A comparison by Hennig *et al.* (1991) showed significantly higher digestibility coefficients for organic matter, crude carbohydrates and nitrogen-free-extract when determined using ileo-rectal anastomosis (end-to-side) than ileo-caecal cannulation. No difference was shown for digestibility of crude protein or fat between the two techniques. Despite these differences in macro-nutrient digestibility, only 11% (20 out of 180) of estimates of amino acid absorption varied by more than 5% between the techniques.

Donkoh *et al.* (1989) reported no significant difference in digestibility coefficients for nitrogen, lysine, threonine, histidine or methionine when ileal digesta were collected by either a simple T-piece cannulation or by the slaughter method (digesta collected from terminal 20 cm of the ileum).

2.4.3 Indigestible markers

Some techniques which have been developed for collecting ileal digesta only permit partial sampling. It is therefore necessary to include an indigestible marker (which can be readily assessed in digesta by the laboratory) in the experimental diet at a known concentration. Laboratory assay of ileal digesta for this marker will allow the concentration of each nutrient in the digesta to be calculated, and then related to the ileal digestibility (absorption) of the nutrient from the experimental diet. Ileal digestibility may be calculated using the following relationship:

$$\text{Ileal digestibility} = \frac{\text{dietary nutrient} - (\text{ileal nutrient} \times \text{dietary marker/ileal marker})}{\text{dietary nutrient}}$$

It is clear from this relationship, that the accuracy with which ileal digestibility is calculated, is dependant upon the accuracy with which the indigestible marker mimics the passage of the test nutrient through the digestive tract of the pig to the point of collection.

Indigestible markers such as barium sulphate (detected radiographically), chromic oxide and ferric oxide (detected chemically and visually), acid insoluble ash, polyethylene glycol (detected chemically), stained food particles and beads or pellets of various materials (recovered mechanically from a slurry of the faeces, and counted), have been used to determine nutrient digestibility. The accuracy with which these various markers reflect digestibility of the experimental feedstuff is largely related to: a) the rate and uniformity of passage of digesta throughout the digestive tract, b) the composition of the diet, and c) the site at which the collection is made.

Use of markers to determine the rate and uniformity of food passage through the gastrointestinal tract has several limitations. Argenzio and Southworth (1974; as cited in Kidder and Manners, 1986) found that particles of different sizes travel at different speeds through the digestive tract, and that fluid in the digesta may travel faster than solid matter. Reports have shown a range of 15-24 hours between ingestion of a marker and initial appearance in the faeces of growing and mature pigs. Other workers have found longer passage times (24-30 hours) with suckled piglets (Kidder and Manners, 1986).

The duration between when a marker is ingested, and when faeces become free of it, is quite variable (40-90 hours) and may be due to diet characteristics and/or the sensitivity of the detection method (Kidder and Manners, 1986). Upon ingestion, markers may be diluted with existing gut contents, leading to uneven passage through the gastrointestinal tract. Studies by Carillo and Benavides (1971; cited by Kidder and Manners, 1986) found that 5% and 95% of the chromic oxide fed to 40kg pigs, was excreted within 18.8 and 66.7 hours respectively in a molasses diet, and 23.3 and 63.8 hours respectively in a maize diet.

Chromic oxide (Cr_2O_3) has been used widely in porcine digestibility studies. The extreme insolubility associated with chromic oxide makes it very suitable as an indigestible marker, however this characteristic also makes laboratory assays quite difficult (Kidder and Manners, 1986). Reports of complete chromium recovery have appeared in the literature, however, many techniques for recovery also exist, making between methodology comparisons less valid.

Small scale experiments reported by McCarthy *et al.*(1973) (cited by Kidder and Manners, 1986), using acid insoluble ash, have shown that apparent nutrient digestibility coefficients calculated using ash (which is insoluble in 4N HCl) in feed and faeces was closer to coefficients determined in the same animals by total collection than when chromic oxide was used as a marker.

Other studies (Jongbloed, 1993) have compared two dietary levels (0.5 g kg^{-1} and 10 g kg^{-1}) of chromic oxide with HCl-insoluble ash. It was found that for feeds, the coefficient of variation for both sampling and chemical analysis was about one half at the lower dietary concentration of chromic oxide than at the higher level, and that for the chemical analysis of faeces, the coefficient of variation was also halved. When the diluted chromic oxide treatment was compared with HCl-insoluble ash, variances of digestibility coefficients for dry matter, nitrogen, calcium and phosphorous were lower for the chromic oxide treatment than for HCl-insoluble ash.

