ($P < 0.01$ to 0.05) for the relative weights of liver and gizzard, and the relative digesta weight of the crop and proventriculus. The liver weight of birds fed legume diets (except for peas) had higher relative empty weight of gizzard than that of no legume diet.

The main effect of meat meal was observed to be significant ($P < 0.01$ to 0.05) for the relative weights of liver, pancreas and small intestine, and the relative digesta weight of the small intestine. Overall, birds fed meat meal diets had lower ($P < 0.05$) relative weights of liver, pancreas and small intestine and relative digesta weight of small intestine than those fed diets with no meat meal diets. Legume x meat meal interaction was not significant ($P > 0.05$) for any digestive tract traits, except for the relative digesta weight in the crop. Digesta weight in the crop of birds fed faba bean diets containing meat meal was higher ($P < 0.05$) than those fed faba bean diets without meat meal.

6.5. Discussion

The performance of birds fed diets containing faba beans, white lupins and peas during the 21 day grow-out period of the present floor pen trial confirms the results obtained in the cage trial reported in Chapter 5. The weight gain and feed efficiency obtained in both studies with birds fed diets containing 200 g/kg of grain legumes are in agreement with published data shown by the earlier researchers (Bekrić *et al*., 1990; Olver and Jonker, 1997; Perez-Maldonado, 1997; Bennet, 2002). However, as reported in Chapter 5, the deleterious effects due to the inclusion of lupins and peas at a level of 200 g/kg in broiler diets were also observed in some studies (Perez-Maldonado *et al*., 1999; McNeill et al., 2004; Viveros *et al*., 2007).

It was evident that during 7 d of the trial period, the weight gain of broilers fed the wheat-soy diet containing faba beans and white lupins was significantly lower than those fed wheat-soy-meat meal diet which contained these legume seeds. However no differences were observed in this parameter over the 21-day and 35-day feeding periods. The lower weight gain shown by young birds fed wheat-soy-meat-meal diet containing faba beans and white lupins was probably caused by the lower feed intake in this group compared to those fed wheat-soy diet which contained these legume seeds.

Table 6.7. Influence of dietary treatments on the digestive tract size of broilers [1]

	Legumes								Pooled SEM[4]	Main effects						Probability, P <		
	No legume		Faba beans		White lupins		Peas			Legumes				MM				
	-MM[2]	+MM	-MM	+MM	-MM	+MM	-MM	+MM		No legume	Faba beans	White lupins	Peas	-	+	Legumes	MM	MM x Legumes
Relative organ weight (g/kg BW)																		
Hearth	4.30	3.85	4.02	4.17	4.10	3.95	4.04	3.90	0.12	4.07	4.09	4.02	3.97	4.11	3.97	NS	NS	NS
Liver	19.9	18.3	18.7	18.9	21.3	20.4	20.8	18.6	0.60	19.1^{b}	18.8^{b}	20.8^{a}	19.7^{ab}	20.1^{a}	19.0^{b}	**	*	NS
Spleen	1.01	0.869	0.809	0.806	0.954	0.938	0.936	0.879	0.06	0.938	0.816	0.946	0.907	0.931	0.873	NS	NS	NS
Pancreas	1.46	1.25	1.47	1.44	1.40	1.31	1.41	1.33	0.05	1.37	1.43	1.35	1.35	1.44^{a}	1.31^{b}	NS	**	NS
Relative empty organ weight																		
Crop	1.93	1.80	2.01	2.00	2.04	2.11	1.87	1.74	0.10	1.87	2.00	2.07	1.87	1.96	1.95	NS	NS	NS
Proventriculus	2.01	1.90	1.94	1.91	1.90	1.93	2.02	1.67	0.10	1.96	1.92	1.91	1.88	1.94	1.89	NS	NS	NS
Gizzard	6.20	6.49	6.85	7.53	6.80	6.84	6.54	6.43	0.28	6.17^{b}	7.26^{a}	6.83^{a}	6.72^{ab}	6.57	6.93	**	NS	NS
Small Intestine[3]	13.3	12.2	13.2	12.2	13.7	12.3	12.8	11.6	0.42	12.7	12.6	13.0	12.7	13.2^{a}	12.3^{b}	NS	**	NS
Caeca	0.908	0.834	0.842	0.793	0.875	0.834	0.838	0.806	0.04	0.871	0.818	0.855	0.822	0.866	0.816	NS	NS	NS
Relative length (cm/kg BW)																		
Proventriculus	1.70	1.62	1.63	1.68	1.71	1.74	1.64	1.69	0.05	1.66	1.66	1.73	1.66	1.67	1.69	NS	NS	NS
Gizzard	1.85	1.85	1.80	1.87	1.90	1.90	1.82	1.89	0.05	1.85	1.83	1.90	1.86	1.84	1.87	NS	NS	NS
Small intestine	71.0	71.3	72.6	72.3	71.7	69.5	73.2	71.6	1.93	71.6	71.1	71.5	73.4	72.4	71.4	NS	NS	NS
Caeca	7.94	7.71	7.77	7.89	7.61	7.68	7.83	8.06	0.29	7.87	7.80	7.67	8.14	7.94	7.80	NS	NS	NS
Relative digesta content (g/kg BW)																		
Crop	4.75^{cd}	4.34^{cd}	3.73^{d}	9.83^{a}	6.85^{bc}	9.32^{ab}	4.53^{cd}	4.88^{cd}	1.33	5.50^{b}	7.55^{ab}	8.56^{a}	5.35^{b}	6.02	7.45	*	NS	**
Proventriculus	0.934	0.811	0.943	1.07	1.23	1.36	1.04	1.17	0.15	0.872^{b}	1.00^{ab}	1.30^{b}	1.02^{ab}	1.04	1.06	*	NS	NS
Gizzard	1.23	1.89	1.68	2.08	2.19	2.42	1.59	1.68	0.30	1.55	1.86	2.30	1.68	1.67	2.03	NS	NS	NS
Small Intestine	40.1	36.2	40.1	38.9	43.4	36.3	39.2	37.9	1.63	37.5	38.5	39.0	39.4	40.0^{a}	37.2^{b}	NS	**	NS
Caeca	1.85	1.76	1.89	1.98	1.67	1.88	1.65	1.48	0.19	1.81	1.76	1.77	1.66	1.78	1.72	NS	NS	NS

^{a,b,c,d} Means in a row with different superscripts differ ($P < 0.05$).

[1]Each value represents the mean of 12 birds

[2]MM = Meat Meal

[3]Small intestine = duodenum + jejunum + ileum.

[4]SEM = Pooled standard error of mean

No differences in weight gain were observed in birds over the 35-day trial period, indicating that the older birds had a higher tolerance, especially with respect to non-starch polysaccharides in the grain legumes (Table 4.2), than the younger birds.

Birds fed diets without meat meal gave better weight gain and feed per gain than those fed diets containing meat meal (Table 6.6). The lower performance of birds fed diets with meat meal may be reflective of the quality of meat meal used in the present study. The wide variability in the contents and digestibility of amino acids in meat meal is well documented (Ravindran *et al*., 2005). Thus the published digestible amino acid data used in the diet formulation may have overestimated the actual digestible amino acid values of the meat meal sample used in the present study.

The litter quality of the birds over 35-day period of the trial was not affected by dietary treatments. These results are somewhat different from those observed in the cage trial reported in Chapter 5. In the cage trial, excreta quality was improved by feeding diets containing faba beans. The reasons for this discrepancy are unclear.

6.6. Conclusions

Weight gain, feed per gain, excreta score and carcass recovery of broilers were not affected by all the dietary treatments during the 35 day grow-out period. The present data, along together with those reported in Chapter 5, demonstrate that, when the diets are balanced in terms of metabolisable energy and digestible amino acids, the inclusion of faba beans, white lupins and peas at 200 g/kg could successfully support good production performance of broilers. It also appeared from the results of the present experiment that older birds had a better tolerance, especially with respect to non-starch polysaccharides in grain legumes, than the younger birds.

CHAPTER 7

Comparison of methodologies to determine apparent ileal amino acid digestibility of feed ingredients for broilers

7.1. Abstract

The influence of method of determination (direct vs. difference method) on the apparent ileal digestibility coefficient of amino acids in two cereals (maize and wheat), two grain legumes (Australian sweet lupins and peas) and soybean meal was investigated in the present study. In the direct method, the test ingredients were incorporated as the sole source of dietary protein in the assay diets. The assay diets used in the difference method were formulated by substituting cereals, legumes and soybean meal for 50, 25 and 50% (w/w), respectively, of a maize-soy basal diet. Each diet contained 3 g/kg titanium dioxide as an indigestible marker and offered *ad libitum* to four replicate cages of broilers (4 birds/cage) from d 28 to d 35 post-hatching. On day 35, the digesta contents were collected from the terminal ileum and apparent ileal digestibility coefficient of amino acids were calculated using marker ratios. The influence of the method of determination on the apparent ileal digestibility coefficient was found to vary amongst the feed ingredients. For maize and wheat, the digestibility values of arginine, isoleucine, lysine and valine determined with the difference method were higher ($P < 0.05$ to 0.01) than that of the direct method. The digestibility values of arginine, isoleucine, leucine, phenylalanine, threonine and valine of Australian sweet lupins determined by the difference method were higher ($P < 0.05$ to 0.01) than those determined with the direct method. For peas, the digestibility values of isoleucine, threonine and valine determined with the direct method was considerably lower ($P < 0.05$ to 0.01) than those determined with the difference method. Histidine, threonine, cystine and proline digestibilities of soybean meal determined with the difference method were higher ($P < 0.05$ to 0.01) than those determined with the direct method. When the effect of methodology on all five ingredients were analysed together, the main effect of method was found to be significant ($P < 0.05$ to 0.001) for the apparent ileal digestibility coefficient of most amino acids. Overall, the apparent ileal digestibility coefficient of amino acids of feed ingredients determined with the difference method was higher than those determined by the direct method, suggesting that the use of the direct method may underestimate the apparent ileal digestibility of amino acids in low and medium protein ingredients.

7.2. Introduction

Published data on the apparent ileal digestibility coefficient of amino acids of feed ingredients for poultry show large differences between ingredients and even in samples within the same ingredients. In addition to inherent ingredient-related and bird-related factors, this variability is also associated with methodological differences including assay diets, choice of digesta marker, method of euthanasia, site of digesta collection and assay methodology.

Two different methods, namely, the direct method and the difference method have been used in order to determine the amino acid digestibility of feed ingredients for poultry (Lemme *et al*., 2004). The 'direct method' is the most common method used to measure amino acid digestibility of food ingredients, largely because of the simplicity of the assay diet and calculations (Ravindran and Bryden, 1999). The test ingredient represents the sole source of amino acids in a dextrose or starch-based assay diet fortified with minerals and vitamins. Calculation of the digestibility coefficient assumes that the amino acid digestibility of the diet is representative of that of the feed ingredient. However, this assumption is not always true and it can lead to a slight error in calculation because the diet itself triggers the secretion of some endogenous amino acid. In addition, the relative size of error will increase as the amino acid content in the test diet decreases (Lemme *et al*., 2004)

In the difference method, the test diet comprises of a mixture (usually 50:50) of the basal and the test ingredients (Lemme *et al*., 2004). The digestibility in the test ingredient(s) was calculated using the difference in digestibility between the two assay diets and the contribution level of the nutrient in the diets. It is assumed that there is no interaction between the basal diet and the test ingredient.

The aims of the present study was to examine the influence of the method of determination (direct vs. difference) on the apparent ileal digestibility coefficient of amino acids in two cereals (maize and wheat), two grain legumes (Australian sweet lupins and peas) and soybean meal for broilers.

7.3. Materials and methods

All experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC 05/20 and 05/21) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

7.3.1. Ingredients: Two cereals (maize and wheat), two grain legumes (Australian sweet lupins cv Wallan and peas cv Santana) and a commercial soybean meal were used. Wheat and maize represented low protein ingredients, and peas and Australian sweet lupins represented ingredients with moderate protein levels. Legume seeds, with hulls, were ground in a hammer mill to pass through a 3-mm sieve prior to inclusion into the diets.

7.3.2. Birds and housing: Day-old male broilers (Ross 308), obtained from a commercial hatchery, were raised in floor pens and fed a commercial broiler starter diets (230 g/kg crude protein) until day 21. Feed and water were available at all times. The temperature was maintained at 32^{o}C during the first week and gradually decreased to approximately 23^{o}C by the end of the third week. Ventilation was controlled by a central ceiling extraction fan and wall inlet ducts. On day 21, 256 birds of uniform body weight were selected and randomly assigned to 44 cages (4 birds per cage). The birds were offered a commercial broiler finisher diet (180 g/kg crude protein) until the introduction of assay diets on day 28. On day 28, four replicate cages were randomly assigned to each assay diet.

7.3.3. Diets: In the direct method, the test ingredients were incorporated as the sole source of dietary protein in the assay diet (Table 6.1). For cereals, the assay diets contained (per kg) 938 g of test cereals, 20 g of vegetable oil and 39 g of mineral and vitamin supplements. Whilst for protein meals, the assay diets were based on dextrose (Dextrose monohydrate; Starch Australasia Ltd, Tamworth, NSW, Australia) and the test ingredient. The proportion of dextrose and the test feedstuff were varied such that 180 g crude protein/kg was provided in each protein meal assay diet. In the difference method, the assay diets were formulated by substituting the cereal and soybean meal for 50% (w/w) of a maize-soy basal diet, whereas the legumes were substituted for 25% (w/w) of the basal diet (see Table 4.1 in Chapter 4-Section 4.3.3)**.** Titanium dioxide was added at 3 g/kg to all diets as an indigestible marker.

Table 7.1. Composition (g/kg air dry basis) of assay diets -direct method

Ingredient	Maize	Wheat	Australian sweet lupins	Peas	Soybean meal
Test ingredient	938	938	450	720	416
Dextrose	-	-	451	181	525
Soybean oil	20	20	60	60	20
Sodium bicarbonate	2	2	2	2	2
Dicalcium phosphate	19	19	19	19	19
Limestone	13	13	10	10	10
Salt	2.0	2.0	2.0	2.0	2.0
Trace mineral –vitamin premix[1]	3.0	3.0	3.0	3.0	3.0
Titanium dioxide	3	3	3	3	3

[1]See Table 4.1.

