THE INFLUENCE OF DOMESTICATION AND ENVIRONMENT ON THE VALUE OF LUPINS (*LUPINUS* SPP.) AS A FEED FOR RUMINANTS

by

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SUMMARY

Lupins have significant potential in Australian agriculture due to their high protein concentration and their adaptation to a range of soil types. Lupins also can fix nitrogen and provide a disease 'break' in cereal rotations, which contribute to their value in cropping systems. The lupin seed (*L. angustifolius*) is widely used as a supplementary feed for ruminants during the summer-autumn period and are a useful protein supplement for pigs and poultry in Australia. However, *L. angustifolius* does not perform well on fine-textured and/or alkaline soils, which occur on over 9 million hectares in the cereal zones of SA and WA. To solve this problem, plant breeders have commenced a selection program to domesticate *L. pilosus* and *L. atlanticus* which are more suited to these types of soils. At this early stage of domestication of *L. pilosus* and *L. atlanticus*, *L. angustifolius* was used as a benchmark to determine changes in seed structure and chemical composition which may result from breeding and selection of these two lupins. On the other hand, *L. pilosus* and *L. atlanticus* could be like *L. cosentinii* which is essentially a wild type adapted to southern Australian soil conditions and a valuable feed for sheep in this area.

Domestication had significant influences on both the seed size and seed coat structure of lupins and no significant impact on seed yield. Recently released cultivars (1987 and 1988) of *L. angustifolius* had smaller seeds with a thicker seed coat than those released in 1971 (Chapter 3), but the yield of these cultivars was not significantly higher than that of the cultivars released in early years. *L. atlanticus*, *L. cosentinii* and *L. pilosus* had similar seed yields to *L. angustifolius* under similar growing conditions with a May sowing in 1995 at Turretfield, South Australia (Chapter 4), but they had much bigger seeds than the domesticated lupin (*L. angustifolius*) (Chapter 3 and 7). Selection for softseeded seeds resulted in a reduction of seed coat thickness in *L. angustifolius*, but thick seed coats were positively related to seed size of wild lupins (Chapter 3). The change in seed size and seed coat structure could result in poor adaptation to the environment and sensitivity to diseases, and hence yield loss.

With large amounts of *L. angustifolius* being fed to animals in Australia, nutrient content is a crucial factor for its utilisation efficiency by animals. Domestication of *L. angustifolius* from 1971 (Uniharvest) to 1988 (Gungurru, Warrah and Yorrel) had no significant influence on N, ADF, NDF and mineral content except for seed S content (Chapter 5). A reduction of seed S content of cultivars released in 1988 compared to that of the cultivar released in 1976 could affect animal production when it is integrated over the 1.13 million tonnes (estimated by Edwards in 1994) being used by the intensive livestock industry. On the other hand, there was considerable variation in nutrient content (N, fibre and minerals) between lupin species (Chapter 5 and 7). *L. cosentinii* had a higher N, seed coat fibre, Mn, P and S content than *L. angustifolius* which could contribute to it being an excellent feed for ruminants, and its higher Mn and P content can be also benefit plant growth and development, resulting in high seed yields.

Growing conditions also play an important role in the yield, yield components and chemical composition of lupins. June sowing at Turretfield in 1995 significantly decreased the numbers of seeds and pods per plant and reduced yield by 29% when compared to a May sowing. June sowing also decreased the kernel N content of lupins, but had no significant effects on ADF and NDF content. Seeds grown in 1994 were much smaller than those grown in 1993 (26.7 vs 46.6 g/100 seed), had low nitrogen production of per 100 seed weight (1.6 vs 2.1 g N/100 seed) and low levels of Fe, Mn, Cu, Zn, K, P and S (Chapter 6). Location had no significant influence on seed N content. However high levels of seed ADF, NDF and lower levels of seed Mn occurred in seeds grown at Yeelanna compared to seeds grown at Minnipa Research Centre (Chapter 6). The significant changes both in seed yield and nutrient content (eg N and mineral) under different growing conditions could cause a fluctuation in animal production, particularly when over 200,000 tonnes of *L. angustifolius* seeds and 200,000 to 350,000 tonnes of *L. cosentinii* seeds are grazed annually by sheep in WA.