Both liquid (Co-EDTA, 5 g kg^{-1}) and solid (Cr-NDR, 4 g kg^{-1}) phase markers were used by Jongbloed (1991) to compare duocenal, ileal and overall digestibility of dry matter, phytic acid and phosphorous in pigs. It appears from this study that by using Cr-NDR the apparent digestibility of dry matter, total P, and phytic acid measured in the duodenum were somewhat overestimated, whereas by using Co-EDTA the relative measurements were underestimated. Ileal digestibility coefficients for each nutrient were lower in a maize/soya-bean diet using Co-EDTA as a marker, whereas in a

tapioca/maize-gluten diet the values were similar for each marker. Overall digestibility of dry matter and total phosphorous in both diets were also lower when using Co-EDTA as a marker. The results obtained by Jongbloed (1991) were inconclusive regarding the appropriate marker for use in determining ileal digestibility.

Kohler *et al.* (1990) used both chromic oxide and titanium dioxide as solid phase markers and Co-EDTA as a liquid phase marker in a conventional (maize/barley/wheat) diet, a pectin-rich diet, a crude fibre-rich diet and a semisynthetic diet. Chromium recoveries were higher in both the semisynthetic (mean = 98.5%) and conventional diets (mean = 91.6%) than either the pectin-rich (mean =77.8%) or crude fibre-rich (mean = 72.5%) diets. The recovery of Co-EDTA was higher than chromium recovery in all but the semisynthetic diets, in which case recoveries were similar. Titanium recovery was only determined for the semisynthetic diet, however, in this case recoveries were less than for either of the other markers. These studies indicate that the recovery rate of chromium depends upon the diet used. Diets high in crude fibre and pectin yield a lower recovery. Co-EDTA recovery also appears related to dietary fibre and pectin levels, however recoveries indicate some benefits through the use of Co-EDTA as a marker in conventional, pectin-rich and crude-fibre-rich diets.

2.4.4 Endogenous secretions and microbial biomass

Endogenous digestive secretions and cells sloughed from the lining of the small intestine (Batterham, 1992b) and bacterial loss of amino acids are of considerable importance when estimating ileal amino acid digestibility in the pig. Bacterial protein contributes less to the amino acid content of digesta collected at the terminal ileum than endogenous amino acids, however, both endogenous secretions and bacterial biomass are significant contributors to estimates of amino acid digestibility at the terminal ileum. Endogenous losses and bacterial biomass have been shown to contribute 30-90% of the total nitrogen in ileal digesta, depending upon the feedstuff being assessed (Low, 1990).

Many factors have been cited to influence endogenous protein secretion, including dry matter and protein intake (Krawielitzki, 1977; cited by Kidder and Manners, 1986), protein quality and structure (Gebhardt et al., 1981; cited by Kidder and Manners, 1986), level and composition of crude fibre (non-starch polysaccharides increase endogenous secretions) and the content of anti-nutritional factors (Sauer and Ozimek, 1986). Endogenous secretions are highly variable, and in many cases are feedstuff and diet specific. Often endogenous secretions attributable to an ingredient will vary when other ingredients are combined with it in a diet. When formulating diets, it is important to realise that endogenous secretions are amino acids which are unavailable to the pig if not absorbed by the time they reach the distal end of the small intestine. These secretions are nutrient "loss" which vary according to the feedstuff used. On this basis, a sound argument exists for attributing endogenous loss (of amino acids) to individual feedstuffs as a nutritional "cost" of using that feedstuff.

Endogenous amino acid secretions have been traditionally determined by measuring the amount of each amino acid in the ileal digesta of pigs offered a protein-free diet ("direct" method), or by aid of regression to zero amino acid intake using a series of levels of the test source of amino acids (Sauer and Ozimek, 1986)("indirect" method). More recent methods have been developed, in which lysine side chains in dietary protein are guanidinated to form homoarginine. Homoarginine is digested and absorbed by the pig but is not used for protein synthesis (until transformed to lysine) and does not reappear in endogenous secretions. Estimated endogenous secretions have been shown to be lower in pigs offered protein-free diets compared to pigs intravenously infused with a balanced blend of amino acids, indicating that protein status of the pig will also affect endogenous secretions (Low, 1990).

2.4.5 "Apparent" vs "true" vs "real" ileal digestibility

Apparent ileal digestibility of amino acids may be defined as the difference between the quantity of an amino acid consumed by a pig, and the content of the amino acid

within digesta collected at the terminal ileum. Apparent digestibility is therefore measuring the digestibility of amino acids in a feedstuff less the total contribution of amino acids by endogenous secretion and bacterial biomass (Batterham, 1992b).

As discussed earlier, endogenous secretions vary from feedstuff to feedstuff and are affected by many parameters (dietary fibre, protein status of the animal, other dietary ingredients etc.). The amount and amino acid composition of endogenous secretion will therefore have a significant affect on the apparent digestibility of amino acids in a particular feedstuff, and furthermore, this estimate may vary considerably from experiment to experiment as a result of varying methodology.