7.3.4. Collection of ileal digesta: On day 35 post-hatching, digesta were collected from the lower half of the ileum and processed as described in Chapter 4 (Section 4.3.5).

7.3.5. Chemical Analysis: The procedures to determine the contents of nitrogen, amino acids and titanium dioxide were carried out as described in Chapter 4 (Section 4.3.6.1), Chapter 3 section 3.3.3.5, and Chapter 4 (Section 4.3.6.6), respectively.

7.3.6. Calculations: The apparent ileal digestibility coefficient of amino acids determined with the difference method was calculated as described in Chapter 4 (Section 4.3.7.2). The AIDC of amino acids determined with the direct method was calculated as follows:

$$\text{AIDC} = \frac{\text{(AA / Ti) diet} - \text{(AA / Ti) ileal}}{\text{(AA/Ti) diet}}$$

Where, (AA / Ti) diet = ratio of amino acid to titanium in the diet, and
(AA / Ti) ileal = ratio of amino acid to titanium in the ileal digesta.

7.3.7. Statistical analysis: Student t-test (SAS, 1997) was used to compare ileal amino acid digestibility values for each ingredient determined by direct and difference methods. Two-way analysis of variance was also used in order to determine the main effects and interaction of method and ingredient on amino acid digestibility using the General Linear Model procedure of SAS (1997). Differences were considered significant at $P < 0.05$.

7.4. Results

The amino acid concentrations of the test ingredients are summarised in Table 7.2.

Table 7.2. Amino acid concentration (g/kg dry matter) of feed ingredients assayed

	Maize	Wheat	Australian sweet Lupins	Peas	Soybean meal
Dry matter					
Indispensable amino acids					
Arginine	4.39	6.55	31.8	22.0	35.1
Histidine	2.92	3.29	8.51	6.46	19.7
Isoleucine	3.25	4.16	11.0	9.68	19.9
Leucine	11.4	8.90	19.2	17.5	35.3
Lysine	2.93	3.81	15.4	17.3	28.6
Methionine	2.04	2.32	2.55	2.59	6.68
Phenylalanine	4.76	6.45	10.4	10.9	22.2
Threonine	3.28	3.91	12.5	8.97	18.7
Valine	4.35	5.95	11.5	10.5	21.0
Dispensable amino acids					
Alanine	6.82	5.14	11.1	10.0	19.8
Aspartic acid	6.58	8.03	27.2	28.6	51.1
Cystine	1.85	2.60	5.48	3.10	7.00
Glycine	3.59	5.78	11.8	10.4	18.0
Glutamic acid	18.2	42.0	59.3	39.6	85.1
Proline	9.42	12.7	9.88	9.61	23.5
Serine	3.93	5.63	10.9	10.3	19.6
Tyrosine	3.62	4.55	9.66	8.30	16.5

The influence of methodology on the apparent ileal amino acid digestibility coefficient of amino acids in maize, wheat, Australian sweet lupin, peas and soybean meal is summarised in Tables 7.3 to 7.8. In general, apparent ileal digestibility coefficient of amino acids in cereals and grain legumes determined with the difference method were higher than those determined with the direct method. However, the differences were significant ($P < 0.05$) only for some amino acids. The mean apparent ileal digestibility coefficients of amino acids in maize, wheat, Australian sweet lupins, peas and soybean meal determined by the difference method were 0.862, 0.858, 0.857, 0.829, and 0.881, respectively. The corresponding coefficients determined by the direct method were 0.833, 0.812, 0.793, 0.791, and 0.855, respectively.

For maize, the differences were significant ($P < 0.05$ to 0.01) for arginine, isoleucine, lysine, phenylalanine, valine, and aspartic acid (Table 7.3), with the apparent ileal digestibility coefficient of these amino acids being higher when determined by the difference method. Significant difference ($P < 0.05$), in favour of the difference method, was also found in the mean digestibility coefficient of indispensable amino acids. The overall mean of apparent ileal digestibility coefficient of amino acids of maize determined with the difference method was not different ($P > 0.05$) to that of the direct method.

Table 7.3. Comparison of the apparent ileal digestibility coefficients in maize determined with the direct and difference methods[1]

	Method		Significance
	Direct	Difference	
Indispensable amino acids			
Arginine	0.868 ± 0.010	0.965 ± 0.013	**
Histidine	0.807 ± 0.015	0.800 ± 0.013	NS
Isoleucine	0.839 ± 0.012	0.908 ± 0.022	*
Leucine	0.898 ± 0.005	0.902 ± 0.018	NS
Lysine	0.805 ± 0.010	0.930 ± 0.019	**
Methionine	0.893 ± 0.006	0.896 ± 0.014	NS
Phenylalanine	0.900 ± 0.007	0.947 ± 0.013	*
Threonine	0.693 ± 0.022	0.699 ± 0.020	NS
Valine	0.830 ± 0.010	0.874 ± 0.008	*
Mean	0.837 ± 0.010	0.880 ± 0.012	*
Dispensable amino acids			
Alanine	0.883 ± 0.007	0.892 ± 0.012	NS
Aspartic acid	0.803 ± 0.015	0.876 ± 0.017	*
Cystine	0.745 ± 0.011	0.721 ± 0.021	NS
Glycine	0.772 ± 0.018	0.820 ± 0.012	NS
Glutamic acid	0.898 ± 0.005	0.935 ± 0.020	NS
Proline	0.883 ± 0.005	0.846 ± 0.027	NS
Serine	0.775 ± 0.014	0.798 ± 0.015	NS
Tyrosine	0.866 ± 0.010	0.854 ± 0.016	NS
Mean	0.828 ± 0.011	0.843 ± 0.015	NS
Overall mean[2]	0.833 ± 0.010	0.862 ± 0.014	NS

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$)
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

For wheat, the differences were significant ($P < 0.05$ to 0.01) for arginine, isoleucine, lysine and valine (Table 7.4), with the apparent ileal digestibility coefficient of these amino acids being higher when determined by the difference method. Significant differences ($P < 0.05$) were found in the mean apparent ileal digestibility coefficient of indispensable amino acids in wheat determined with both methods. The overall mean apparent ileal digestibility coefficient of amino acids of wheat determined with the difference method was significantly higher ($P < 0.05$) than that of the direct method.

Table 7.4. Comparison of the apparent ileal digestibility coefficients in wheat determined with the direct and difference methods[1]

	Method		
	Direct	Difference	Significance
Indispensable amino acids			
Arginine	0.774 ± 0.028	0.857 ± 0.011	*
Histidine	0.764 ± 0.019	0.816 ± 0.014	NS
Isoleucine	0.821 ± 0.011	0.886 ± 0.006	*
Leucine	0.842 ± 0.014	0.883 ± 0.010	NS
Lysine	0.750 ± 0.022	0.852 ± 0.009	**
Methionine	0.859 ± 0.011	0.883 ± 0.010	NS
Phenylalanine	0.893 ± 0.011	0.942 ± 0.010	NS
Threonine	0.697 ± 0.014	0.747 ± 0.015	NS
Valine	0.804 ± 0.018	0.858 ± 0.008	*
Mean	0.801 ± 0.016	0.858 ± 0.008	*
Dispensable amino acids			
Alanine	0.765 ± 0.023	0.810 ± 0.008	NS
Aspartic acid	0.762 ± 0.019	0.860 ± 0.010	**
Cystine	0.811 ± 0.011	0.791 ± 0.042	NS
Glycine	0.773 ± 0.021	0.829 ± 0.004	*
Glutamic acid	0.931 ± 0.006	0.953 ± 0.005	*
Proline	0.914 ± 0.007	0.913 ± 0.017	NS
Serine	0.789 ± 0.017	0.829 ± 0.010	NS
Tyrosine	0.854 ± 0.010	0.871 ± 0.008	NS
Mean	0.825 ± 0.014	0.857 ± 0.011	NS
Overall mean[2]	0.812 ± 0.015	0.858 ± 0.009	*

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$)
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

The apparent ileal digestibility coefficient values of amino acids in Australian sweet lupins determined with the direct and the difference methods are presented in Table 7.5. Significant differences ($P < 0.05$ to 0.01) were observed for arginine, isoleucine, leucine, phenylalanine, threonine, valine, proline and serine, with the apparent ileal digestibility coefficient of these amino acids being higher when determined by the difference method. Significant differences ($P < 0.05$ to 0.01) were found in the means of dispensable and indispensable amino acid digestibilities. The overall mean of apparent ileal digestibility coefficient of amino acids of Australian sweet lupins determined with the difference method was higher ($P < 0.05$ to 0.01) than that of the direct method.

Table 7.5. Comparison of the apparent ileal digestibility coefficients in Australian sweet lupins determined with the direct and difference methods[1]

	Method		Significance
	Direct	Difference	
Indispensable amino acids			
Arginine	0.900 ± 0.008	0.949 ± 0.007	**
Histidine	0.755 ± 0.013	0.797 ± 0.0028	NS
Isoleucine	0.784 ± 0.014	0.863 ± 0.019	*
Leucine	0.798 ± 0.013	0.905 ± 0.022	**
Lysine	0.831 ± 0.008	0.857 ± 0.008	NS
Methionine	0.787 ± 0.010	0.741 ± 0.047	NS
Phenylalanine	0.817 ± 0.010	0.919 ± 0.023	**
Threonine	0.765 ± 0.017	0.819 ± 0.011	*
Valine	0.764 ± 0.014	0.840 ± 0.024	*
Mean	0.800 ± 0.012	0.854 ± 0.014	*
Dispensable amino acids			
Alanine	0.790 ± 0.014	0.847 ± 0.021	NS
Aspartic acid	0.782 ± 0.011	0.834 ± 0.022	NS
Cystine	0.790 ± 0.010	0.844 ± 0.031	NS
Glycine	0.784 ± 0.013	0.834 ± 0.022	NS
Glutamic acid	0.869 ± 0.010	0.923 ± 0.021	NS
Proline	0.733 ± 0.017	0.873 ± 0.048	*
Serine	0.717 ± 0.014	0.862 ± 0.024	**
Tyrosine	0.814 ± 0.012	0.863 ± 0.018	NS
Mean	0.785 ± 0.011	0.860 ± 0.014	**
Overall mean[2]	0.793 ± 0.012	0.857 ± 0.013	**

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$)
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

The apparent ileal digestibility coefficient of amino acids in peas determined with the direct and the difference methods are presented in Table 7.6. There were no significant differences ($P > 0.05$) in apparent ileal digestibility coefficient of amino acids of peas determined with these two methods, except for isoleucine, valine and threonine, with the apparent ileal digestibility coefficient of these amino acids being higher when determined by the difference method. In general, the mean of the apparent ileal digestibility coefficient of amino acids of peas determined with the difference method were numerically higher than those based on the direct method, but this was not significant.

Table 7.6. Comparison of the apparent ileal digestibility coefficients in peas determined with the direct and difference methods[1]

	Method		Significance
	Direct	Difference	
Indispensable amino acids			
Arginine	0.888 ± 0.015	0.920 ± 0.013	NS
Histidine	0.784 ± 0.012	0.774 ± 0.047	NS
Isoleucine	0.788 ± 0.016	0.846 ± 0.016	*
Leucine	0.803 ± 0.017	0.844 ± 0.023	ns
Lysine	0.863 ± 0.015	0.891 ± 0.010	NS
Methionine	0.785 ± 0.017	0.826 ± 0.043	NS
Phenylalanine	0.821 ± 0.019	0.865 ± 0.025	NS
Threonine	0.746 ± 0.009	0.782 ± 0.013	**
Valine	0.774 ± 0.015	0.837 ± 0.015	*
Mean	0.806 ± 0.014	0.843 ± 0.016	NS
Dispensable amino acids			
Alanine	0.791 ± 0.014	0.840 ± 0.017	NS
Aspartic acid	0.813 ± 0.015	0.846 ± 0.018	NS
Cystine	0.605 ± 0.019	0.612 ± 0.054	NS
Glycine	0.796 ± 0.012	0.812 ± 0.021	NS
Glutamic acid	0.858 ± 0.013	0.911 ± 0.020	NS
Proline	0.770 ± 0.016	0.847 ± 0.028	NS
Serine	0.756 ± 0.014	0.813 ± 0.022	NS
Tyrosine	0.812 ± 0.017	0.827 ± 0.024	NS
Mean	0.775 ± 0.013	0.813 ± 0.020	NS
Overall mean[2]	0.791 ± 0.013	0.829 ± 0.018	NS

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$)
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

Method of determination had no effect on the apparent ileal digestibility coefficient of indispensable amino acids in the soybean meal (Table 7.7), except for histidine ($P < 0.001$) and threonine ($P < 0.05$), cystine ($P < 0.01$) and proline ($P < 0.05$). The apparent ileal digestibility coefficient values of these amino acids were higher when determined by the difference method. No significant differences ($P > 0.05$) were observed in the apparent ileal digestibility coefficient values of the means of dispensable and indispensable amino acids in soybean meal determined with both methods. The overall mean of apparent ileal digestibility coefficient of amino acids in soybean meal determined by the direct method was similar to that of the difference method.

Table 7.7. Comparison of the apparent ileal digestibility coefficients in soybean meal determined with the direct and difference methods

Amino acids	Method		Significance
	Direct	Difference	
Indispensable amino acids			
Arginine	0.919±0.007	0.918±0.008	NS
Histidine	0.836±0.007	0.910±0.006	***
Isoleucine	0.868±0.007	0.891±0.012	NS
Leucine	0.865±0.006	0.876±0.014	NS
Lysine	0.896±0.006	0.915±0.009	NS
Methionine	0.899±0.008	0.898±0.021	NS
Phenylalanine	0.880±0.007	0.888±0.014	NS
Threonine	0.807±0.009	0.855±0.014	*
Valine	0.867±0.018	0.879±0.011	NS
Mean	0.871±0.007	0.892±0.010	NS
Dispensable amino acids			
Alanine	0.856±0.008	0.869±0.013	NS
Aspartic acid	0.853±0.007	0.865±0.014	NS
Cystine	0.671±0.019	0.809±0.021	**
Glycine	0.827±0.008	0.853±0.012	NS
Glutamic acid	0.900±0.006	0.901±0.013	NS
Proline	0.843±0.006	0.887±0.014	*
Serine	0.860±0.004	0.869±0.021	NS
Tyrosine	0.883±0.007	0.895±0.012	NS
Mean	0.837±0.007	0.869±0.012	NS
Overall mean[2]	0.855±0.007	0.881±0.011	NS

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$).
[1]Each value represents mean of four replicates (4 birds per replicate).
[2]Average digestibility of 17 amino acids.