The potential of wild lupins (*L. atlanticus* and *L. pilosus*) as supplementary feed for sheep was determined by *in sacco* and *in vivo* methods. The parameters included

degradability in the rumen, DMD *in vivo* and feeding value. The similar degradability *in sacco*, DMD and feeding value of wild lupins and domesticated lupins at 150 g/head/day levels of supplementation to sheep may indicate that the higher levels of anti-nutritional factors of wild lupins are unlikely to affect their utilisation efficiency at the levels of supplement commonly provided for ruminants. Thus selection of very low levels of alkaloids in varieties will probably be of low priority for ruminant production.

Overall, the changes that have occurred with *L. angustifolius* over the past 40 years of breeding and selection would appear to offer a useful model for how the domestication of *L. pilosus* and *L. atlanticus* may progress. If there are no significantly different agronomic problems, these species seem to offer the opportunity of initially providing feed for ruminants via either direct grazing or as a seed supplement over large areas of farmland in southern Australia. As breeding progresses towards reduced alkaloid concentration, the seed also has the potential for increasing monogastric production.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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ABBREVIATIONS

ADF	Acid Detergent Fibre
AED	Apparent Energy Digestibility
AND	Apparent Nitrogen Digestibility
CP	Crude Protein
DM	Dry Matter
DMD	Digestibility of Dry Matter
DMI	Dry Matter Intake
GE	Gross Energy
GEI	Gross Energy Intake
HI	Harvest Index
LW	Live Weight
MRC	Minnipa research Centre
N	Nitrogen
NDF	Neutral Detergent Fibre
NI	Nitrogen Intake
NSP	Non-starch-polysaccharides
SA	South Australia
ST	Sowing Time
WA	Western Australia

CHAPTER 1
GENERAL INTRODUCTION

Lupins (mainly *Lupinus angustifolius*) are largely used as supplementary feed during the summer-autumn period for ruminants and as a protein resource for pigs and poultry in Australia. It is estimated that around 1.13 million tonnes of lupins are used in intensive livestock production in Australia (Edwards 1994) and over 200,000 tonnes of *L. angustifolius* grain and 200,000 to 350,000 tonnes of *L. cosentinii* seeds are possibly grazed directly by sheep each year in Western Australia (Murray 1994). Although there are many lupin species, *L. angustifolius* dominates traded quantities of grain in Australia.

However, there are still some problems encountered when lupins are used as a feed. For example, low levels of sulphur-containing amino acids and high levels of oligosaccharides and NSP are limiting factors for monogastrics. Highly degradable nitrogen in the rumen and a poor ratio of nitrogen to sulphur for optimum microbial growth and a low ratio of calcium and phosphorus may all limit the value of lupins for ruminants. *L. angustifolius* does not perform well on calcareous soils (White 1990 and Tang 1994). This partly results in the supply and the demand of lupins in different states in Australia being out of balance. To solve these problems, domestication of *L. atlanticus* and *L. pilosus* is probably a solution as these lupins can grow on calcareous soil (Buirchell and Cowling 1992 and Egan and Hawthorne 1994).

However, domestication of grain legumes generally results in a number of simultaneous changes, including morphological and anatomical, physiological and chemical, functional and 'behavioural' (Plitmann and Kislev 1989). Such genetic changes in plants can result in greater variation in adaptation to environments and changes in resistance to diseases and pests. For example, the reduction of anti-nutritional factors in grain legumes might result in increased susceptibility to disease and predators (Gladstones 1970 and Johns 1994). Such changes could reduce the

quantity and quality of grain for animals. Environmental factors also have strong effects on plant growth, grain yield and grain nutrient content. For example, protein content of *L. angustifolius* varied from 27.2% to 37.6% in Australia (Petterson and Mackintosh 1994a). These variations could have a significant effect on animal production because of the large amount of lupins being used by animals.

With new species being considered that may greatly extend the range and scale of the lupin crop, an understanding of the impact of domestication and environment on parameters that might be changed in the process, and thus impact on animal nutrition becomes essential.