Apparent digestibility values which are corrected for endogenous contribution of amino acids to the digesta (using any of the methods discussed earlier for estimation of endogenous loss) results in an estimate of digestibility known as "true" digestibility. True digestibility is the proportion of the ingested amino acids in a feedstuff which is actually digested and absorbed from the feedstuff. True digestibility values are calculated on the assumption that endogenous secretion is unaffected by feedstuff, dietary fibre or other parameters, however this assumption, as discussed earlier can be questioned (deLange *et al.*, 1989).

"Real" digestibility is a term which refers to a more recent improvement to the methodology associated with determining true digestibility. This method involves radioactive labelling of a feedstuff with the stable-isotope ^{15}N, which provides a clear differentiation between non-digested dietary and endogenous protein (deLange *et al.*, 1989). Real digestibility coefficients are therefore derived from a more definitive estimate of total endogenous secretion than the techniques used to calculate true digestibility. ^{15}N labelling is the only method available for determining the endogenous protein secretions when protein-containing diets are used. The ^{15}N technique is limited in that only total endogenous protein secretion can be determined. Assumptions of

amino acid composition of the endogenous protein must be made in order to calculate real digestibility. An accurate estimate of amino acid composition of endogenous protein secretion is crucial for accurate calculation of real digestibility using the ^{15}N technique.

Experiments by Krawielitzki *et al* (1977; cited by Sauer and Ozimek, 1986) in which dietary protein was labelled with the ^{15}N isotope, demonstrated that traditional estimates of endogenous protein loss were underestimating secretion (Sauer and Ozimek, 1986). He found that the apparent vs true (direct method) vs real protein digestibility for soyabean, barley and corn were 86 vs 90 vs 97%, 77 vs 85 vs 96% and 74 vs 85 vs 97% respectively.

True digestibility is a fundamental property of a feedstuff, and for this reason (if endogenous losses are accurately determined) is an excellent comparison of absolute nutrient digestibility between feedstuffs. However, apparent digestibility coefficients, when determined in diets of similar composition to those used in practice, will provide clear estimates of the net digestible nutrient benefit to the pig, and hence provide a more practical comparative measure of the nutritive value of available feedstuffs than true digestibility.

2.4.6 Factors affecting determination of apparent ileal digestibility

2.4.6.1 Dietary protein content and plasma amino acid status of the pig

It has been demonstrated that the apparent digestibility of amino acids increases exponentially as the dietary protein contribution from the test ingredient increases. In the case of soyabean meal (Sauer *et al.*, 1989), it was shown that this continued until a dietary crude protein (from soyabean meal) of 15-16% was attained (figure 6). This improvement in apparent digestibility is explained by reference to endogenous protein secretions as a proportion of the total amino acid content of the digesta. As the overall concentration of amino acids contained in the experimental diet decreases,

endogenous secretions and microbial biomass will contribute proportionally more amino acids to the ileal digesta than the indigestible amino acids contributed by the test ingredient. In this case, apparent ileal digestibility of amino acids will tend to decrease. This situation is further compounded when test ingredients of high digestibility are included at low levels in experimental diets.

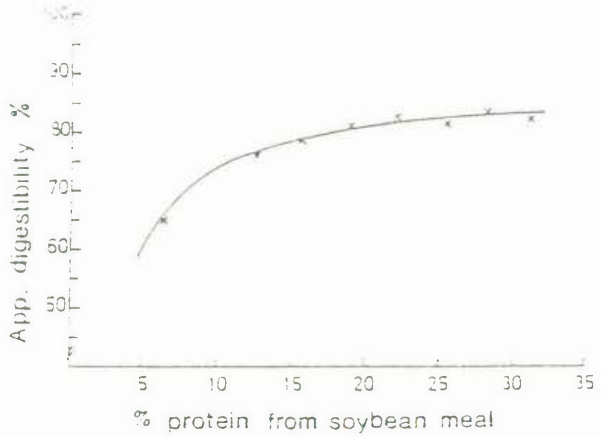

Figure 6. Apparent protein digestibility of soyabean meal at differing dietary protein levels (Sauer et al., 1989)

The plasma protein status of the pig will also affect endogenous amino acid secretion. deLange *et al.* (1989) found that endogenous protein decreased significantly (18.5 to 12.7 g kg^{-1} dry matter intake) when pigs were intravenously infused with a mixture of amino acids. No significant decrease in individual amino acid secretion was observed except for proline, however up to 7.4% difference in threonine composition of the endogenous secretions were noted between pigs offered a protein-free diet with and without intravenous infusion of amino acids.