When data from all ingredients were analysed together (Table 7.8), the main effect of these methods was found to be significant ($P < 0.05$ to 0.001) for the apparent ileal digestibility coefficient of most amino acids. With the exception of methionine and tyrosine, the digestibility coefficient of amino acids determined with the difference method was higher ($P<0.05$) than those determined with the direct method.

The main effect of ingredients was significant ($P < 0.05$ to 0.001) for the apparent ileal digestibility coefficient of all amino acids, except for glycine. The digestibility coefficient of histidine, lysine and threonine of soybean meal was higher ($P < 0.05$) than that of grain legumes. Maize, wheat and soybean meal had similar ($P > 0.05$) apparent ileal digestibility coefficient of isoleucine, methionine and valine, and these values were higher ($P < 0.05$) than those of sweet lupins and peas. The average digestibility coefficient of indispensable amino acids of maize and soybean meal was higher ($P < 0.05$) than that of wheat sweet lupin and peas. In the case of dispensable amino acids, the average apparent ileal digestibility coefficient of cereals and soybean meal was observed to be higher ($P < 0.05$) than that of grain legumes.

Significant interactions ($P < 0.05$ to 0.001) between ingredients and methods were only found in arginine, leucine, lysine, cystine, serine and proline. The digestibility coefficient of arginine of maize, wheat and sweet lupins determined with the difference method was higher ($P < 0.05$) than that of the direct method. The digestibility coefficient of leucine of sweet lupins determined with the difference method was found to be higher ($P < 0.05$) than that of the direct method. The apparent ileal digestibility coefficient of leucine (sweet lupins) and lysine (maize and wheat) determined with the difference method were markedly higher ($P < 0.05$) than that of the direct method. Cystine digestibility coefficient in SBM determined with the difference method was higher that of the direct method. The digestibility coefficients of proline and serine in sweet lupins and peas determined with the difference method was found to be higher than those determined with the direct method.

Table 7.8. Influence of dietary treatments on the apparent ileal digestibility coefficients of amino acids[1]

	Ingredients										Pooled SEM	Main effects							Probability, P <		
	Maize		Wheat		Sweet lupins		Peas		SBM			Ingredients (I)					Methods (M)		I	M	I x M
	Dir	Diff	Dir	Diff	Dir	Diff	Dir	Diff	Dir	Diff		Maize	Wheat	Sweet lupin	Peas	SBM	Dir	Diff			
IAA[2]																					
Arg	0.868^{de}	0.965^{a}	0.774^{f}	0.857^{e}	0.900^{cd}	0.949^{ab}	0.888^{cde}	0.920^{bc}	0.919^{bc}	0.918^{bc}	0.013	0.916^{a}	0.816^{b}	0.925^{a}	0.904^{a}	0.918^{a}	0.870^{b}	0.921^{a}	***	***	***
His	0.807	0.800	0.764	0.816	0.755	0.797	0.784	0.774	0.836	0.910	0.021	0.803^{b}	0.790^{b}	0.776^{b}	0.779^{b}	0.873^{a}	0.789^{b}	0.819^{a}	***	*	NS
Ile	0.839	0.908	0.821	0.886	0.784	0.863	0.788	0.846	0.868	0.891	0.014	0.874^{a}	0.854^{a}	0.823^{b}	0.817^{b}	0.879^{a}	0.820^{b}	0.879^{a}	**	***	NS
Leu	0.898^{a}	0.902^{a}	0.842$^{bc}_{d}$	0.883^{ab}	0.798^{d}	0.905^{a}	0.803^{cd}	0.844^{bc}	0.865^{ab}	0.876^{ab}	0.013	0.900^{a}	0.862^{b}	0.851^{bc}	0.823^{c}	0.871^{ab}	0.841^{b}	0.882^{a}	***	***	*
Lys	0.805^{f}	0.930^{a}	0.750^{g}	0.852^{e}	0.831^{ef}	0.857^{de}	0.863^{cde}	0.891$^{bc}_{d}$	0.896^{abc}	0.915^{ab}	0.013	0.867^{bc}	0.801^{d}	0.844^{c}	0.877^{b}	0.906^{a}	0.829^{b}	0.889^{a}	***	***	***
Met	0.893	0.896	0.859	0.883	0.787	0.741	0.785	0.826	0.899	0.898	0.023	0.895^{a}	0.871^{a}	0.764^{b}	0.806^{b}	0.899^{a}	0.845	0.849	***	NS	NS
Phe	0.900	0.947	0.893	0.942	0.817	0.919	0.821	0.865	0.880	0.888	0.015	0.923^{a}	0.918^{a}	0.868^{bc}	0.843^{c}	0.884^{b}	0.862^{b}	0.912^{a}	***	***	NS
Thr	0.693	0.699	0.697	0.747	0.765	0.819	0.746	0.782	0.807	0.855	0.015	0.696^{c}	0.722^{c}	0.792^{b}	0.764^{b}	0.831^{a}	0.742^{b}	0.781^{a}	***	***	NS
Val	0.830	0.874	0.804	0.858	0.764	0.840	0.774	0.837	0.867	0.879	0.016	0.852^{a}	0.831^{ab}	0.802^{b}	0.805^{b}	0.842^{ab}	0.788^{b}	0.857^{a}	*	***	NS
Mean	0.837	0.880	0.801	0.858	0.800	0.854	0.806	0.843	0.871	0.892	0.012	0.859^{a}	0.830^{b}	0.828^{b}	0.824^{b}	0.882^{a}	0.823^{b}	0.866^{a}	***	***	NS
DAA[3]																					
Ala	0.883	0.892	0.765	0.810	0.790	0.847	0.791	0.840	0.856	0.869	0.015	0.888^{a}	0.788^{c}	0.818^{a}	0.815^{bc}	0.863^{a}	0.817^{b}	0.851^{a}	***	***	NS
Asp	0.803	0.876	0.762	0.860	0.782	0.834	0.813	0.846	0.853	0.865	0.015	0.839^{ab}	0.810^{b}	0.808^{b}	0.829^{ab}	0.859^{a}	0.802	0.856^{a}	*	***	NS
Cys	0.745$^{bc}_{d}$	0.721^{cd}	0.811^{ab}	0.791^{abc}	0.790^{abc}	0.844^{a}	0.605^{e}	0.612^{e}	0.671^{de}	0.809^{ab}	0.027	0.733^{b}	0.801^{a}	0.817^{a}	0.609^{c}	0.740^{b}	0.724	0.755	***	NS	*
Gly	0.772	0.820	0.773	0.829	0.784	0.834	0.796	0.812	0.827	0.853	0.015	0.796	0.801	0.809	0.804	0.840	0.790^{b}	0.830^{a}	NS	***	NS
Glu	0.898	0.935	0.931	0.953	0.869	0.923	0.858	0.911	0.900	0.901	0.013	0.916^{ab}	0.942^{a}	0.896^{bc}	0.804^{c}	0.901^{bc}	0.891^{b}	0.925^{a}	**	***	NS
Pro	0.883^{ab}	0.846^{b}	0.914^{a}	0.913^{a}	0.733^{c}	0.873^{ab}	0.770^{c}	0.847^{b}	0.843^{b}	0.887^{ab}	0.022	0.865^{b}	0.913^{a}	0.803^{c}	0.809^{c}	0.865^{b}	0.829^{b}	0.873^{a}	***	**	**
Ser	0.775^{cd}	0.798^{bcd}	0.789$^{bc}_{d}$	0.829^{ab}	0.717^{e}	0.862^{a}	0.756^{de}	0.813^{bc}	0.860^{a}	0.869^{a}	0.016	0.787^{b}	0.809^{b}	0.789^{b}	0.784^{b}	0.865^{a}	0.779^{b}	0.834^{a}	***	***	**
Tyr	0.866	0.854	0.854	0.871	0.814	0.863	0.812	0.827	0.883	0.895	0.014	0.860^{b}	0.863^{ab}	0.839^{bc}	0.819^{c}	0.889^{a}	0.846	0.862^{a}	***	NS	NS
Mean	0.828	0.843	0.825	0.857	0.785	0.860	0.775	0.813	0.837	0.869	0.014	0.835^{ab}	0.841^{ab}	0.822^{b}	0.794^{c}	0.852^{a}	0.810^{b}	0.848^{a}	**	***	NS
Overall mean[4]	0.833	0.862	0.812	0.858	0.793	0.857	0.791	0.829	0.855	0.881	0.013	0.848^{ab}	0.835^{abc}	0.825^{bc}	0.810^{c}	0.856^{a}	0.812	0.858	**	***	NS

* (P < 0.05); ** (P < 0.01); *** (P<0.001); NS (P > 0.05).

[1]Each value represents mean of four replicates (4 birds per replicate).

[2]IAA=Indispensable amino acids

[3]DAA= dispensable amino acids

[4]Average digestibility of 17 amino acids.

7.5. Discussion

The results show that the method of determination influenced the apparent ileal digestibility of amino acids in the feed ingredients assayed. The difference between digestibility coefficients determined with the direct and the difference methods varied depending on the feed ingredient and the amino acid considered.

In general, the apparent ileal digestibility coefficient of amino acids determined with the direct method for all ingredients was lower than those determined with the difference method. However, significant differences were only observed for some indispensable amino acids. Differences were seen for arginine, isoleucine, lysine, and valine in maize and wheat; phenylalanine in maize; arginine, isoleucine, leucine, lysine, phenylalanine, threonine and valine in Australian sweet lupins; and isoleucine, threonine and valine in peas. In soybean meal, differences were only observed for histidine and threonine. Direct comparison of the present findings with published data is difficult because there are no published data which compare methods of determination of amino acid digestibility of feed ingredients for broiler chickens. However, the present findings are in agreement with those of Fan and Sauer (1995a) who determined the apparent ileal digestibility values of barley and canola meal in growing pigs by the direct and difference methods, and reported that amino acid digestibility values in feed ingredients with low protein content are underestimated by the direct method.

The underestimation of amino acid digestibility coefficients in ingredients with low and moderate protein contents by the direct method may be partly explained by the greater proportions of amino acids from endogenous sources, relative to amino acids of dietary origin, in digesta at low dietary amino acid intakes (Ravindran and Bryden, 1999; Ravindran *et al*., 2005). The most abundant amino acids in the ileal endogenous protein of chickens are glutamic acid, aspartic acid, threonine, proline, serine and glycine (Ravindran *et al*., 2004). These amino acids are found in high concentrations in intestinal and pancreatic secretions, and mucoproteins (Corring and Jung, 1972; Juste, 1982; Lien *et al*., 1997).

Fan *et al*. (1994) reported that the ileal amino acid digestibility values of an ingredient increased when the dietary amino acid content increased. When the direct method is employed, one can expect that the dietary levels of some of the amino acids, which include the limiting ones, are lower than their respective upper limit levels. As a result, small differences in the dietary contents of these amino acids will elicit relatively large changes in their apparent ileal digestibilities. The higher inclusion level of cereals and grain legumes in the test diets formulated with the direct method might be expected to further aggravate this

effect. For this reason, use of the difference method may be appropriate in order to determine the apparent ileal amino acid digestibility coefficients in low and medium protein feed ingredients. Similar suggestions have been proposed by Fan and Sauer (1995b) based on studies with growing pigs.

The differences in the mean amino acid digestibility values obtained with the direct and difference methods for maize, wheat, lupin, peas and soybean meal were 3.5, 5.7, 8.1, 4.8 and 3.0%, respectively. Based on these data, the ingredient most influenced by the methodology used to determine amino acid digestibility was Australian sweet lupins, followed by wheat and peas. The discrepancies may be due to the differences in NSP content of these ingredients. It has been previously shown that the high intestinal digesta viscosity caused the viscous NSP has negative effects on the digestion of nutrients, via: (i) reducing the passage rate, (ii) reducing the mixing of digestive enzymes with substrate nutrients, (iii) increasing secretion of endogenous enzymes, which increases the endogenous losses, (iv) increasing secretory response of mucus, which may increase resistance for transport of nutrients through the unstirred water layer adjacent to the epithelial surface by increasing mucus layer thickness, and/or (5) interacting with gut microflora (Smits and Annison, 1996).

The digestibility coefficients of individual amino acids in wheat, maize and soybean meal obtained by the direct method in the current study were consistent with previous published data (Ravindran *et al*., 1999, 2005; Huang *et al*., 2006, 2007). Direct comparison of the apparent ileal digestibility coefficient values of food ingredients determined by the difference method is difficult because most of the published apparent ileal digestibility coefficient values were determined by the direct method.

7.6. Conclusions

In conclusion, it is evident that the influence of methodology of determination on the apparent ileal digestibility coefficient of amino acids was found to vary amongst feed ingredients. The application of the direct method in amino acid digestibility measurement of feed ingredients resulted in an underestimation of the apparent ileal digestibility coefficient of some amino acids in the ingredients tested. Based on these findings, it may be suggested that the difference method is more suitable for the determination of apparent ileal digestibility coefficient of amino acids in low and medium protein ingredients for broilers.