Therefore, the objectives of this work were to determine:

1. the changes in seed structure and chemical composition of *L. angustifolius* with breeding and selection,
2. the effect of environmental factors on the seed structure, yield and chemical composition of lupins,
3. the variation in seed structure and chemical composition between lupin species and within species, and
4. the nutritive value of wild lupin seed and domesticated lupin seed for ruminant animals, specifically sheep.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The importance of lupins as a viable protein source for the livestock industry and as a possible fibre source for the human food industry is becoming increasingly apparent due to the following advantages:

1. the protein content of lupins is similar to soy beans and higher than that of most other seed legumes,
2. lupins contain very high levels of dietary fibre, low levels of starch and lignin,
3. lupins can be grown in poor soil which may not be suitable for other high-production crops,
4. lupins can fix nitrogen which contributes to their value in crop rotations, and
5. lupins used for monogastric feed, only require grinding or crushing; no processing is needed for ruminants.

Despite these advantages, there are still some problems encountered when lupins are used as a feed. For example, lupins like other legume crops are low in sulphur-containing amino acids, contain high levels of oligosaccharides and NSP which can limit their utilisation by animals, especially monogastrics. Feeding trials (Dixon and Hosking 1992, and Margan 1994) have shown poor performance of animals and low utilisation efficiency of nutrients in diets with relatively high levels of lupins.

The feeding value of lupins is affected by the nutrient composition. The nutritional components play an important role in the feeding value of lupins and are affected by stage of domestication, and managerial and environmental factors during growth. Previous research has shown that the nutrient content varies between species and within species. For example, Petterson and Mackintosh (1994b) reported that protein

content ranges from 25% to 45% in the genus *Lupinus*. Growth conditions also have a large impact on the nutritional profile of the seed. Normally, sweet lupin varieties contain very low levels of alkaloids ranging from 0.2 to 0.3 g/kg (Hill 1977 and Ruiz *et al.* 1977), but under certain agronomic and seasonal conditions, alkaloids can be as high as 0.3 to 1.3 g/kg (Godfrey *et al.* 1985). Breeders have developed new cultivars, which are more suitable for stockfeed. For example, the registered cultivars of *L. angustifolius* contain very low alkaloids compared with their parents (Gladstones 1989). However, breeders mainly consider the seed yield, toxicity, resistance to diseases and adaptation to environments, while more general nutritional components which may change during breeding and selection are not specifically taken into account in the breeding program.

The improvement of the feeding value of lupins has concentrated on nutrient supplementation where there are deficiencies, and mechanical treatment including crushing, dehulling and heating. Addition of enzymes to monogastric diets may also improve the utilisation of lupins. In the long term, the composition could be improved by breeding. This literature review will concentrate on:

1. the influence of breeding and selection on the chemical composition of lupins,
2. the effect of environmental factors on the nutritive value of lupins, and
3. the nutritive value of lupins to animals.

Narrow-leafed lupins *L. angustifolius* will provide the central focus due to an active history of breeding over the last 30-40 years. The more recently domesticated species *L. pilosus* and *L. atlanticus* will be used to determine changes that may occur as breeding and selection progress.

2.2 Lupins in Australia

2.2.1 Lupin breeding in Australia

Breeders have made a great effort in domestication of *Lupinus angustifolius* especially in Western Australia (Gladstones 1982b, Gladstones 1989, Gladstones 1990, Landers 1991, Cowling 1994 and Gladstones 1994). This was marked by the release of the first cultivar of *L. angustifolius* cv. Uniwhite in 1967. Uniwhite had important agronomic characteristics such as white flowers, white and soft seeds, reduced pod shattering, late maturity and low seed alkaloid content (Gladstones 1982b and Landers 1991). Following Uniwhite, Uniharvest and Unicrop were released in 1971 and 1973 respectively and had increased resistance to pod shattering. In addition, Unicrop was an early-flowering cultivar. This completed the first phase of lupin breeding in Australia (Gladstones 1994).

The second breeding phase mainly concentrated on grey leaf spot and anthracnose resistance (Gladstones 1994). As a result, cultivars Marri, Yandee and Chittick were released with increased disease-resistance. Yandee and Chittick were early-flowering. When cropping, there were some problems with the early flowering cultivars because they were susceptible to frost in cold districts or developed large vegetative plants with a low harvest index. The late flowering Marri is low yielding and is prone to lodging. In addition, lupinosis became another problem for sheep grazing lupin stubble where the fungus *Diaporthe toxica* develops. To solve these problems, wild genotypes with a phomopsis resistance gene were crossed with cultivars such as Illyarrie to produce the cultivars Gungurru and Yorrel which have some resistance to phomopsis. These cultivars also have good yields and low alkaloid contents. Gungurru rapidly became the dominant variety in most agricultural areas in Western Australia in the late 1980's (Gladstones 1989).