2.4.6.2 Feed intake

Ileal collections using cannulation are often performed under restricted feeding conditions, as this tends to reduce blockage of the device. Sauer *et al.*(1989) reported no difference in ileal digestibility (ileo-caecal re-entrant cannula) of amino acids by barrows within the growth phase 50 to 70 kg when offered a barley-soyabean diet (19.4% crude protein) at either 1.4, 2.1 or 2.8% of body weight. Haydon *et al.* (1984) offered a 16% sorghum-soyabean diet at either *ad libitum*, 3 or 4.5% of body weight to pigs fitted with a simple T-cannula . Those pigs fed restrictively were offered the diet at 12 hour intervals. Pigs fed *ad libitum*, consumed feed equal to approximately 6% of body weight per day. Neither feeding method nor level greatly affected amino acid digestibility, however, coefficients tended to decrease as feeding level decreased.

2.4.6.3 "Direct" vs "difference" method of calculating digestibility

Where possible, apparent ileal digestibility of amino acids is determined by offering pigs a diet in which the test protein is the only source of amino acids in the diet (van Weerden *et al.*, 1987). This is known as the "direct" method and may be used when the experimental diet is comprised primarily of the test feedstuff, or in diets where a high proportion of non-protein feedstuff (such as sucrose) is included with the test protein. The latter of these cases is useful when assessing protein meals which contain anti-nutritional factors such as tannins (Anderson, 1985) and trypsin inhibitors. Anti-nutritional compounds have been shown to hamper amino acid digestibility, and/or make the feed unpalatable if offered at a high inclusion rate in the diet.

The "indirect" method is used when two or more protein feedstuffs are included in the experimental diet. For example, when the crude protein level of a test feedstuff is low, the apparent ileal digestibility of its constituent amino acids will be underestimated (as discussed above), unless another higher protein ingredient is also included in the diet. When using this technique a dietary treatment containing only the second feedstuff

must be included in the experiment so that digestibility of the test feedstuff may be calculated by difference (Sauer *et al.*, 1989).

When using either the direct or indirect method, it is important that the test ingredient be included at maximum rates. Some experiments (van Weerden *et al.*, 1987) indicate a small (3%) but significant effect on amino acid digestibility (calculated by the indirect method) when test feedstuffs of low digestibility are included at 10% of the diet compared to 30%.

Imbeah *et al.* (1988) reported a significantly ($P<0.05$) higher ileal digestibility of lysine in barley-canola meal diets (0.70 vs 0.62) when assessed by direct than indirect methods respectively, however their studies generally demonstrated that the digestible amino acid composition of a complete diet may be predicted from the amino acid digestibility of the individual ingredients.

2.4.6.4 Dietary fibre

Dietary fibre has been shown to increase the number of intestinal mucosal cells which are sloughed off (Bergner, 1991) and to enhance mucous production (Schneeman *et al.*, 1982) leading to greater losses of endogenous amino acids.

Some components of dietary fibre are capable of adsorbing amino acids and peptides, thereby withholding them from absorption. The extent to which this occurs appears related to the degree of lignification (Sauer and Ozimek, 1986). Lignin, a polymer of phenylpropyl alcohols and acids, is insoluble and hydrophobic in nature. Decreased digestibility (3.5-16.3% (Dierick *et al.*, 1983; cited by Sauer and Ozimek, 1986)) of amino acids associated with lignin may be due to hydrophobic binding of amino acids (Shah *et al.*,1982: cited by Sauer and Ozimek, 1986). In vitro studies (Schneeman, 1978) suggest that fibre may also adsorb trypsin and chymotrypsin, as shown by decreased activity of these enzymes. In addition , substances such as pectins, which

are broadly classified as fibre, may form gels, thereby obstructing the access of digestive enzymes to amino acids (Low, 1985).

2.4.6.5 Degree of grinding

Hermann (1987) demonstrated a significant improvement in digestibility of amino acids in wheat and barley as particle size decreased, however no improvement was found under the same circumstances for field beans, lupins or soyabean meal. Similar studies by both Sauer *et al.* (1977) and Owsley *et al.* (1981) using wheat and sorghum also demonstrated a positive improvement in digestibility when particle size was reduced.

2.4.6.6 Site of sampling

Apparent ileal digestibility of amino acids is generally determined at the terminal end of the ileum. Deviation from this sampling site may lead to erroneous results depending upon the test feedstuff. Alimon and Farrell (1980) studied the disappearance of amino acids from the upper and lower half of the small intestine in pigs. This study demonstrated that as the ileal digestibility (assessed at the terminal ileum) of lysine in feedstuffs decreased, the proportion of lysine which disappeared in the lower half of the small intestine increased. This work implies that site of sampling will have more effect on ingredients of low ileal digestibility.