CHAPTER 8

Influence of dehulling on the apparent metabolisable energy and ileal amino acid digestibility of grain legumes for broilers

8.1. Abstract

The present experiment was designed to investigate the influence of dehulling on the nitrogen-corrected apparent metabolisable energy and the apparent ileal amino acid digestibility of faba beans, Australian sweet lupins and peas. The experimental diets included a maize-soybean meal basal diet and six legume diets. The legume diets were developed by substituting legumes (whole or dehulled) for 25% (w/w) of a maize-soy basal diet. All diets contained 3 g/kg titanium dioxide as an indigestible marker. Each diet was offered *ad libitum* to four replicate cages of broilers (4 birds/cage) from d 28 to 35 post-hatching. Total collection of excreta was carried out during the last four days in order to determine the AME. On day 35, digesta contents were collected from the terminal ileum and apparent ileal digestibility coefficient of amino acids were calculated using marker ratios. Dehulling decreased ($P < 0.01$ to 0.001) soluble, insoluble and total non-starch polysaccharides of all legumes. The starch content, ileal starch digestibility and AME of peas were not ($P > 0.05$) affected by dehulling. The starch content and digestibility increased ($P < 0.001$) in faba beans after the removal of hulls and the AMEn values were improved ($P < 0.01$) by 15.3% after dehulling. Dehulling increased ($P < 0.05$) AMEn of Australian sweet lupins. The improvements observed in the AMEn in faba beans and lupins may be due to the decrease in non-starch polysaccharides of these legumes after dehulling. In faba beans, the improvements in starch content and digestibility may have been other contributing factor. Dehulling provided a product with higher amino acid concentration than the whole seed. The apparent ileal digestibility coefficient of most amino acids for all three legume species were unaffected ($P > 0.05$) by the removal of hulls. These results suggest that dehulling of grain legumes would be nutritionally beneficial and probably economical in view of the improved amino acid concentrations and available energy values.

8.2. Introduction

Grain legumes have many desirable characteristics for the feeding of non-ruminant animals. However, the major factor which limits the use of these legume seeds in practical diets is the existence of naturally occurring anti-nutritional factors, including non-starch polysaccharides, tannins and trypsin inhibitors, which have adverse effects on nutrient digestibility and absorption.

The removal of hulls has been evaluated as an effective way of reducing the content of anti-nutritional factors and also a way to improve the nutritional value of grain legumes. However, the effectiveness of dehulling in reducing the anti-nutritional factors in grain legumes is largely dependent on the type of anti-nutritional factors. Tannins, for example, can be effectively reduced by dehulling (Van der Poel *et al*., 1992; Alonso *et al*., 2000a), whereas trypsin inhibitors and lectins, which are present in the seed cotyledon, cannot be removed by physical processing (Longstaff and McNab, 1991; Alonso *et al*., 2000b). Crude protein, crude fat and amino acid concentrations have been shown to increase. The concentrations of crude fibre, acid detergent fibre and neutral detergent fibre decrease after dehulling (Breytenbach, 2005; Olkowski *et al*., 2005).

The effects of dehulling of grain legumes on energy utilisation in broilers have been investigated by several researchers (Brenes *et al*., 1993a,b; Breytenbach, 2005; Suchy *et al*., 2006), but the level of improvement was dependent on the legume species and cultivar. Brenes *et al.* (1993a), for example, showed that dehulling considerably improved AME of high-tannin peas, but it had no effect in low-tannin peas. Breytenbach (2005) demonstrated that the AMEn of Australian sweet lupins increased by 2.3% after dehulling. In a study by Brenes *et al*. (1993b), it was shown that the dehulling of lupins increased AME by 18%. Published data on the effects of dehulling on the amino acid digestibility of grain legumes for broilers are scarce.

The objective of the present study was to investigate the effects of dehulling on the apparent metabolisable energy and apparent ileal amino acid digestibility of faba beans, Australian sweet lupins and peas for broiler chickens.

8.3. Materials and methods

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC 05/20 and 05/21) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

8.3.1. Ingredients: Seeds from three legume species, namely, faba beans (cv PGG Tic), Australian sweet lupin (cv Wallan) and peas (cv Santana), were assayed. The seeds were dehulled by using a stone mill (Manawatu milling Ltd., Fielding) and then the hulls were separated by the use of sieves and forced air. Prior to inclusion into assay diets, whole and dehulled seeds were ground, in a hammer mill, to pass through a 3-mm sieve.

8.3.2. Birds and housing: A total of 112 four-week-old male broiler (Ross 308) chickens were used in the present study. The housing and experimental designs were similar to those described in Chapter 4 (Section 4.3.2).

8.3.3. Diets: A basal diet based on the maize and soybean meal was formulated (Chapter 4, Table 4.1). Six assay diets were then formulated by substituting the legume samples (whole or dehulled) 25% (w/w) of the basal diet. All diets contained titanium oxide (3 g/kg) as indigestible marker.

8.3.4. Excreta collection: Total excreta collection was carried out between day 32 and 35 Post-hatching and the excreta were processed as described in Chapter 4 (Section 4.3.4.)

8.3.5. Collection of ileal digesta: On day 35, digesta from the terminal ileum were collected and processed as described in Chapter 4 (Section 4.3.5).

8.3.6. Determination of hull: kernel ratio: Representative **s**amples (300 grams) of seeds were crushed in a mortar. Hulls and kernel were manually separated on a plate and each component was weighed.

8.3.7. Chemical analysis: The procedures to determine the content of nitrogen, NSP, gross energy, amino acids, titanium dioxide and starch were followed as described in Chapter 4 (Section 4.3.6.1, 4.3.6.3 to 4.3.6.6), Chapter 3 section 3.3.3, Chapter 4 (Section 4.3.6.6), Chapter 4 (Section 4.3.6.2), respectively.

8.3.8. Calculations: The AME and AIDC of amino acids were calculated as described in Chapter 4 (Section 4.3.7.2).

8.3.9 Statistical analysis: Student t-test was used to compare the nutritional values of whole and dehulled grain legumes (SAS, 1997). Differences were considered significant at $P < 0.05$.

8.4. Results

The proportion between the kernels and hulls of the three grain legumes is presented in Table 8.1. Australian sweet lupins had the highest proportion of hulls and peas had the lowest.

Table 8.1 The proportion (g/kg seed) of kernels and hulls of grain legumes

	Kernel	Hull
Faba beans	852	148
Australian sweet lupins	701	299
Peas	911	89

The effect of dehulling on the amino acid concentration of grain legumes is shown in Table 8.2. In general, the concentration of amino acids in all three legumes increased after dehulling. The average increases of amino acid concentrations were higher in Australian sweet lupins (36.3%), intermediate in faba bean (15.2%) and low in peas (5.9%) (Table 8.2).

Table 8.2. Amino acid concentration (g/kg dry matter basis) in faba beans, lupin and peas as influenced by dehulling

	Faba beans (cv PGG Tic)		Improvement (%)	*Lupinus angustifolius* (cv Wallan)		Improvement (%)	Peas (cv Santana)		Improvement (%)
	whole	dehulled		whole	dehulled		whole	dehulled	
Dry matter	882.7	879.3		930.8	895.9		869.3	874.0	
Indispensable amino acids									
Arginine	24.5	29.5	20.6	25.4	36.5	43.8	17.4	19.0	9.3
Histidine	6.6	7.7	16.0	7.9	10.3	30.3	5.6	6.1	8.7
Isoleucine	9.4	11.1	17.9	10.4	14.4	37.6	8.4	8.9	6.3
Leucine	17.7	21.0	18.9	18.8	25.9	37.4	15.0	16.0	7.2
Lysine	15.3	17.4	14.2	14.5	18.6	28.9	16.1	17.0	5.4
Methionine	1.9	2.1	6.8	1.9	2.6	33.2	2.0	2.0	0.5
Phenylalanine	10.3	12.2	18.7	10.9	15.0	38.2	10.5	11.2	6.3
Threonine	8.4	9.7	15.3	10.2	14.1	37.9	7.8	8.1	3.6
Valine	11.4	12.8	12.3	11.8	15.9	35.1	10.0	10.6	5.9
Dispensable amino acids									
Alanine	11.4	12.8	12.3	11.8	15.9	35.1	10.0	10.6	5.9
Aspartic acid	11.1	12.8	15.2	10.9	14.8	34.9	9.7	10.4	6.9
Cystine	28.7	33.7	17.6	28.3	39.2	38.6	26.2	27.5	5.2
Glycine	3.5	3.9	11.8	4.0	5.0	26.8	3.3	3.2	-2.1
Glutamic acid	10.8	12.1	12.2	12.1	16.5	36.7	9.4	10.0	6.7
Proline	43.5	51.2	17.7	57.0	82.0	43.8	38.0	41.1	8.3
Serine	11.6	13.6	17.4	12.2	17.2	41.1	8.6	9.5	9.5
Tyrosine	11.0	12.7	15.1	13.3	18.2	37.4	9.9	10.3	4.5
Total			258.3			616.8			99.5
Average			15.2			36.3			5.9

The removal of hulls decreased ($P < 0.01$ to 0.001) soluble, insoluble and total NSP in all three legumes (Table 8.3). The reductions in soluble and total NSP were highest in faba beans, intermediate in Australian sweet lupins and lowest in peas. Whilst, the largest decrease of insoluble NSP was found in faba beans, followed by peas and finally Australian sweet lupins. Dehulling had no effect ($P > 0.05$) on the content and ileal digestibility of starch in peas, whereas the starch content and digestibility increased ($P < 0.001$) in faba beans (Table 8.3, 8.4 and 8.6). Starch content and starch digestibility of faba beans increased by 13.7 and 12.6%, respectively, by dehulling.

Table 8.3 Effects of dehulling on soluble, insoluble and total non-starch polysaccharides (NSP) and starch content (g/kg DM basis) of grain legumes

Nutrient	Whole	Dehulled	Significance
Soluble NSP[1]			
Faba beans	20.0± 0.10	14.1± 0.05	***
Australian sweet lupins	31.5± 0.02	19.3± 0.09	***
Peas	19.2± 0.15	15.1± 0.10	**
Insoluble NSP[1]			
Faba beans	185± 0.05	84.0± 0.08	***
Australian sweet lupins	463± 0.10	240± 0.07	***
Peas	141± 0.10	77.1± 0.13	***
Total NSP[1]			
Faba beans	205± 0.05	98.0± 0.08	***
Australian sweet lupins	495± 0.10	259± 0.08	***
Peas	160± 0.07	92.2± 0.09	***
Starch[1]			
Faba beans	40.8 ± 0.35	46.4 ± 0.06	***
Peas	46.4 ± 0.12	48.1 ± 0.07	NS

*** ($P < 0.001$); ** ($P < 0.01$); NS ($P > 0.05$)
[1] Each value represents mean ± SE of two replicates

Significant differences ($P < 0.01$ to 0.001) were observed between whole and dehulled faba beans in terms of AME, AMEn and ileal starch digestibility (Table 8.4). Dehulling improved the AME and AMEn values of faba beans by 15.3 and 15.4%, respectively. In terms of ileal amino acid digestibility, significant differences ($P < 0.01$ to

0.05) were only observed for cystine, histidine and proline. After the removal of hulls, cystine, histidine and proline digestibilities increased by 33.2, 21.0 and 20.3 %, respectively.

Table 8.4. Influence of dehulling on AMEn, AME and apparent ileal digestibility coefficients of starch and amino acids in faba beans for broilers[1]

	Whole	Dehulled	Significance
AMEn (MJ/kg DM)	10.46±0.27	12.06±0.14	**
AME (MJ/kg DM)	11.13±0.28	12.84±0.16	**
Ileal starch digestibility	0.795 ± 0.021	0.895 ± 0.006	***
Ileal digestibility coefficient			
Indispensable amino acids			
Arginine	0.911±0.022	0.912±0.009	NS
Histidine	0.703±0.038	0.850±0.021	*
Isoleucine	0.848±0.043	0.828±0.020	NS
Leucine	0.853±0.043	0.862±0.021	NS
Lysine	0.913±0.042	0.878±0.015	NS
Methionine	0.857±0.126	0.939±0.122	NS
Phenylalanine	0.864±0.050	0.856±0.017	NS
Threonine	0.835±0.051	0.814±0.023	NS
Valine	0.833±0.062	0.845±0.020	NS
Mean of indispensable amino acids	0.846±0.033	0.866±0.030	NS
Dispensable amino acids:			
Alanine	0.890±0.021	0.866±0.034	NS
Aspartic acid	0.855±0.038	0.861±0.019	NS
Cystine	0.632±0.033	0.842±0.099	*
Glycine	0.812±0.017	0.846±0.009	NS
Glutamic acid	0.902±0.031	0.906±0.009	NS
Proline	0.706±0.049	0.849±0.022	**
Serine	0.857±0.035	0.846±0.015	NS
Tyrosine	0.842±0.049	0.839±0.016	NS
Mean of dispensable amino acids	0.812±0.035	0.857±0.028	NS
Overall mean[2]	0.830±0.044	0.862±0.029	NS

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$)
[1] Each value represents mean ± SE of four replicates
[2] Overall mean of 17 amino acids

Dehulling improved ($P < 0.01$ to 0.05) the AME (6.29 MJ/kg vs 8.20 MJ/kg) and AMEn (5.82 MJ/kg vs 7.39 MJ/kg) of Australian sweet lupins (Table 8.5). However, the apparent ileal amino acid digestibility coefficients were not affected ($P > 0.05$) by the removal of hulls.

Table 8.5. Influence of dehulling on AMEn, AME and apparent ileal amino acid digestibility coefficient of Australian sweet lupins for broilers[1]

	Whole	Dehulled	Significance
AMEn (MJ/kg DM)	5.82±0.12	7.39±0.34	**
AME (MJ/kg DM)	6.29±0.16	8.20±0.31	*
Ileal digestibility coefficient			
Indispensable amino acids			
Arginine	0.861±0.010	0.884±0.011	NS
Histidine	0.668±0.029	0.743±0.010	NS
Isoleucine	0.771±0.034	0.777±0.026	NS
Leucine	0.772±0.048	0.762±0.029	NS
Lysine	0.772±0.024	0.796±0.021	NS
Methionine	0.707±0.060	0.673±0.068	NS
Phenylalanine	0.747±0.031	0.764±0.031	NS
Threonine	0.718±0.036	0.739±0.022	NS
Valine	0.753±0.040	0.754±0.027	NS
Mean of indispensable amino acids	0.752±0.035	0.766±0.027	NS
Dispensable amino acids			
Alanine	0.729±0.043	0.718±0.043	NS
Aspartic acid	0.688±0.039	0.752±0.019	NS
Cystine	0.663±0.041	0.674±0.048	NS
Glycine	0.734±0.026	0.766±0.019	NS
Glutamic acid	0.825±0.016	0.848±0.018	NS
Proline	0.705±0.049	0.743±0.013	NS
Serine	0.777±0.022	0.799±0.027	NS
Tyrosine	0.780±0.028	0.789±0.024	NS
Mean of dispensable amino acids	0.738±0.033	0.761±0.026	NS
Overall mean[2]	0.745±0.034	0.764±0.027	NS

* ($P < 0.05$); ** ($P < 0.01$); NS ($P > 0.05$)
[1] Each value represents mean ± SE of four replicates
[2] Overall mean of 17 amino acids

No significant (P > 0.05) differences were found in AME, AMEn and apparent ileal digestibility coefficient of starch and amino acid of whole and dehulled peas (Table 8.6).