More recently, breeders have been attempting to overcome the problem of poor early pod setting. Their approach has involved redesign of the plant structure to reduce the

number of branches. This reduces the competition faced by flowers on the main stem, allowing more pod set on the main stem and hence higher yield and higher harvest index (Delane *et al.* 1986b, Seaton and Sedgley 1986, Delane and Gladstones 1988 and Landers 1991). However, there was evidence that higher yield potential for reduced-branch cultivars only appeared when these cultivars were grown on sandy soils with a low water-holding capacity in a short growing season environment. In other environments, yield had been disappointing due to higher susceptibility to brown leaf spot and less resistance to phomopsis stem blight (Hamblin *et al.* 1986, Delane and Gladstones 1988 and Heenan 1994). Therefore selection for good pod setting on the main stem which contributes mainly to high seed yield, became one of the major aims of lupin breeding (Delane and Gladstones 1988). Danja, which has improved pod setting on the main stem, gave consistently higher yields than Yandee (by 11% in 30 out of 38 sites). In summary, breeding objectives for *L. angustifolius* included selection for low alkaloid content in seed, improved agronomic features such as permeable seed coat, non-shattering pods, drought tolerance, lodging resistance, early flowering, early maturity, disease resistance and high seed yield.

2.2.2 Production

Lupins are an increasingly important crop in Australia and more than half of the total lupin production in the world occurs in Australia (Landers 1991). By the year 2000, it is predicted that two million tonnes of lupins will be produced in Australia, of which 500,000 tonnes will be used in domestic consumption and possibly 1.5 million tonnes exported (Coffey 1994). Most of this production is used for livestock feed. In Australia, Edwards (1994) estimated the possible use of lupin seed for pigs, poultry, dairy, cattle and feedlot beef feed as around 1.129 million tonnes. Despite the heavy use in intensive livestock production, Murray (1994) also estimated that over 200,000 tonnes *L. angustifolius* seed and around 200,000 to 350,000 tonnes of *L. cosentinii* seeds are consumed by sheep. Up to 5 times this weight in plant residues is possibly grazed by sheep each year in Western Australia. Only a small proportion of lupins is sold for human consumption. However, there is a trend towards lupins being accepted as valuable protein and fibre sources for human foods,

especially in Middle Eastern and Asian countries. Another potential area for lupin use is in aquaculture, as an alternative protein source (Edwards and van Barneveld 1998).

2.2.3 Problems

Although there are many lupin species, only *L. angustifolius* is grown in large quantities in Australia. However, *L. angustifolius* as livestock feed has some limitations. For example, *L. angustifolius* does not perform well on fine-textured and/or alkaline soils (Buirchell 1994, Cowling 1994, Egan and Hawthorne 1994 and Tang 1994). This partly results in the supply and the demand of lupins in different regions in Australia being out of balance. For example, in Queensland, potential lupin usage in the livestock industry is 254,000 tonnes, but there is no lupin cultivation, while Western Australia produces for an increasing export market (Edwards 1994).

There is considerable variation in nutrient contents of lupins, especially in protein content both between species and within species (Petterson and Mackintosh 1994a,b). This variation is a likely result of the variable environments in which lupins are grown. It is difficult to formulate a balanced diet for livestock without understanding the impact of the environment on nutrient content of feed seed.

L. angustifolius as commonly used in Australia, contains low levels of sulfur-containing amino acids and high levels of non-starch-polysaccharide and oligosaccharide, and has low lysine availability, which may be limiting factors for non-ruminants. Even for ruminants, the poorly balanced ratio of N and S, Ca and P, and the high rumen degraded protein are limiting factors for their production (Dixon and Hosking 1992, and Murray 1994).