2.4.7 Use of apparent ileal digestibility values in diet formulation

Amino acid nutrition of pigs contributes significantly to the cost of pig production in Australia. It is therefore critical to provide diets of optimal amino acid composition. In this respect, it is more efficient to formulate diets on the basis of digestible rather than total amino acids, "particularly when considering amino acids which are likely to be limiting" (Sauer and Ozimek, 1986).

Many factors have been shown to influence apparent ileal digestibility of amino acids in dietary ingredients. Those of particular importance include, a) factors which influence endogenous secretion, b) the effect of combined dietary feedstuffs, and c) the effect of different methodology in obtaining the values which are published in the literature. Published values for a wide range of feedstuffs using the same technique at the same facility provide a valuable comparative reference for nutritionists (Rhône-Poulenc, 1989).

Apparent ileal digestibility of amino acids provides a valuable comparative measure of the relative value of one feedstuff to another, and when used as a parameter in diet formulation results in diets of differing composition which are closer in nutritive value than diets formulated on a total amino acid basis.

2.4.8 Alternative species as a model for amino acid digestibility in the pig

To persons unfamiliar with techniques used for determining of amino acid digestibility in pigs, many of the methods outlined in this review may be considered harmful to the pig. Research facilities are under increasing pressure to modify techniques in response to concerns of the animal ethics lobby. Furthermore, the cost of experimentation with pigs is high, whilst availability of funds is becoming scarce. For these reasons there is considerable interest in development of techniques which are rapid, accurate and less costly than conventional studies.

Digestive physiology of the rat is similar to that of the pig and a number of studies have been performed to determine whether the rat would make a useful, low-cost, biological model for pig nutrition. Moughan and Donkoh (1991) and others have shown a close correlation between mean apparent ileal digestibility of lysine (within 3%) and other amino acids in barley, meat and bone meal and a compound diet between the rat and the pig. Further work with the rat may enable preliminary feedstuff appraisal to be conducted with this species prior to experimentation with pigs.

2.5 NUTRITIVE VALUE OF *Lupinus angustifolius* (cv. Gungurru) FOR GROWING PIGS

2.5.1 Chemical composition of lupins

A comprehensive review of chemical analyses for lupins has been compiled by Horton *et al.* (1990), a summary of which is included in Table 3 together with some additional assays of *L. angustifolius* cv. Gungurru lupin-seed meal, de-hulled lupin-seed meal and lupin-seed hulls) (Fernandez and Batterham, 1992; Wigan *et al.*, 1994).

L. angustifolius (cv. Gungurru) is a recognised low alkaloid (sweet) lupin (Table 3). Alkaloids are toxins which lupins (and other plants) produce as a natural deterrent to insects, birds and other predators. Alkaloids, if present impart a bitter taste, leading to reduced voluntary feed intake by pigs.

Table 3. Chemical composition (g/kg, air-dry basis) of lupin-seed meal (lupins), de-hulled lupin-seed meal (kernels), and lupin-seed hulls (hulls) as reported by various workers

	L. angustifolius	*L. angustifolius* (cv. Gungurru)				
Composition	*Lupins	** Lupins	** Kernels	**Hulls	***Lupins	***Kernels
Crude protein	345	311	405	56	288	389
Crude fat	63	46	76	15	52.5	69
Fibre						
- crude	165	172	51	619	183	50
- ADF	223	—	—	—	185	47
- NDF	252	313	110	747	260	78
S-NSP	—	150	197	76	—	—
I-NSP	—	315	130	709	—	—
Alkaloids	0.2	0.07	0.13	<0.01	0.17	0.24
Gross energy	20.2	17.9	19.3	15.8	18.2	19.0
Lysine	17.3	15.9	20.0	3.9	13.8	17.6

*Crude protein = N x 6.25 ADF = Acid detergent fibre. NDF = Neutral detergent fibre. S-NSP=Soluble non-starch polysaccharides. I-NSP=Insoluble non-starch polysaccharides. Gross energy = MJ/kg *=Horton et al. (1990). **=Fernandez and Batterham (1992). ***=Wigan et al.(1993).*

Lupins do not contain any known anti-nutritional factors and for this reason are normally offered uncooked and coarsely crushed to pigs. Despite these advantages *L. angustifolius* is generally restricted to a maximum of 30% in diets for growing pigs. Beyond this level, reduced daily feed intake has been noted as a constraint in commercial production.