Table 8.6 Influence of dehulling on AMEn, AME and apparent ileal digestibility coefficients of starch and amino acid of peas for broilers[1]

	Whole	Dehulled	Significance
AMEn (MJ/kg DM)	12.30±0.57	11.90±0.48	NS
AME (MJ/kg DM)	12.84±0.61	12.43±0.54	NS
Ileal starch digestibility	0.815±0.026	0.829 ± 0.032	NS
Ileal digestibility coefficient			
Indispensable amino acids			
Arginine	0.876±0.011	0.882±0.037	NS
Histidine	0.792±0.023	0.774±0.076	NS
Isoleucine	0.760±0.043	0.787±0.066	NS
Leucine	0.799±0.039	0.806±0.074	NS
Lysine	0.855±0.023	0.864±0.047	NS
Methionine	0.847±0.052	0.859±0.111	NS
Phenylalanine	0.819±0.021	0.812±0.061	NS
Threonine	0.725±0.035	0.748±0.066	NS
Valine	0.773±0.040	0.776±0.074	NS
Mean of indispensable amino acids	0.805±0.032	0.812±0.068	NS
Dispensable amino acids			
Alanine	0.807±0.046	0.884±0.059	NS
Aspartic acid	0.781±0.026	0.804±0.057	NS
Cystine	0.718±0.012	0.705±0.053	NS
Glycine	0.772±0.025	0.788±0.047	NS
Glutamic acid	0.861±0.014	0.876±0.045	NS
Proline	0.791±0.039	0.849±0.063	NS
Serine	0.746±0.038	0.800±0.046	NS
Tyrosine	0.789±0.049	0.866±0.061	NS
Mean of dispensable amino acids	0.783±0.031	0.821±0.054	NS
Overall mean[2]	0.795±0.031	0.816±0.061	NS

NS (P > 0.05)
[1] Each value represents mean ± SE of four replicates
[2] Overall mean of 17 amino acids

8.5. Discussion

Apparent metabolisable energy: Marked improvements in AMEn were observed with the dehulling of lupins (30.0%) and faba bean (15.3%). The magnitude of improvement in AMEn of dehulled lupin meal was higher than that reported by Breytenbach (2005), but the AMEn values of whole and dehulled lupins determined in the present study were lower than those previously reported (8.61 to 8.81 MJ/kg). In terms of AME value, our value (6.29

MJ/kg) for whole lupin seeds was lower than the value of 6.71 MJ/kg determined by Prinsloo (1993 cited by Berytenbach, 2005). No comparable data are available on the effects of dehulling on the energy value of faba beans. In the case of peas, the finding that the removal of hull had no effect on AME was similar to that reported by Brenes *et al*. (1993a).

The improvements in energy utilisation in lupins and faba beans are probably due to the elimination of the indigestible, high fibrous hull, leading to the improvements in nutrient dense product and improvements in nutrient digestibility (see Tables 8.1 and 8.3). The largest proportion of hulls observed in the present study was in the lupin seeds (299 g/kg seed), followed by faba beans (148 g/kg seed) and peas (89 g/kg seed). However, the greatest decrease in soluble, insoluble and total non-starch polysaccharide was observed in faba beans, followed by lupins and peas. Brillouet and Riochet (1983) reported that significant amounts of lupin non-starch polysaccharide are located in the seed pericarp with cellulose, hemicellulose and pectins as the main non-starch polysaccharide component. Faba bean hulls consist of structural polysaccharides, largely of cellulose and some hemicellulose (Longstaff and McNab, 1991). Thus, the improvements in the AME of lupins and faba beans after dehulling could be explained, at least in part, by the removal of the fibre component and, a reduction in soluble and total non-starch polysaccharides.

The Australian sweet lupins contained negligible amount of starch (< 1%), but was rich in fat (Table 4.2) which serves as the main energy source in this legume seed. In faba beans, starch was the main contributor to available energy. The starch content and starch digestibility of faba beans were increased by 13.7 and 12.6%, respectively, following the removal of hulls.

Apparent ileal amino acid digestibility: In contrast to the beneficial effects on metabolisable energy, dehulling had no effect on the ileal digestibility of most amino acids. The exceptions were the digestibility of cystine, proline and histidine in faba beans, which were improved with the removal of the hulls. This finding may be related to the reduction in tannin content in the dehulled meal. It has been shown that tannins have a high affinity to interact with proline and histidine in proteins (Jansman *et al*., 1993a). Longstaff and McNab (1991) reported that broiler chicks fed diets containing faba bean hulls rich in condensed tannins had lower apparent digestibility of amino acids, particularly of methionine and cystine, which was attributed to an increase in the excretion of inactivated enzymes and glycoproteins of the gastrointestinal mucosa. Jansman *et al*. (1993b) showed that digestibility of all amino acids decreased linearly with increasing levels of tannin-rich faba

bean hull extracts. The amino acids which were affected to the greatest degree were proline and glycine. A linear positive relationship was also observed between the level of hull extract and the secretion of proline-rich proteins by the parotid glands.

8.6. Conclusions

In conclusion, dehulling resulted in a more nutrient dense product and a reduction in non-starch polysaccharides in faba beans, Australian sweet lupins and peas. Dehulled seeds of faba beans and peas had higher amino acid concentration and starch content. In faba beans, dehulling improved the ileal digestibility of starch and the AME. Removal of hulls had no effect on the starch digestibility and AME of peas. In general, apparent ileal amino acid digestibility coefficients of the three legumes were not influenced by the removal of hulls. Overall, the present data suggest that dehulling of grain legumes would be nutritionally beneficial and probably economical in view of the improved amino acid concentrations and available energy values.

CHAPTER 9
Influence of extrusion on the nutritional value of peas

9.1. Abstract

The influence of extrusion cooking on the nutritive value of peas was evaluated in *in vitro* and *in vivo* assays. In the *in vitro* assay, peas were included either as raw or extruded under combinations of two moisture conditions (19 and 22%) and three temperatures (120, 140 and 170^{o}C). The *in vivo* study included a maize-soy basal diet and three assay diets which contained either raw or two extruded pea samples. The assay diets were developed by substituting 25% of the basal diet (w/w) with raw and extruded peas. The results showed that extrusion markedly ($P < 0.05$ to 0.0001) influenced the contents of crude protein, non-starch polysaccharides, starch and trypsin inhibitor, but it had no effect ($P > 0.05$) on fat and ash contents. In general, the soluble non-starch polysaccharides and trypsin inhibitor contents of most extruded pea samples were higher ($P < 0.05$) than those of raw peas, but the insoluble and total non-starch polysaccharides decreased ($P < 0.05$) with extrusion. Moisture x temperature interaction was found to be significant ($P < 0.05$ to 0.001) in all parameters, except for fat, ash and starch. However for crude protein, there was no effect ($P > 0.05$) of barrel temperature on low feed moisture (19%), but at high moisture level (22%), the crude protein of extruded peas increased ($P < 0.05$) with the barrel temperature. Extrusion increased ($P < 0.05$) the *in vitro* starch digestibility of peas, but decreased ($P < 0.05$) the *in vitro* protein digestibility. In the *in vivo* assay, extrusion cooking increased ($P < 0.05$) the apparent ileal starch digestibility, but it had no effect ($P > 0.05$) on the apparent ileal protein digestibility and the apparent metabolisable energy of peas. These findings suggest that, under the conditions of the present study, extrusion cooking was not beneficial to improving the nutritive values of peas for broilers.

9.2. Introduction

Extrusion cooking is a process where the feed is subjected to mixing, shearing, and heating under high pressure before the extrudate is forced through a die (Sørensen *et al*., 2002). During this process, the feed may undergo reactions which could be beneficial, if nutrient availability is improved or detrimental if nutrients are destroyed or altered to become resistant to digestion.

Extrusion cooking may influence the nature of feed components by changing physical (e.g. particle size), chemical (e.g. starch gelatinization, inactivation of anti nutrients) and nutritional (e.g. nutrient digestibility) properties (Alonso *et al*., 2000a; El-Hady and Habiba, 2003; Diaz *et al*., 2006). Camire (2000a) reported that five general physicochemical changes can occur during extrusion cooking: binding, cleavage, loss of native conformation, recombination of fragments and thermal degradation. In addition, the composition of feed materials could be altered by physical losses such as leakage of fat and, evaporation of water and volatile compounds at the die.

The degree of change in feed constituents depends on a number of factors such as the type of ingredient or diet, particle size, type of extruder and the extruder conditions (e.g. moisture content, screw speed, barrel temperature, die diameter, feed rate, screw compression ratio, residence time, torque and pressure, energy input and pH) and type of reactants present, such as water, lipids, carbohydrate and proteins (Björck and Asp, 1983; Ilo *et al*., 1996; Grela *et al*., 2001; Anguita *et al*., 2006).

Appropriate processing temperature is critical for the elimination of heat-labile anti-nutritional factors found in legume seeds. In full-fat soybeans, Björk and Asp (1983) reported that trypsin inhibitor activity was reduced with increasing extrusion temperature and moisture content. At constant temperature, inactivation increased with the residence time and moisture content. In contrast, some studies have shown that trypsin inhibitor activity and some anti-nutrients such as tannins in peas and lupins were not inactivated, but even increased, after extrusion (Alonso *et al*., 2001; Masoero *et al*., 2005; Prandini *et al*., 2005).

In terms of amino acids, an increase in extruder temperature, screw compression ratio and screw speed has been reported to increase lysine degradation, whilst an increase in moisture content and die diameter had the opposite effect (Björck and Asp, 1983). Over-processing will also lower amino acid digestibility since amino acids may be destroyed or become unavailable due to the formation of indigestible complexes between reducing sugars and free amino groups in proteins.

On the other hand, extrusion has also been to shown to have positive effects on the digestibility of protein *in vitro* (Alonso *et al*., 2000b; El-Hady and Habiba, 2002), fat (Dänicke *et al*., 1998; Lichovnikova *et al*., 2004), amino acids (Lichovnikova *et al*., 2004) and starch (Alonso *et al*., 2000b; Masoero *et al*., 2005; Diaz *et al*., 2006) of grain legumes. The enhancement in nutrient digestibility after extrusion was probably due to the inactivation of enzymes and anti-nutritional factors, denaturation of native protein and gelatinisation of starch (Alonso *et al*., 1998; El-Hady and Habiba, 2003; Sheriff and Sajeev, 2005). In addition, extrusion inactivates or kills the microbes, thus rendering the feed material sterile and stable. The objectives of this study were to examine the effects of extrusion cooking on the chemical composition, nutrient digestibility and apparent metabolisable energy of peas.

9.3. Materials and Methods

9.3.1. Processing: Round seeded peas, purchased from a commercial supplier, were ground in a hammer mill to pass through a 3 mm sieve and then extruded in a twin-screw co-rotating self wiping extruder Clextral BC 21 (Firminy Cedex, France) with length/diameter ratio of 25, screw speed up to 600 rpm and outer screw diameter of 25 mm (Figure 9.1). The screw configuration from feed section to die consisted of three sections with forward elements. The first section had 4 elements (each 50mm length with 3 screw flights and 13 mm pitch); the second zone consisted 5 elements (each 50mm in length having 4 screw flights and 10 mm pitch); and the third zone had 5 elements (each 50mm in length with 6 screw flights and 7 mm pitch) The total length of the screw was 700 mm with 14 elements in three zones. The extruder was equipped with a bulk solids metering feeder (KTRON T20, Switzerland). A round die (3.0 mm diameter), equipped with a cutting device set at 130rpm, was used.

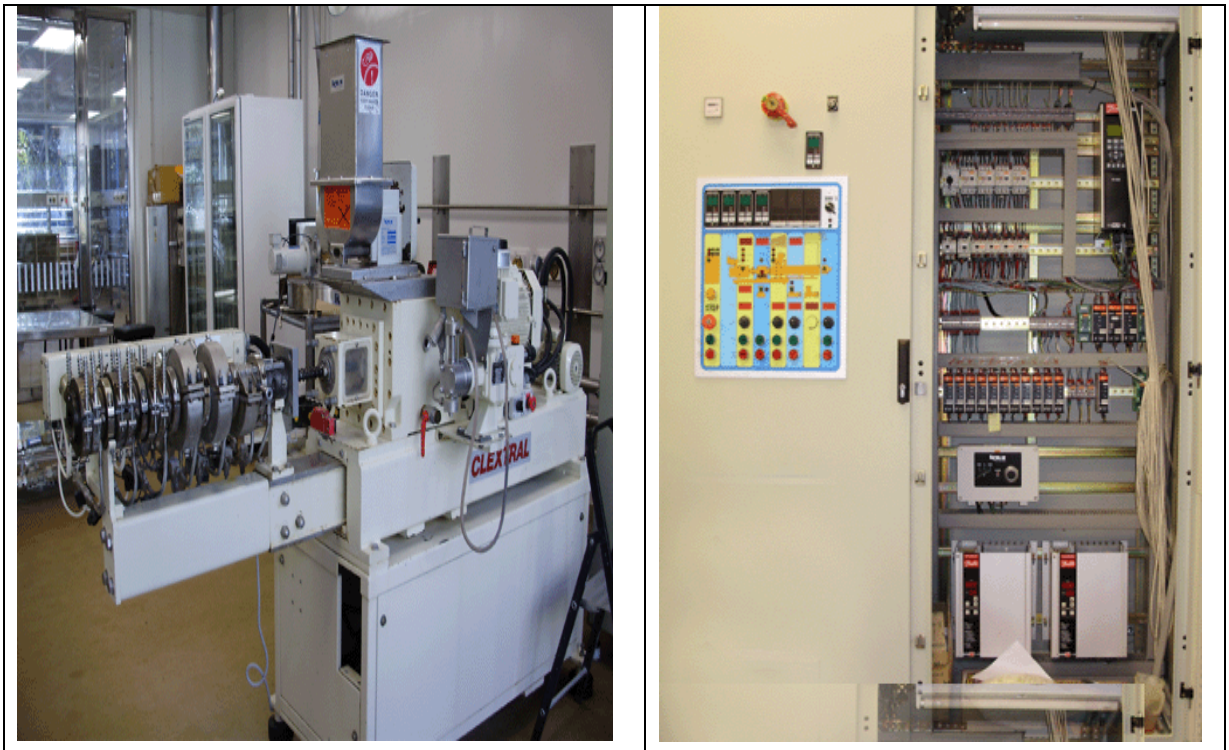


Figure 9.1. Extruder Clextral BC 21

Extrusion of peas was performed at three temperatures (120, 140 and 170^{o}C) and two moisture levels (19 and 22%) (Figure 9.2). The desired moisture levels were obtained by adding water prior to the extruder section by means of a pump. The water feed rate for obtaining the final moisture content of 19% was 0.50 kg/h, while 0.75 kg/h was used to achieve 22% final moisture content.