2.3 Nutritive value of lupins

The chemical composition of low alkaloid sweet lupins has been reviewed by Hill (1977), Hill (1986), Patterson and Mackintosh (1994a,b). There is no doubt that sweet lupins have a desirable chemical composition, and so are highly accepted by ruminants and widely used in pig and poultry rations in Australia. While chemical composition is an important first step in evaluating the nutritive value of lupins, the availability of nutrients is more important for animal production. The feed quality of lupins has been discussed by King (1990), van Barneveld and Hughes (1994), van Barneveld *et al.* (1995) and Edwards and van Barneveld (1998) for non-ruminants, and by Dixon and Hosking (1992), Hough and Jacobs (1994), Murray (1994) and Edwards and van Barneveld (1998) for ruminants. These authors suggest that lupins are a good protein supplement when fed at certain levels in the diet. Poor performance of animals did occur when high levels of lupins were included in the diet. Possible reasons for poor performance included:

1. imbalance in nutrient content *eg* low in sulphur-containing amino acids and high in complex carbohydrate content for non-ruminants, and
2. protein is readily degraded in the rumen, poor ratio of nitrogen and sulphur for optimum microbe growth, and low ratio of calcium and phosphorus may all limit the value of lupins for ruminants.

2.3.1 Chemical components

Protein and amino acids Lupins have high and variable protein content from 28% in *Lupinus angustifolius* to 47.6% in *L. luteus* (Hill 1977), but true-protein nitrogen is of considerable importance for animal feeding. As proteins are broken down into their constituent amino acids before being absorbed, what is important is not the total nitrogen content, but total amounts of different amino acids and their availability. If the profile of amino acids in feed could match the requirements of animals, the feed should be well utilised by animals. This balance of nutrients is particularly important in ration formulation in intensive animal industries. The imbalances shown in Table

2.1 for pigs result in reduced amounts of lupins in the diet or for inputs of specific amino acid supplements.

Table 2.1 Amino acid concentration* in the protein of legumes compared with the 'ideal' patterns of amino acids for pigs

	Seed legume			'Ideal' patterns of amino acids (% of lysine)		
Amino acid	Pea	Lupin	Soy bean	5 to 20kg	20 to 50kg	50 to 100kg
Lysine**	109	78	97	100	100	100
Methionine	51	41	76	30	30	30
Cystine	63	65	69	60	65	70
Methionine+cystine	56	53	71	65	67	70
Threonine	84	80	94	18	19	20
Tryptophan	74	68	115	60	60	60
Valine	103	91	110	100	100	100
Isoleucine	105	110	119	32	32	32
Leucine	110	107	123	48	48	48
Tyrine+phenylalanine	134	121	141	95	95	95
Histidine	124	126	135	68	68	68
Arginine	465	501	326	42	36	30

* Expressed as a percentage of the requirement for that individual amino acid by the growing pig.

** lysine set at 6.4 g/16 g N.

Source: Batterham (1993).

Lupins can only provide 41% and 78% of the methionine and lysine required by growing pigs. Other studies (Gladstones 1970, Hill 1977, Hove and King 1978, Hill 1986 and Murray *et al.* 1990) also suggested that methionine is the first limiting amino acid in lupin seed protein. However, the amino acid profile for ruminants may not be as critical as that for non-ruminants because lupin protein is highly degraded in the rumen and ruminants can obtain essential amino acids from microbes. However, sulphur-containing amino acids are still important for wool growth (Lee and Williams 1993).

Carbohydrates Lupins contain around 50% carbohydrate which is composed mainly of NSP and oligosaccharides, with small amounts of disaccharides and monosaccharides, and low starch and lignin content. For example, NSP and oligosaccharide content of three recently released cultivars (Gungurru, Yorrel and Danja) of *L. angustifolius* are listed in Table 2.2.

Table 2.2 The composition of the carbohydrates in the cotyledons and hulls of the seeds of three cultivars of *L. angustifolius* (g/kg DM)

		Cotyledons			Hull		
		Gungurru	Yorrell	Danja	Gungurru	Yorrell	Danja
NSP							
	Total	294	293	314	856	885	891
	Soluble	46	43	44	50	49	50
Starch		6	6	7	4	3	4
Klason lignin		8	9	9	15	12	13
Oligosaccharides							
	Raffinose[a]	31	29	30	4	3	4
	Stachyose	46	51	44	<1	<1	<1

a. Includes verbascose.