2.5.2 Oligosaccharide composition of cultivated lupins and digestion by pigs

Oligosaccharides are energy-rich carbohydrates, which are present in many leguminosae at variable levels. Galactosyl-sucrose oligosaccharides are members of the raffinose family of oligosaccharides. They consist of raffinose, stachyose, verbascose and ajugose, and are related by having one or more alpha-D-galactopyranosyl groups in their structure (Rackis, 1975; Cristofaro *et al.*, 1974) (figure 7). D-galactosyl groups are found naturally joined to sugars such as D-glucose, sucrose, certain polysaccharides, and to a few non-sugars such as glycerol and inositol (French, 1954; cited by Cristofaro *et al.*, 1974).

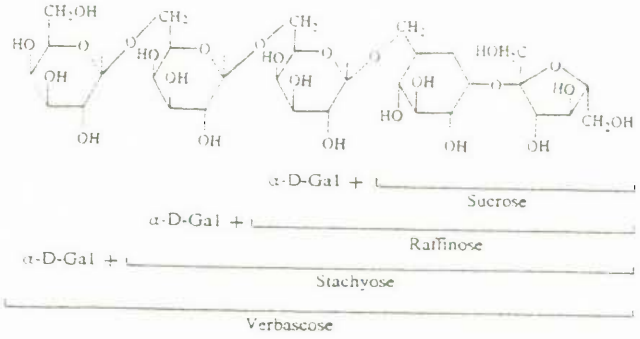

Figure 7. Structural relationships of the raffinose family oligosaccharides (Saini and Gladstones, 1986)

Galactosyl-sucrose oligosaccharides are present in cultivated lupin-seeds at concentrations between 10 and 23% of dry matter (Saini and Gladstones, 1986). Variable oligosaccharide content between and within species of lupin have been reported, making it difficult to accurately predict the oligosaccharide content in a lupin sample without laboratory assay.

The nitrogen-free-extract (NFE) (which is calculated by difference) found for most feedstuffs represents the digestible carbohydrate content of the feedstuff. The absolute NFE of lupins has been reported within the range 25-45% of dry matter (Deschamps, 1958; cited by Hill, 1977 , however lupins contain little or no starch. The highest reported concentration (73 g/kg) being found in a sample of *L. angustifolius* kernels by Fountain (1973; cited by Hill, 1977). The balance of the NFE in lupins is likely to be oligosaccharides and some NSP.

Raffinose family of oligosaccharides occur at higher levels in cultivated lupin species (up to 19%) than in many other food legume seeds (typically 5 -7%) (Saini and Gladstones, 1986). Of the raffinose family of oligosaccharides present in lupins, stachyose is the predominant sugar (approx. 53% of the total sugar content, Trugo *et al.*, 1988, see table 4). Stachyose and verbascose are the least desirable members of the raffinose family owing to their greater influence on flatus formation (Saini and Gladstones, 1986) than the lower molecular weight raffinose. An interesting characteristic of the cultivated species of *L. angustifolius* relative to the wild species is the shift which has occurred in the composition of the NFE towards a higher proportion of sucrose, and within the oligosaccharides from verbascose to the simpler and lower molecular weight molecule, raffinose. The intestinal mucosa of pigs lack the enzyme alpha-1,6-galactosidase which is required to cleave the alpha-linked galactose units present in these oligosaccharides before enzymic digestion is possible (Rackis, 1975; Trugo *et al.*, 1988).

Table 4. Component galactosyl-sucrose oligosaccharide composition (% of dry matter) of Lupinus angustifolius and Lupinus angustifolius seed-coat and complete seed.

	Seed-coat	Complete seed		
	Saini and Gladstones (1986)	Saini and Gladstones (1986)	Macrae and Zand-Moghaddam (1979)	Trugo *et al.* (1988)
Raffinose	21.0	4.4	0.8	1.5
Stachyose	46.6	9.2	11.1	5.2
Verbascose	0	1.3	1.5	2.0
Total alpha-galactosides	67.6	14.9	13.4	8.7

Whilst pigs are unable to enzymatically utilise the energy contained in oligosaccharides, microbial fermentation in the hindgut (and to a much lesser extent in the small intestine), yields volatile fatty acids which are absorbed through the mucosa and provide a valuable energy source to the pig. The bacteria responsible for this fermentation have been isolated (Calloway, 1966; cited by Cristofaro *et al.*, 1974) and identified (Richards *et al., 1968*; cited by Cristofaro *et al.*, 1974) as *Clostridium perfringens*. Other products of microbial fermentation include quantities of carbon dioxide, hydrogen and methane. High production of these gases leads to flatulence, nausea, cramps (Macrae and Zand-Moghaddam, 1979) and reduced pH in the lower intestinal tract (Rackis, 1975). The amount and composition of expelled gases will vary depending upon type, location and abundance of intestinal micro-organisms possessing alpha-galactosidase activity in the digestive tract of the pig (Rackis, 1975).