The optimum temperatures of the seven extruder sections from the feeder end were 50, 60, 70, 80, 100, 100 and 140^{o}C. The extruded materials were then allowed to cool to room temperature.

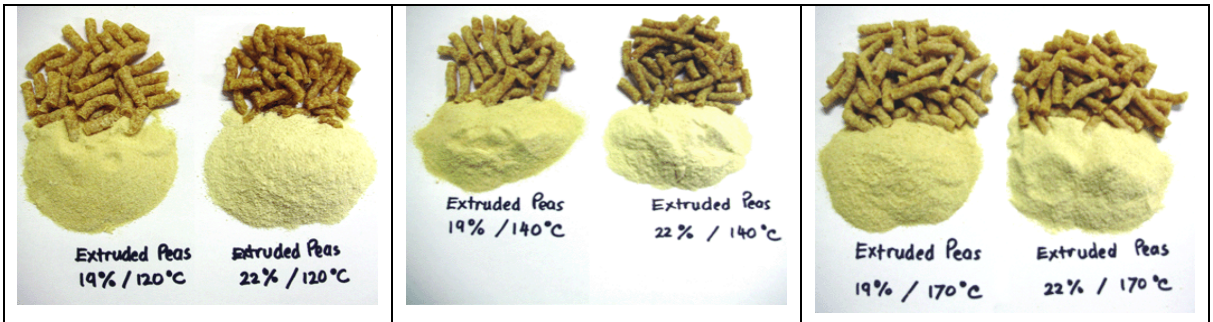


Figure 9.2. Pea extrudates

9.3.2. Experimental design:

9**.3.2.1. *In vitro* study:** Seven treatments consisting of raw-untreated peas and six extruded pea samples were assayed. Each treatment was replicated three times. The extruded materials were ground in a hammer mill to pass through a 0.5 mm sieve and then subjected to *in vitro* protein (Monro, J., Crop and Food Research Inc, New Zealand) and starch digestibility assays (Mishra et al., 2008). The procedures used in these *in vitro* assays are described in Appendix 1. *In vitro* protein and starch digestibilities were calculated using the following formula:

$$\text{Nutrient digestibility coefficient} = \frac{\text{(g nutrient sample- g nutrient residue)}}{\text{g nutrient sample.}}$$

9.3.2. 2. *In vivo* study

The experimental procedures were approved by the 'Massey University Animal Ethics Committee' (MUAEC 05/20 and 05/21) and complied with the 'New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes'.

9.3.2.2.3. Birds and housing: A total of 64 four-week-old male broilers (Ross 308) were used in the present study. The arrangement of birds and housing was similar to that described in Chapter 4 (Section 4.3.2).

9.3.2.2.4. Diets: Four treatment diets consisting of a maize-soy basal diet and three test diets containing raw and extruded pea meals were assayed. The pea meals were extruded at 140^{o}C and at two moisture level (19 and 22%). These processing conditions were selected since these were found to show the best nutritional properties in the *in vitro* study. The extruded peas were ground in a hammer mill to pass through a 3 mm sieve. The test diets were then formulated by substituting the raw and extruded pea meals for 25% (w/w) of the basal diet (Chapter 4, Table 4.1). All diets contained titanium oxide (3 g/kg), as an indigestible marker.

9.3.2.2.5. Excreta collection: The excreta collection was conducted as described in Chapter 4 (Section 4.3.4.).

9.3.2.2.6. Collection of ileal digesta: The digestibility assay procedures were the same as described in Chapter 4 (Section 4.3.5).

9.3.3. Chemical analysis: All analyses were conducted at least in duplicates and the results are presented on the dry matter basis.

9.3.3.1. Proximate analysis: Proximate analysis was conducted, as described in Chapter 4 (Section 4.3.6.4).

9.3.3.2. Starch and NSP: Starch and NSP contents were analysed, as described in Chapter 4 (Section 4.3.6.2 and 4.3.6.3, respectively).

9.3.3.3. Gross energy: Gross energy content was analysed, as described Chapter 4 (Section 4.3.6.4.).

9.3.3.4. Titanium oxide content: Titanium oxide analysis was conducted as described in Chapter 4 (Section 4.3.6.6).

9.3.3.5. Trypsin inhibitor: Trypsin inhibitor activity was determined, as described in Chapter 4 (Section 4.3.6.7).

9.3.4. Calculations

9.3.4.1. AME and AMEn: AME and AMEn values were calculated, as described in Chapter 4 (Section 4.3.7.1).

9.3.4.2. Digestibility: The apparent ileal digestibility of protein and starch was calculated, using titanium as the indigestible marker, as shown below:

$$\text{AIDC of diet} = \frac{\text{(Nutrient / Ti) diet} - \text{(Nutrient / Ti) ileal}}{\text{(Nutrient/Ti) diet}}$$

$$\text{AIDC of legume} = \frac{(\text{AIDC of legume diet} \times \text{nutrient in legume diet}) - (\text{AIDC of basal diet} \times 0.75 \times \text{nutrient in basal diet})}{0.25 \times \text{nutrient in test ingredient}}$$

Where, (Nutrient / Ti) diet = ratio of nutrient (protein or starch) to titanium in diet, and
(Nutrient / Ti) ileal = ratio of nutrient (protein or starch) to titanium in ileal digesta.

9.3.5. Statistical Analysis

The data from *in vitro* study were analysed by both one-way and two-way analysis of variance (ANOVA), using the General Linear Model procedure of SAS (1997). The data from *in vivo* study was subjected to one-way ANOVA. Differences were considered to be

significant at $P < 0.05$ and significant differences between means were separated by the Fisher's Least Significant Difference test.

9.4. Results

The chemical composition of the peas was significantly ($P < 0.05$) affected by extrusion, except for crude fat and ash (Table 9.1). Within extruded samples, the main effects (moisture and temperature conditions) and the interaction effect were significant ($P < 0.05$ to 0.0001) for most parameters, the exceptions being crude fat, ash, and starch contents.

The moisture content of the peas extruded with 19% / 120^{o}C and 22% / 120^{o}C operating conditions were higher ($P < 0.05$) than that of raw peas, whilst the other extruded peas (19% / 140^{o}C; 22% / 140^{o}C; 19% / 170^{o}C and 22% / 170^{o}C) had lower ($P < 0.05$) moisture content compared to raw peas. The crude protein content of extruded pea samples were similar ($P > 0.05$) to that of raw pea meal. A significant decrease ($P < 0.05$) in crude protein content, after extrusion, was only found in peas extruded at 22% / 120^{o}C operating condition.

The main effects of feed moisture and barrel temperature and the interaction between feed moisture and barrel temperature on crude protein content were found to be significant ($P < 0.05$ to 0.01). The crude protein content was increased ($P < 0.05$) by increasing the barrel temperature in the high moisture level (22%), whereas in the low feed moisture (19%), the crude protein content of peas extruded at 120 and 170^{o}C temperature did not differ ($P > 0.05$) from each other.

The effects of extrusion treatments on non-starch polysaccharides components were inconsistent, but the general effect of extrusion was to increase soluble non-starch polysaccharides and lower insoluble non-starch polysaccharides. In general, the total non-starch polysaccharides content was influenced ($P < 0.05$) by the extrusion. Within extruded samples, the main effects (feed moisture and barrel temperature) and the interaction on soluble non-starch polysaccharides were significant ($P < 0.05$). The soluble non-starch polysaccharides content was increased ($P < 0.05$) by increasing the barrel temperature in the high moisture level (22%). However, the increase was not significant ($P > 0.05$) between peas extruded at 140 ^{o} and 170 ^{o}C temperature.

There was an interaction ($P < 0.01$) between feed moisture x barrel temperature on insoluble and total non-starch polysaccharides. The insoluble non-starch polysaccharides was decreased by increasing the barrel temperature in the high moisture level (22%), whereas in the low feed moisture (19%), the insoluble non-starch polysaccharides of peas extruded at

140 and 170^{o}C temperatures did not differ ($P > 0.05$). Starch content was unaffected ($P > 0.05$) by extrusion. Trypsin inhibitor activity was influenced ($P < 0.05$) by extrusion cooking. Contrary to expectations, the trypsin inhibitor activity was increased ($P < 0.05$) by most extrusion treatments. A decrease ($P < 0.05$) of trypsin inhibitor activity was only observed in peas extruded at 19% / 120^{o}C. The feed moisture x barrel temperature interaction was found to be significant ($P < 0.01$) for trypsin inhibitor activity. The trypsin inhibitor activity of peas extruded at 120 and 170^{o}C in both low (19%) and high feed moisture (22%) was comparable ($P > 0.05$), but the observed values were higher than that of 140 ^{o}C.

In vitro protein digestibility (IVPD) values of all extruded peas were lower ($P < 0.01$) than that of raw peas. The highest reduction in IVPD was in treatment 22% / 120^{o}C. Feed moisture had no effect ($P > 0.05$) in IVPD, but there was an interaction ($P < 0.05$) between moisture content and barrel temperature on IVPD. The IVPD of peas extruded at 140 and 170 ^{o}C in high feed moisture (22%) did not differ ($P > 0.05$) from each other, but these values were higher than that determined for peas extruded at 120^{o}C. No differences ($P > 0.05$) were found between the IVPD of peas extruded at 120 and 140 ^{o}C, or between the IVPD of peas extruded at 120 and 170 ^{o}C in the low feed moisture (19%).

Extrusion cooking improved ($P < 0.0001$) *in vitro* starch digestibility (IVSD) (Table 9.1). The magnitude of improvement ranging from 56.9% was in treatment 22% / 170^{o}C to 59.5% for treatment 22% / 120^{o}C. The main effects of feed moisture and barrel temperature and the interaction between feed moisture and barrel temperature on IVSD were significant ($P < 0.05$ to 0.01). At the high moisture level (22%), there was a downward tendency ($P < 0.05$) of IVSD as the barrel temperature increased, but the IVSD of peas extruded at 120 and 140^{o}C was found to be similar ($P > 0.05$). On the other hand, at the low moisture level (19%), the IVSD of peas at 120, 140 and 170^{o}C did not differ ($P > 0.05$) from each other.

Table 9.1. The effect of extrusion treatments on the chemical composition (g/kg DM) and *in vitro* nutrient digestibility (%) of peas [1]

Extrusion parameter		Total moisture	Crude protein	Crude fat	Ash	Non-starch polysaccharides			Starch	Trypsin inhibitor (TIU/mg DM)	*In vitro* digestibility coefficient	
Feed moisture (%)	Barrel temperature (^{o}C)					Soluble	Insoluble	Total			Protein	Starch
Raw[2]	Raw	118^{c}	230^{abc}	25	31	23^{b}	177^{a}	200^{ab}	465	0.23^{c}	0.828^{a}	0.547^{d}
19[2]	120	129^{a}	234^{a}	27	32	18^{c}	168^{c}	186^{e}	462	0.29^{b}	0.796^{bc}	0.860^{b}
19[2]	140	99^{g}	226^{c}	25	31	25^{b}	172^{c}	197^{c}	460	0.25^{c}	0.807^{b}	0.862^{ab}
19[2]	170	112^{d}	232^{ab}	26	33	28^{a}	174^{b}	202^{a}	466	0.28^{b}	0.790^{cd}	0.858^{b}
22[2]	120	127^{b}	214^{d}	26	32	24^{b}	174^{b}	198^{bc}	462	0.38^{a}	0.778^{d}	0.872^{a}
22[2]	140	102^{f}	229^{bc}	26	31	28^{a}	166^{d}	194^{d}	461	0.19^{d}	0.794^{bc}	0.864^{ab}
22[2]	170	108^{e}	233^{ab}	26	32	29^{a}	156^{d}	185^{e}	463	0.24^{c}	0.802^{bc}	0.845^{c}
Pooled SEM		0.42	1.28	0.44	0.55	0.96	0.87	0.89	1.73	0.005	0.005	0.003
ANOVA[3]												
Feed moisture (M)		***	***	NS	NS	***	**	**	NS	***	NS	**
Barrel temperature (T)		***	**	NS	NS	*	***	***	NS	***	*	**
M x T		***	***	NS	NS	*	***	***	NS	***	*	*

[a,b,c] Means in a column with different superscripts differ ($P < 0.05$).

*Significant at $P < 0.05$; ** Significant at $P < 0.01$; ***Significant at $P < 0.001$.

[1]Each value represents the mean of three determinations.

[2]Analysed as one-way ANOVA

[3]Analysed as a two-way ANOVA

The effects of extrusion on the energy availability and nutrient digestibility in broiler chickens are presented in Table 9.2. No differences ($P > 0.05$) were noted between the AME, AMEn and ileal protein digestibility of raw untreated peas and pea extrudates. However, starch digestibility coefficients were markedly ($P < 0.05$) increased by extrusion treatments with 19% moisture having a similar ($P > 0.05$) starch digestibility coefficient to that with 22% moisture.