Source: Evans and Cheung (1993) and Evans (1994).

NSP and oligosaccharides may be the main factors limiting the use of lupins by non-ruminants. van Barneveld and Hughes (1994) suggested that NSP might interfere with the action of enzymes and influence microbial activity, hence reduce the efficiency of lupins as a monogastric feed. The sugars in the oligosaccharide family (raffinose, stachyose and verbascose) were poorly digested owing to the lack of the enzyme α-galactosidase in the intestinal mucosa of monogastric animals (Carre *et al.* 1985). Increased amounts of oligosaccharides can cause flatulence in monogastrics (Cristofaro *et al.* 1974 and Reddy *et al.* 1989). However, this group of sugars is a valuable energy source for ruminants due to its slow fermenting character resulting in a low risk of lactic acidosis.

Anti-nutritive factors Most seed legumes contain some secondary metabolites, which play important defence roles in the plants. These metabolites reduce the nutritive value of plants due to detrimental effects on mammalian digestion and metabolism. The main anti-nutritional components in lupins are alkaloids and nearly 70 different quinolizidine alkaloids have been found to be present in lupins of wild and domesticated species (Keeler 1989). The major alkaloids are spartein, lupanin, hydroxylupanin, angustifolin and lupinin (Nowacki 1960 and Hill 1977). The main actions of alkaloids appear to be on the central nervous system and the reduction of intake due to the bitter taste of the alkaloids. Some symptoms such as lack of appetite, fever and difficult or laboured breathing may occur when animals are poisoned (Ruiz *et al.* 1977). The amounts of other anti-nutritional factors such as phytate phosphorus, tannin and trypsin inhibitors were found to be low (Savage and Hill 1986). When compared with those of other commercial seeds, such as soy beans, these inhibitors are unlikely to reduce the nutritive value of sweet lupin seeds (Hill 1986).

The alkaloid content is influenced by both genetic and environmental factors (Buirchell *et al.* 1994, Harris 1994 and Sipsas 1994). For example, the major alkaloids in *L. atlanticus*, *L. pilosus* and *L. cosentinii* are epilupinine, multiflorine and small amounts of sparteine, and these lupins also contain higher concentrations of alkaloids than that of *L. angustifolius*. Lupanine and 13-hydroxylupanine are the main alkaloids for *L. angustifolius*.

Other components Most lupin species also contain relatively high levels of oil when compared with other legumes except for soy beans and peanuts. Values range from 4% in *L. angustifolius* to 23% in *L. mutabilis* (Hill 1977, Williams 1979, Gladstones 1982a and Hill 1986). The mineral composition of lupin seeds is usually not important as deficiencies can be balanced by the addition of mineral mix to the diets. It may be important, however, if any element is present at a toxic level. For example, *L. albus* can accumulate manganese from the soil (Hill 1986). This suggests that intensive livestock producers need to determine nutrient contents of lupins before they are included in diets.

2.3.2 Chemical composition related to animal production

Ruminants Lupins (mainly *L. angustifolius*) are widely used as supplementary feed for ruminants in Australia during the Summer-Autumn period, especially for weaners, pregnant ewes and dry sheep due to the lack of high quality feed. Generally, lupin supplementation has a positive effect on intake, LW change and wool growth (Thompson and Curtis 1990, Dixon and Hosking 1992 and Murray 1994). However, the efficiency of lupins as a supplement largely depends on the quality of roughage on offer and the amount of seed supplied. When lupin seed was supplied to sheep fed low to medium quality roughage, LW changes were usually linearly correlated to the level of supplement up to at least 15g lupin seed per kg LW (Dixon and Hosking 1992). There was evidence that low levels of supplementation stimulated the intake of roughage whereas at high levels of supplementation roughage intake decreased. Rowe *et al.* (1994) compared barley, oats and lupins as supplements for cattle fed hay and found that hay intake linearly decreased as increasing amounts of all seeds were fed. Intake of chaffed wheaten hay was increased by 4% when sheep were fed 100 g/day lupin seed supplement and decreased by 14% at 400 g/day lupin supplement (Margan 1994). Similar results were also reported by Curtis and Mavrantonis (1990), Foot *et al.* (1983), Roberts *et al.* (1979), Butler (1981), Kenney (1986) and Smith and Warren (1986).