2.5.3 Non-starch polysaccharide composition of lupin-seed hulls

Hulls represent approximately 23 to 26% of the entire seed weight (Saini and Gladstones, 1986). The composition and effect of hulls on digestion of nutrients by the pig is of primary concern in studies regarding the nutritive value of whole lupins. The

digestive characteristics of oligosaccharides in the seed hull may be considered similar to that of oligosaccharides present in the kernels.

Bailey *et al.* (1974) has examined the composition (table 5) of lupin seed hulls (*L. angustifolius*) and found that faecal digestibility of nitrogen by the rat was significantly ($P<0.05$) depressed (by 10.4, 17 and 28.6%) as the dietary inclusion of lupin-seed hulls increased from 0% (to 20, 40, and 60% respectively). Bailey *et al.* (1974) also reports an increase in voluntary feed intake of rats when offered diets containing hulls at varying levels and a non-significant ($P<0.10$) 12% improvement in the growth rate of rats offered the basal diet plus 20% hulls.

Table 5. Composition (% of dry matter) of lupin-seed hulls (L. angustifolius).

Fraction	Composition (Bailey *et al.*, 1974)
Soluble sugars	0.74
Pectic substances	27.7
Hemicellulose	12.7
Cellulose	50.9
Lignin	0.37

Studies using pigs (Taverner, 1975) (39.7 to 47.3 kg live weight), found that the digestibility of crude fibre (66.1%) in lupin-based diets was significantly greater than in either wheat (44.2%) or wheat/soyabean (45.9%) diets. Practically all of the crude fibre content of lupins is found in the seed coat. The seed coat is very low in lignin (Table 5) (the least digestible of all NSP) which may explain the high digestibility of crude fibre in lupin-seed meal.

Cellulose is a primary component of lupin-seed hulls. Drochner (1991) has reported that hindgut fermentation of cellulose is a slow process (particularly in the presence of

lignin) which is related to transit time, adaptation of the pig (age, microbial population/species) and particle size.

In contrast to cellulose, pectins are highly digestible in the hindgut of the pig (80-90%)(Drochner, 1991).

2.5.4 Recent experiments with *L. angustifolius*

Conflicting reports regarding the nutritive value of lupin-seed meal and de-hulled lupin-seed meal relative to soyabean for pigs have been published in Australia. Godfrey (1986) concluded that dehulled lupin-seed meal is of equivalent nutritional quality to that of soyabean meal for growing pigs. However, the dietary treatments used in these experiments were high in lysine as a proportion of digestible energy (0.75 g total lysine/MJ of digestible energy) and as a result may not have been sensitive enough to identify differences between the treatments. Another possible explanation for this result is absence of the highly fibrous seed-coat which may decrease nutrient digestibility. Based on this latter explanation, Fernandez and Batterham (1992) conducted a number of experiments to further study this aspect of digestibility.

Sucrose-based diets containing lupin-seed meal (lupins), de-hulled lupinseed meal (kernels), soyabean meal (SBM) and SBM plus lupinseed hulls (hulls) were designed. Gross energy digestibility (GED), digestible energy (DE) and apparent ileal digestibility (AID) of lysine were determined, and these results were used to formulate diets enabling comparison of performance of pigs fed either lupins, kernels, SBM or SBM plus hulls. Each of these diets was formulated to contain similar AID lysine/MJ of DE and was offered restrictively to pigs through the growth phase 20-45kg (Fernandez and Batterham, 1992).

They found that the apparent ileal digestibility of lysine was improved from 0.81 in lupins to 0.87 in the kernels by de-hulling. It was also found that the ileal digestibility of

lysine in the SBM treatment decreased from 0.85 to 0.76 when lupin hulls were added. In terms of gross energy digestibility it was shown that de-hulling significantly improved the value of kernels (0.86) relative to lupins (0.74) and that addition of hulls depressed gross energy digestibility in SBM plus hulls (0.75) relative to SBM (0.88). Despite improvements in apparent nutrient digestibility by de-hulling, empty-body-weight gain was lower in pigs offered diets containing kernels (475g/day) and SBM (465g/day) relative to hulled lupins (529g/day), whilst pigs offered SBM plus hulls produced gains superior to those offered the SBM (506 vs 465 g/day). The only difference in protein deposition was between pigs fed kernels (71g/day) and lupins (78g/day) (Table 6).