9.5. Discussion

Chemical composition: The results show that extrusion cooking of peas modified the chemical composition, except for crude fat and ash contents. The lack of effect of extrusion on the fat and ash contents in peas is in agreement with the findings of Alonso *et al*. (2001). In contrast, Diaz *et al*. (2006) reported that the fat and ash contents of peas were increased by 61 and 4%, respectively, following extrusion. The observed discrepancy may be due to the differences in the extruder type used. In the present study, a twin-screw extruder type was used, whilst in the study by Diaz *et al*. (2006), a single-screw extruder type was used. As reported by Björk and Asp (1983), the type of extruder is an important factor which affects the degree of modification in nutritional properties. Extrusion conditions are also important, but it was difficult to compare the effects of this aspect, because Diaz *et al*. (2006) did not clearly describe the conditions used in their study.

Crude protein content was not influenced by the extrusion treatments, the exception being the 22% / 120^{o}C treatment where the protein content was found to decrease. These findings were in general agreement with Alonso *et al*. (2000b). The reason for the decrease in crude protein content in the 22% / 120^{o}C treatment was unclear.

The increase in soluble non-starch polysaccharide with extrusion was in agreement with previous studies (Björck and Asp, 1983; Østergard *et al*., 1989; Vasanthan *et al*., 2002) and this may be attributed to the conversion of part of the insoluble non-starch polysaccharide to soluble non-starch polysaccharide. Lue *et al*. (1991) explained that the changes in the dietary fibre profile of grain flours after extrusion occur via the formation of starch which is resistant to enzymatic attack and also the macromolecular degradation of fibre increases its solubility.

Table 9.2 The effect of extrusion on the apparent metabolisable energy (AME, MJ/kg DM)), nitrogen-corrected AME (AMEn, MJ/kg DM), and apparent ileal digestibility coefficient (AIDC) of protein and starch of peas for broilers[1]

Extrusion parameter		AME	Pooled SEM	AMEn	Pooled SEM	AIDC of protein	Pooled SEM	AIDC of starch	Pooled SEM
Feed moisture (%)	Barrel temperature (^{o}C)								
Raw	Raw	12.3	0.64	11.7	0.54	0.828	0.085	0.865[b]	0.05
19	140	11.9	0.38	11.1	0.38	0.938	0.050	1.015[a]	0.02
22	140	12.2	0.74	11.1	0.58	0.803	0.058	0.986[a]	0.02

[1]Each value represents the mean of four replicates (4 birds / replicate).

[a,b]Means in a column with different superscripts differ ($P < 0.05$).

Extrusion cooking had no effect on the starch content. This finding disagreed with the previous studies (Prandini *et al*., 2005; Diaz *et al*., 2006) which showed a decrease in the starch content of peas extruded with a single-screw extruder. This variability was probably due to the difference in methodology, especially the type of extruder used. In the present study, a twin-screw extruder was used, whereas a single-screw extruder was used in previous studies. Perez-Navarrete *et al*. (2006) reported that a decrease in the starch content of extruded products was probably due to the formation of newly indigestible starch, which makes it difficult to be extracted by enzymes.

The improvement of trypsin inhibitor activity of peas after extrusion (except at 22% / 140^{o}C) was an unexpected result. These findings were in contrast with those reported by the previous researchers (Van der Poel, 1992; Kearns, 1994; O'Doherty and Keady, 2001; Diaz *et al*., 2006). In the study by Van der Poel (1992), the trypsin inhibitor activity content of pea cultivars (round- and wrinkle seeded peas) were reduced by extrusion at different processing temperatures (106 to 140^{o}C) and moisture contents (14 to 33%). However, the degree of inactivation was dependent on the processing condition and the cultivar used. The trypsin inhibitor activity inactivation of round-seeded peas was almost complete under the different processing conditions investigated, whereas the trypsin inhibitor activity in wrinkle-seeded pea was inactivated only at a higher temperature.

The increase of trypsin inhibitor activity determined in most extruded samples in our study may be due to the presence of trypsin-like protease activity (Domoney and Welham 1992; Domoney *et al*. 1993; James *et al*., 2005). It may be that since trypsin cleaves N-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA) on the carbonyl side of arginine to render a yellow solution (free p-nitro aniline), the trypsin-like activity observed could be due to a compound that also cleaves onto the carbonyl side of the arginine residue (James *et al*., 2005). The compound responsible for this trypsin-like activity may not be degraded by heat, unlike trypsin inhibitor activity, and thus it may appear as an augment in trypsin inhibitor activity in heat-treated or extruded samples.

***In vitro* and *in vivo* protein digestibility:** Extrusion resulted in significant reductions (2.5 to 6.5%) in the *in vitro* protein digestibility of peas. However, in the *in vivo* study, extrusion had no effect on ileal protein digestibility. This discrepancy suggests that the *in vitro* assay used was not a good tool for the assessment of protein digestibility in the animal. In addition, *in vitro* values are estimates and could not always precisely determine the real (biological) value of a nutrient.

The reduction in *in vitro* protein digestibility values obtained in the present study was in contrast with those reported by Alonso *et al*. (2000b). This discrepancy may be due to the differences in cultivar and methodology. It is also possible that the lack of improvement in the protein digestibility of pea protein after heat treatment could be due to protein aggregation (Alonso *et al*., 2000b; Wang, 2000; Meng *et al*., 2002; Carbonaro *et al*., 2005) and Maillard reaction (non-enzymatic browning-thermal cross-linking) (Vasanthan *et al*., 2002).

Nielsen *et al*. (1988) showed that complete degradation of heated legume proteins (phaseoline, vicilin, glycinin, and beta-conglycinin) did not occur even after 60 minutes of incubation. Unlike phaseolin, the other legume proteins (vicilin, glycinin, and beta-conglycinin) were found to be less completely digested by a variety of proteases in the denatured state than in the native state. Clemente *et al*. (2000) found that low digestibility of globulins has been related to their compact structure and intracellular location which hinder the susceptibility to proteolysis.

***In vitro* and *in vivo* starch digestibility:** The improvement of *in vitro* starch digestibility of peas after extrusion was in consistent with the published results by Alonso *et al*. (2000a). The improvement of starch digestibility both *in vitro* and *in vivo* in peas after extrusion was probably due to gelatinisation which increases the accessibility of starch to endogenous enzymes.

Native granule starch, which consists predominantly of α-glucan in the form of amylose and amylopectin, is hydrolysed slowly by α-amylase and amyloglucosidase compared with gelatinised starch in processed foods. When native starches are heated in excess water, the crystalline structure is disrupted and water molecules form hydrogen bonds to the exposed hydroxyl groups of amylose and amylopectin (Ratnayake *et al*., 2002; Tester *et al*., 2004). This causes an increase in granule swelling and solubility. Granule structure is completely lost and a thin paste or gel is formed, which makes the starch completely digestible by starch hydrolysing enzymes.

Ileal starch digestibility in the 19% / 140^{o}C treatment was calculated to exceed 100% (Table 9.2). This error may be due to the method of determination employed. The difference method was used in this study in order to calculate ileal digestibility and it assumes that there is no interaction between the basal diet and the test ingredient.

Apparent metabolisable energy: In the present study, extrusion cooking had no effect on the AME and AMEn of peas. In contrast, Breytenbach (2005) reported that the AME value of Australian sweet lupin decreased (8.61 vs. 7.52 MJ/kg) after extrusion with a single-screw extruder at a barrel jacket temperature of 120 ^{o}C. This decrease was attributed to

the increased bulkiness that occurred during expansion which leads to a decreased feed and energy intake. In contrast, Prinsloo (1993 cited by Breytenbach, 2005) demonstrated that the AME value of Australian sweet lupins for adult cockerels was increased by 11.5% (6.71 vs. 7.58 MJ/kg) by extrusion with a barrel temperature of 120 ^{o}C. These findings suggest that the extent of modification in the nutritional value of a feed ingredient via extrusion was dependent on the type of extruder, processing condition, type of ingredient and type of chicken.

It is noteworthy that although the ileal starch digestibility of peas markedly increased after extrusion, the apparent metabolisable energy values were unaffected. It is difficult to provide an explanation for this finding. Starch digestibility was determined at the ileal level, whereas the AME was determined at the excreta level. It is well documented that microbial activity in the hindgut of chickens has a marked influence on the utilisation of nutrients, including starch (Bedford, 1996; Wiseman, 2006).

9.6. Conclusions

In conclusion, extrusion cooking markedly influenced the chemical composition of peas. Extrusion increased ($P < 0.05$) the *in vitro* and *in vivo* starch digestibility of peas, but decreased ($P < 0.05$) the *in vitro* protein digestibility. However, improvements in starch digestion did not translate into any beneficial effect in terms of energy availability in peas for broilers.

CHAPTER 10
General Discussion

10.1. Introduction

The search for new protein sources for the New Zealand poultry industry has become urgent in recent years, due to a possible future ban on the use of animal protein meals and the escalating price of soybean meal in the world market. All soybean meal used in local feed formulations is imported and research into alternative protein sources which can partially or fully replace soybean meal is necessary in order to reduce the dependency of the local feed industry on imported ingredients.

The nutritional evaluation of grain legumes for poultry has been extensively researched elsewhere and (as reviewed in Chapter 2) a large volume of information is available on their nutritive value. However, these data are inadequate for accurate feed formulation under local conditions because of the well documented effects of cultivars, soil, climate and agronomic factors on the composition and utilisation of nutrients. No local data are currently available on the potential nutritional value of grain legumes for poultry. The overall aim of the research studies reported in this thesis was to evaluate various aspects of the feeding value of locally grown grain legumes. Based on agronomic potential, the focus of the research was on peas, faba beans and lupins (Chapters 3 to 9).

The general findings of this thesis suggest that grain legumes can be used as partial replacements for soybean meal and meat meal in practical broiler diets with no deleterious effects on bird performance. It was also found that energy availability in faba beans and lupins was improved by the removal of hulls. On the other hand, dehulling and extrusion had no beneficial effects on the nutritional value of peas. Future studies are warranted in order to evaluate the use of other technologies, such as enzymes, to further improve the nutritional and feeding values of grain legumes for birds. However, the economical aspects should be considered before any technology is employed.

10.2. Nutrient characteristics and protein quality of grain legumes

Data reported in Chapter 3 showed that there were no cultivar differences in the proximate and non-starch polysaccharide composition of chickpeas, peas and white lupins. Differences were only observed between the two cultivars of Australian sweet lupins. The proximate

composition of grain legumes is within the range reported in the literature (Jood *et al*., 1998; Perez-Maldonado *et al*., 1999; Ravindran *et al*., 2005).

Lupins were found to contain high levels of protein and fat, but their high non-starch polysaccharides contents would limit their inclusion levels in poultry diets. The moderate fat and starch contents of chickpeas and the high starch content of peas make these legume seeds good energy sources for poultry. The current findings confirmed that legume proteins are deficient in lysine, methionine, cystine and threonine. However, cereal proteins generally have higher concentrations of these amino acids and they will counter these deficiencies either partially or completely, depending on the diet formulation. Furthermore, the low cost of crystalline forms of methionine, lysine and threonine will also make it possible to balance practical diets for these amino acids.

No differences were observed in the protein quality, measured as protein efficiency ratio, between cultivars of the different grain legume species. The poor protein efficiency ratio, and higher relative pancreatic weights and mortality rate in chicks fed raw soybean diets are suggestive of the presence of high concentrations of anti-nutritional factors, especially protease inhibitors, in raw soybeans. On the other hand, the low mortality and the relative pancreatic weights in birds fed raw chickpeas, peas or lupins indicate that anti-nutritional factors levels found in the chickpea, pea and lupin cultivars grown in New Zealand are low.

10.3. Determination of apparent metabolisable energy and apparent ileal digestibility coefficient of amino acids in grain legumes for broilers

The data from Chapter 4 showed that the variability in metabolisable energy values between different legumes largely reflected the differences in starch, fat and non-starch polysaccharide contents. The lower apparent metabolisable energy value in the broad bean cultivar of faba bean may be due to the lower starch and relatively high non-starch polysaccharide contents in this cultivar compared to other cultivars. The apparent metabolisable energy values of faba beans and peas were higher than that of lupins, but lower than that of soybean meal. The nitrogen-correction apparent metabolisable energy of faba beans and peas, however, were comparable to that of soybean meal. The high non-starch polysaccharide content, especially the soluble fraction, was the major factor contributing to the low apparent metabolisable energy values in both lupin species. Soluble non-starch polysaccharide are known to increase digesta viscosity, modify the intestinal mucosa and change regulation of intestinal hormones, leading to reduced digestion and utilisation of nutrients (Smits and Annison, 1996; Choct,

1997). Considerable differences in the apparent ileal digestibility coefficient of amino acids were found between legume species, but the apparent ileal digestibility coefficient of amino acids was found to be similar in cultivars within each legume. The apparent ileal digestibility coefficient of amino acids of both lupins and peas were comparable to that of soybean meal. The variability in observed amino acid digestibility between species may be due to differences in amino acid concentrations and the levels of anti-nutritional factor, especially protease inhibitors and tannins.

10.4. The effects of feeding diets with grain legumes on performances and gross morphology of gastro-intestinal tract of broilers

Data in Chapter 5 showed that feeding broiler starters with maize-soybean based diets containing 200 g/kg faba beans, lupin and peas had no adverse effects on bird performance. These findings are consistent with previous studies (Bekrić *et al*., 1990; Perez-Maldonado *et al*., 1999; Farrell *et al*., 1999). However, in some studies (Steenfeldt *et al*., 2003; Viveros *et al*., 2007), the use of 200 g/kg Australian sweet lupins and white lupins was reported to reduce the growth rate and feed efficiency of broiler starters. Cultivar effects on performance parameters were not significant for any of the grain legumes. The improvements in excreta quality observed in birds fed faba bean diets were probably due to their relatively low NSP content. The effects of legume diets on the relative size of different segments of digestive tract were not consistent. The changes observed in some segments of digestive tract in birds fed grain legumes may be associated with the effects of dietary non-starch polysaccharide.

Data reported in Chapter 6 showed that the performance of birds fed diets which contained faba beans, white lupins and peas during the starter period (1-21 d) in floor pens confirmed the results obtained in Chapter 5. During the first week of the trial, the weight gain of broilers fed wheat-soy diet which contained faba beans and white lupins was significantly lower than those fed wheat-soy-meat meal diet which contained these legume seeds. However, no differences were observed in weight gain over 21 and 35 days of the trial period. The lower weight gain shown by young birds fed wheat-soy-meat-meal diet which contained faba beans and white lupins was due to the lower feed intake in this group compared to those fed a wheat-soy diet which contained these legume seeds.