The high efficiency achieved at low levels of supplementation with low quality roughage based diets was likely due to the deficiency of protein in poor roughage diets. Once this deficiency was overcome by low levels of legume seed supplementation, further increases in the legume seed in the diet did not increase the voluntary intake of the roughage due to direct substitution, hence low efficiency occurred (Minson and Milford 1967). There is also evidence that high levels of lupin supplementation did not further improve the apparent protein and energy digestibilities. For example, Margan (1994) found that 100g/day and 400 g/day lupin supplementation had similar effects on the digestibilities of the diets by increasing the apparent protein digestibility by 38% and the apparent energy digestibility by 16% of the diets at 100g/day, and by 36% and 19% at 400 g/day respectively. In contrast, Curtis *et al.* (1994) reported that the apparent DM digestibilities of diets

was 65.6% for low level lupin supplementation (3:1 wheaten chaff:lupin) compared to 81.4% for high level supplementation (1:3 wheaten chaff:lupin). This difference may be because that the feeding level of lupins was very high in the Curtis *et al.* (1994) experiment, and hence the digestibility of diets was close to that of a sole lupin diet. Petterson *et al.* (1997) have reported this as being about 90%.

Another reason for low efficiency at high levels of supplementation may be because lupin protein is highly degraded in the rumen (Dixon and Hosking 1992, Brand and Franck 1992 and Kibelolaud *et al.* 1993), and the fractional rumen outflow rate of protein supplements decreases as the proportion of dietary concentrate is increased (Ganev *et al.* 1979). The combination of these two factors at high levels of lupin supplementation may contribute to the lower efficiency of lupin utilisation. However, the degradability of lupins was dependent on the particle size when used *in sacco*. For example, Freer and Dove (1984) found that the degradability of lupins was decreased from 0.46 to 0.03 as the particle size of the sample increased from 'fine' (sample ground through a 0.8 mm screen) to 'coarse', (samples passed through a grinding mill without a screen). This suggested that lupin supplementation could be more efficient on farms where roughly crushed seed or whole seed is commonly fed to the animals.

Lupins used as a feed supplement for dairy cows showed a similar effect as that for sheep except that crushed lupin seed was required for dairy cows, as whole seed fed to cows often resulted in a high level of excretion of seed through the digestive tract (Hough and Jacobs 1994). Lupins used as a feed supplement for beef cattle had no advantages compared with cereal seed except in young cattle (Hough and Jacobs 1994 and Jacobs and Tudor 1994). For young cattle, the need is for more undegraded protein which lupins can offer as the rumen function of young cattle is not mature thus allowing more lupin protein to be passed (Hough and Jacobs 1994).

Compared with other seed supplements, lupins are however generally better than cereals for ruminants except for beef cattle (Kenney and Smith 1985, Morcombe and Ferguson 1990, Rowe and Aitchison 1987, Dixon and Hosking 1992, and Murray 1994). Lupins had similar effects to fishmeal on animal production at lower levels of

supplementation, but at higher protein levels, liveweight gain with fishmeal was higher than that with lupin seed (Jacobs and Zorrilla-Rios 1993 cited by Hough and Jacobs 1994). Similar results were reported by Dixon and Hosking (1992), who found liveweight gain of young sheep fed medium quality roughage based diets was increased by supplying fishmeal rather than lupin seed. Compared with soy bean meal supplementation for sheep grazing summer pasture, there was no difference in animal responses to different supplement sources (Foot *et al.* 1983). Lupin supplementation for sheep or cattle grazing low quality stubble or pasture, tended to be better than pea and fababean supplementation in terms of liveweight change (Allden and Geytenbeek 1980a,b, Hawthorne 1980, Hynd and Allden 1986, and Morcombe and Ferguson 1990). In conclusion, lupins are a valuable, convenient and safe supplementary feed for ruminants, but proper management is needed in terms of efficiency, because different species of animals, at different growth stages and different diets used can affect this efficiency.

Monogastrics Lupins are a useful protein supplement for pigs and poultry in Australia due to their high crude protein, amino acid, energy levels and competitive price compared with other protein supplement sources (van Barneveld and Hughes 1994).