Table 6. Digestibility of nutrients (in lupins, kernels and SBM), and growth performance of pigs offered sucrose-based diets containing lupins, kernels, SBM, and SBM plus hulls

	Lupins	Kernels	SBM	SBM plus hulls
Ingredient digestibility				
Apparent ileal digestibility (lysine)	0.81^{a}	0.87^{b}	0.85^{b}	0.76^{a}
	(0.82)	(0.88)		(0.74)
Gross energy digestibility	0.74^{a}	0.86^{b}	0.88^{b}	0.75^{a}
	(0.73)	(0.85)	(0.84)	
Digestible energy (MJ/kg,air-dry)	13.3	16.6	15.2	12.6
	(13.1)	(16.3)	(14.4)	
Growth response				
Empty-body-weight gain (g/day)	529^{b}	475^{cd}	465^{c}	506^{bd}
Protein deposition (g/day)	78^{a}	71^{b}	73^{ab}	77^{ab}
	(77)			(76)
Fat deposition (g/day)	168^{a}	158^{ab}	152^{b}	161^{ab}
Energy deposition (MJ/day)	8.5^{a}	8.0^{ab}	7.8^{b}	8.2^{ab}

Different superscripts within the same row denotes treatment differences (P<0.0.5) (Source: Fernandez and Batterham (1992)). Data provided in parentheses were provided by Dr. Fernandez subsequent to preparation of this thesis and are reproduced here for reasons of clarity only

These experiments were subsequently repeated with new lupins and SBM samples (E.S. Batterham, unpublished), however, the results reported were similar to those found by Fernandez and Batterham (1992) other than, no growth response was observed when hulls were added to the SBM diet.

The response to hulls reported by Fernandez and Batterham (1992) was unexpected, and a further experiment was conducted to determine whether the sugar component of the diets was somehow altering the nutritive value of the ingredients (Batterham, unpublished). This hypothesis was explored by offering wheat-based diets containing lupins or SBM to growing pigs. Under these conditions, pigs offered the SBM diet significantly outperformed those offered lupins in both empty-body-weight gain and protein deposition (698 vs 631 g/day and 116 vs 104 g/day respectively). The wheat-based diets used in this experiment contained higher ileal digestible lysine levels (approximately 0.64 vs 0.36 g/MJ) than the original sucrose-based treatments. This approach reduced the sensitivity of the results to differences in lysine utilisation and focused more clearly on differences in energy utilisation.

These results suggest that either dietary energy source (sucrose or wheat) or some aspect of dietary fibre (originating from wheat and/or lupins or possibly an interaction between the two), may be responsible for the differences in performance which were demonstrated.

2.5.5 Current experiments with *L. angustifolius* cv. Gungurru.

Published literature at the time of writing, is unable to explain the uncertainty surrounding the nutritive value of lupins, or the effect of de-hulling on subsequent nutritive value to growing pigs. Wide variability both between and within species of lupins with respect to nutrient digestibility, oligosaccharide composition (Table 4) and possible interactions with dietary energy source are unexplained.

The current experiments have been designed to further explore the nutritive value of lupins and kernels relative to soyabean meal. These experiments will determine the extent of nutrient digestibility (ileal and faecal) in sucrose-based diets of lupins, kernels, SBM and wheat, which will then be used to formulate "research" style sucrose-based diets, and "commercial' style wheat-based diets. The sucrose diets will be lysine-sensitive and offered to pigs restrictively, whilst the commercial diets will be higher in lysine and offered on an *ad libitum* basis. Growth and carcase retention data derived from this experiment will assist in elucidating the presence or absence of an interaction between dietary energy source (sucrose or wheat) and the nutritive value of lupins and kernels relative to SBM. If an interaction is found to exist, the sites (ileal vs faecal) and extent of nutrient (gross energy, amino acids, ADF and NDF) digestibility in the pig will be determined in an attempt to explain the interaction.

2.5.6 Summary

Lupins are a valuable feedstuff for pig production in Australia. At present there is considerable uncertainty surrounding their nutritive value for pigs, and whether nutritive value is in some way affected by dietary energy source or by dehulling.

Lupins contain high levels of energy-rich carbohydrates, known as oligosaccharides, which are only digestible by the pig through the action of microbial fermentation. Once fermented, however, oligosaccharides yield high quantities of volatile fatty acids (VFA) which are a readily available source of energy to the pig. A greater understanding of oligosaccharide and other non-starch polysaccharide digestion (sites and extent) is required to further understand the nutritive value of lupins.

Lupins have a seed coat which contributes significantly to the crude fibre content of lupin-seed meal. These hulls are highly digestible in the small and large intestine, however, the effect of the non-starch polysaccharide component on nutrient digestion

and subsequent growth of pigs is of primary importance in identifying the nutritive value of lupins.

The experiments conducted here were designed to elucidate some of the factors which affect the nutritive value of lupins for growing pigs, particularly those relating to digestibility of energy.