The better performance of birds fed diets without meat meal was unexpected since the diets were balanced to contain similar levels of energy and digestible amino acids. The lower weight gain of birds fed diets with meat meal may be due to the poor quality of the meat meal used in the present study. The wide variability in the concentration and digestibility of amino

acids is well documented (Parsons, 1986; Ravindran *et al*., 1999). The digestible amino acid matrix values used to formulate meat meal diets may have overestimated the actual digestible amino acid values in the meat meal used. The litter quality of birds over the 35-day period of the trial was not affected by dietary treatments.

10.5. The effects of methodology of determination on apparent ileal digestibility coeffcient of amino acids of feed ingredients

Data reported in Chapter 7 showed that amino acid digestibility values are influenced by the method of determination. The apparent ileal digestibility coeffcient of amino acids in feed ingredients determined with the direct method was lower than those determined with the difference method. When each ingredient separately was analysed separately, the differences were significant only for some indispensable amino acids (Table 7.3 to 7.7). However, when the ingredients were all put together (Table 7.8), the main effect of methodology significantly affected the apparent amino acid digestibility of mostly the amino acids. These findings are in agreement with those of Fan and Sauer (1995a) who determined the apparent ileal amino acid digestibility of barley and canola meal for growing pigs by the direct and difference methods, and they reported that the digestibility values in low protein feed ingredients should be determined by the difference method rather than with the direct method.

The underestimation of apparent ileal digestibility coeffcient of amino acids in low-protein ingredients by the direct method may be explained by the greater proportions of amino acids from endogenous sources, relative to amino acids of dietary origin, in digesta at low dietary amino acid intakes (Ravindran and Bryden, 1999). Fan *et al*. (1994) reported that the apparent ileal amino acid digestibility value increased with the increasing dietary amino acid intakes.

When the direct method is employed, one can expect that the dietary levels of amino acids, especially the limiting ones, will be lower than their respective upper limit levels. As a result, small differences in the dietary contents of these amino acids will elicit relatively large changes in their apparent ileal digestibilities. The higher inclusion level of cereals and grain legumes in the test diets formulated with the direct method may be expected to further exacerbate this effect. The present data, along together with those of Fan and Sauer (1995a), suggests that the difference method should be used in order to determine the apparent ileal digestibility coeffcient of amino acids in low and medium protein ingredients.

10.6. The effects of dehulling on the apparent metabolisable energy and apparent ileal digestibility coeffcient of amino acids of grain legumes for broilers

Data from Chapter 8 showed that the dehulling of grain legumes was beneficial in terms of nitrogen-corrected apparent metabolisable energy (except in peas) and amino acid concentrations. In general, the apparent ileal digestibility coefficient of amino acids was not influenced by dehulling. The marked improvements in the nitrogen-corrected apparent metabolisable energy of lupins (27%) and faba beans (15.4%) may be explained by the reduction in indigestible fibre which resulted in a more nutrient-dense product. These findings are consistent with the proportion of hulls in these legume seeds. The largest proportion of hulls was determined in lupin seeds (299 g/kg seed), followed by faba beans (148 g/kg seed). However, the greatest decrease in soluble, insoluble and total non-starch polysaccharide was observed in faba beans, followed by lupins and peas. The removal of hull had no effect on the apparent metabolisable energy of peas, which is consistent with the lower proportion of hulls (89 g/kg) in pea seeds.

The starch content and starch digestibility of faba beans were increased by 13.8 and 12.6%, respectively, following the removal of hulls. These data suggest that even though dehulling had no effect on the apparent ileal digestibility coefficient of amino acids of faba beans, lupins and peas, it is still nutritionally beneficial and probably economically beneficial in view of the improved amino acid concentrations and energy values.

10.7. The effects of extrusion on the nutritional values of peas

Data reported in Chapter 9 indicated that extrusion markedly changed the proximate and fibre composition of peas, except for fat and ash contents. The effects of extrusion on non-starch polysaccharide components were not consistent, but the general trend was to increase the soluble non-starch polysaccharide and lower the insoluble non-starch polysaccharide. The significant decrease of soluble non-starch polysaccharide of peas after extrusion was only found in the low barrel temperature and low feed moisture. However, The insoluble non-starch polysaccharide decreased by increasing the barrel temperature at the high moisture level. It is known that the changes in the non-starch polysaccharide profile of grain flours after extrusion occurs via the formation of starch resistant to enzymatic attack and the macromolecular degradation of fibre increases its solubility (Lue *et al*., 1991).

Interestingly, dehulling of peas decreased soluble and total non-starch polysaccharide (Table 8.3), whilst extrusion increased soluble non-starch polysaccharide and decreased total non-starch polysaccharide. Contrary to expectations, the trypsin inhibitor activity was

increased by most extrusion treatments. The increase of trypsin inhibitor activity in most extruded samples was probably due to the presence of trypsin-like protease activity (Domoney *et al*. 1993; James *et al*., 2005; Morrison *et al*., 2007).

The improvement in both *in vitro* and *in vivo* starch digestibility after extrusion may be explained by increased gelatinisation which increased the accessibility of starch to endogenous enzymes. It is noteworthy that although the starch digestibility of peas increased after extrusion, the apparent metabolisable energy values were unaffected. It is difficult to provide an explanation for this finding, but the observed anomaly may be related to the site of measurement. Starch digestibility was determined at the ileal level, whereas the apparent metabolisable energy was determined at the excreta level. It is well documented that microbial activity in the hindgut of chickens has a marked influence on the utilisation of nutrients, including starch (Bedford, 1996; Wiseman, 2006). Extrusion decreased the *in vitro* protein digestibility, but it did not affect the ileal protein digestibility. These observations highlight the limitation of *in vitro* assays. Although the *in vitro* test is an easier and rapid technique, it is only an approximation of true nutrient digestibility and it is not as accurate as the *in vivo* method.

10.8. Factors influencing the apparent metabolisable energy, starch, protein and amino acid digestibilities

The research reported in this thesis demonstrates that the apparent metabolisable energy values of grain legumes are affected by a number of factors, including cultivars (except in Australian sweet lupin and peas (Tables 4.7 and 4.9) and species of grain legumes and dehulling. Faba beans and peas, which were rich in starch and low in non-starch polysaccharide content, had higher apparent metabolisable energy values compared to lupin species which had higher fat contents, but they also had high non-starch polysaccharide content. The apparent metabolisable energy values of faba beans and lupins were markedly increased after dehulling. However, the apparent metabolisable energy of peas was not influenced by dehulling or extrusion. The improvement of apparent metabolisable energy of faba beans following dehulling was associated with increased starch digestibility and reduced non-starch polysaccharide levels after dehulling.

It is evident from this thesis that starch digestibility was affected by non-starch polysaccharide levels and feed processing. However, the degree of improvement in starch digestibility was dependent on the type of grain legumes and feed processing applied. For example, dehulling significantly improved starch digestibility in faba beans (Table 8.4), but

not in peas (Table 8.6). The starch digestibility of peas, on the other hand, was increased after extrusion (Tables 9.1 and 9.2). The improvement of starch digestibility in faba bean through dehulling was probably due to the elimination the indigestible fibre component which was mainly found in the hulls. In the case of peas, the improvement of starch digestibility after extrusion may be explained by starch gelatinisation which increased the accessibility of starch to endogenous enzymes. As reported by Weurding *et al*. (2001) and Wang (2005) that due to its crystallinity, native starch is hydrolysed very slowly by α-amylases and amyloglucosidase compared to amorphous starch which is rendered by mechanical, chemical or heat treatments (Tester *et al*., 2004).

On average, the ileal starch digestibility of raw faba beans and peas found in this thesis was about 80%. Weurding *et al*. (2001) reported that on average, the percentage of pea and faba bean starch digested before the ileum and before the posterior ileum was 70-71 and 91-92%, respectively. According to these authors, the incomplete starch digestion from grain legume starch was due to a combination of a slow starch digestion rate and relatively short retention time in the gastrointestinal tract of broiler chickens.

It was also demonstrated in this thesis that ileal digestibility of amino acids was affected by the type of grain legumes and methodology of determination. Factors such as thermal treatment, anti-nutritional factors, susceptibility of protein per se to proteolytic action can contribute to lower protein digestibility in feed ingredients (McNab, 1975; Ikeda *et al*., 1991). Björck and Asp (1983) reported that mild heat treatment of vegetable proteins generally improves digestibility due to inactivation of protease inhibitors and other antiphysiological substances. However, with the increasing severity of heat treatment, protein digestibility will decrease. In contrast, data reported in Chapter 9 demonstrated that ileal protein digestibility of peas was not affected by extrusion.

10.9.Summary and main conclusion

The research presented in this thesis investigated the nutritional value of selected locally grown grain legumes and this evaluation included characterisation of the nutrient profile and protein quality, the effect of cultivar on the apparent metabolisable energy and apparent ileal digestibility coefficient of amino acids for poultry, the effect of method of determination on the apparent ileal digestibility coefficient of amino acids; the feeding value in practical broiler diets; and the effect of the application of feed processing technology. Overall, the present data demonstrate the potential of grain legumes as protein sources in poultry diets.

The legume proteins had balanced amino acid profiles, with the exception of sulphur-containing amino acids (methionine and cystine) and high digestibility of amino acids. The problem of methionine deficiency can easily be solved by supplementing legume- based diets with commercial crystalline amino acids. Grain legumes are also useful sources of energy for broilers based on their starch (in chick peas, faba beans and peas) and fat (in lupins) contents. However, the energy content is not fully available to birds due to their non-starch polysaccharide content. In the present study, dehulling of grain legumes was found to improve the apparent metabolisable energy value of faba bean and lupins. The use of appropriate non-starch polysaccharide enzymes, which target specific non-starch polysaccharide components in grain legumes, may be another option to further improve the apparent metabolisable energy. Currently, however, no commercially successful exogenous enzymes, which target legume non-starch polysaccharide, are available and further studies are warranted in this context.

In conclusion, the findings of this thesis suggest that grain legumes can be used as partial replacements for soybean meal and animal protein meals in practical poultry diets. In our studies, the level of inclusion of legumes was restricted to 200 g/kg. However, higher levels of inclusion may be possible, especially with the use of enzymes and further processing (dehulling and extrusion). In this context, the evaluation of combinations of processing technologies, including particle size, enzyme supplementation, fermentation, dehulling and extrusion, may be useful in to improve the apparent metabolisable energy and amino acid digestibility- and bird performance. However, it should be borne in mind that whatever the processing technology is applied, the economic aspects should be fully considered.

In addition, although the purpose of this research was to evaluate the nutritional value of grain legumes for poultry, the experimental work described in Chapter 4 to 9 was conducted with male Ross 308 chickens. Therefore, it is important to be born in mind that although the results are probable to be applicable to certain extent to other types of female broiler chickens and other poultry, the conclusions drawn are eventually restricted to male broilers. This restriction is based on the published data reported in Chapter 2 that sex and genotype of birds could affect the nutritional value of grain legumes due to their differences in biochemical and physiological functions, which could affect digestion and absorption of nutrients. Consequently, results need to be tested before their application to female broilers and other types of poultry.

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APPENDICES

1. *In vitro* protein digestibility

In vitro protein digestibility was determined using the modified method of DR. J. Monro from Crop and Food Research, New Zealand. The procedures are as following:

1. One g finely ground sample was weighed into 50-mL blue-screw bottle in duplicate.
2. Add 5 ml reverse osmosis water and incubate in a water bath 40 ^{o}C for 30 minutes.
3. Added 0.35 ml 1M HCl, mix (check the pH, 2.7 - 2.9), and this was followed by 1 ml pepsin solution (4560 Unit) (containing 10 mg pepsin).
4. The enzyme mixture is then incubated in water bath (40 ^{o}C) for 1 hour.
5. Add 0.30 mL 1M NaOH to give pH 6 (5.9-6.10) and add 1 ml 5% pancreatin solution, then incubate in 40^{o}C for 2 hours.
6. Add 1 mL 40% sulphosalicylic acid (to precipitate soluble but undigested protein, so that all undigested protein is recovered and a more complete separation of undigested from digested protein is achieved).
7. Vortex, and allow to stand for 30 minutes.
8. After 30 minutes, centrifuge at 2500 g for 20 minutes.
9. Discard supernatant and resuspend in 10 mL 2% sulphosalicylic acid.
10. Centrifuge at 2500 g for 20 minutes.
11. Discard supernatant.
12. Fill tube with 10 mL 95% ethanol.
13. Vortex, and centrifuge 2x (at 2500 g for 20 minutes).
14. Discard supernatant and add 10 ml aceton (do 2x).
15. Mix and then centrifuge at 2500 g for 20 minutes.
16. Discard supernatant and dry residue overnight in water vacuum (65^{o}C).
17. Weigh the residue. The dried residues are then analysed for N for N digestibility.

2. *In vitro* starch digestibility

In vitro starch digestibility was determined using the modified method of Mishra *et al*. (2008). The procedures are as following:

1. One g finely ground sample was weighed into 50-ml blue-screw bottle in duplicate.
2. Add 5 mL reverse osmosis water and incubate in a water bath 40^{o}C for 30 minutes.
3. Add 0.35 ml 1M HCl, mix (check the pH, 2.7 - 2.9), and this was followed by 1 ml pepsin solution (4560 Unit) (containing 10 mg pepsin).
4. The enzyme mixture is then incubated in water bath (40^{o}C) for 1 hour.
5. Add 0.30 ml 1M NaOH to give pH 6 (5.9-6.10).
6. Add 1 ml 5% pancreatin solution, then incubate in 40^{o}C for 2 hours.
7. Centrifuge at 2500 g for 20 minutes.
8. Leave the residue (a) in the tube and decant supernatant to another falcon tube. Measure the amount of supernatant and make to 80% ethanol, then stand for 1 hour.
9. Collect precipitate (b) by centrifuging (2500 g, 20 minutes).
10. Combine residue (a) and precipitate (b), and wash with 80% ethanol (2x) and acetone, then dry in the vacuum oven (70^{o}C, 5 hours).
11. Reweigh the tube. The dried residues were then analysed for starch content of residue (Megazyme starch assay kit) for starch digestibility.