For poultry, growth was not depressed when 15% white lupin was added to turkey diets, but it was depressed significantly when 30% or more white lupin was included in the diets (Halvorson *et al.* 1983). Morris (1973) found that hens on a ration in which *L. angustifolius* replaced half of the meat meal had a higher rate of lay (69%) than for birds given all lupins (58%). For pigs, when all fishmeal in pig diets was replaced with *L. luteus* meal to maintain 17% CP, the growth rate and feed conversion efficiency was reduced (Florence 1965). In addition, King (1981) found that the growth of pigs (22 to 70 kg live weight) was not affected when 10.3% soy beans was replaced by *L. albus* (cv. Hamburg). These results indicate that both growth rate and feed conversion efficiency were decreased with high levels of lupin supplementation (Hill 1977 and King 1981). The poor performance of pigs and poultry may be a result of the following:

(a) The profile of amino acids in lupins may not meet the requirements of monogastrics, especially the low methionine content of lupins (0.59-0.87 g/16 gN) (King 1981, Barnett and Batterham 1981 and van Barneveld and Hughes 1994). In addition, lysine availability for non-ruminants varied with cultivars tested and the methods used in different experiments (Edwards and van Barneveld 1998). Edwards and van Barneveld (1998) reviewed the nutritive value of lupins for pigs and poultry, and suggested that the availability of lysine was significantly higher than that reported by Batterham *et al.* (1984) (0.37 to 0.55). Pigs and poultry were found to differ with poultry having a greater ability to use lysine than pigs.

(b) The efficiency of lupins as a pig and poultry supplement was affected by the composition of the basic diet. For example, body weight gain (g/day) of pigs was 579 for a sugar-based diet and 779 for a wheat-based diet when lupin (cv. Gungurru) seed was supplied in diets (Wigan *et al.* 1993).

(c) The α-galactosides in lupin seeds are not hydrolysed in the gut because chickens do not have the ability to secrete α-galactosidase. These undigested carbohydrates could affect the performance of chickens (Brenes *et al.* 1993). However, unlike birds, pigs are able to digest some galactans as they have a better developed gut microflora than do chickens (Barnett and Batterham 1981). But the work of Just (1981), Taverner *et al.* (1983), van Barneveld *et al.* (1995) and Edwards and van Barneveld (1998) suggested that much of this energy from lupins is absorbed from the hind gut (volatile fatty acids as energy sources), where it is less efficiently utilised by the pig than that from feeds such as wheat, and meat and bone meal which are absorbed from the small intestine (monosaccharide as energy sources).

(d) The other reason for poor performance of monogastrics is the presence of alkaloids in lupins that have a bitter taste and reduce the intake of animals, thus reducing animal production. At high levels of intake, symptoms of alkaloid poisoning can be lack of appetite, fever and difficult or laboured breathing. Low-alkaloid lupin species may be important for monogastric feeding because as little as 0.1% alkaloid content is detrimental to pigs (Hackbarth 1961), and over 0.03% of

alkaloids content in pig diets could reduce the intake, hence reduces the liveweight gain (Edwards and van Barneveld 1998).

2.4 Influence of genetic change and environmental factors on plant characteristics that may influence the nutritive value of plants

2.4.1 Domestication

In general, the changes occurring in grains, grown as crops, during domestication are morphological and anatomical, physiological and chemical, functional and 'behavioural' (Plitmann and Kislev 1989). However, the most obvious changes are the growth habit of most crop plants and the seed coat thickness of seed legumes (Donald and Hamblin 1976, Austin *et al.* 1980, Evans 1980, Lush and Evans 1980, Plitmann and Kislev 1989 and Redden *et al.* 1993). Domestic cultivars had a shorter life cycle, smaller plant size, seeds more permeable to water, higher productivity, and loss of photoperiodic sensitivity and seed dormancy, higher inbreeding and low variability. Changes in chemical content are mainly reduction of toxic substances such as alkaloids or cyanogenic compounds and development of attractive colours and taste. These changes make plants more productive, however often more sensitive to growing conditions. For example, recently released barley cultivars have shorter and lighter stems, hence a small plant which has a higher harvest index due to reduction of the weight of vegetative parts and increasing seed yield (Donald and Hamblin 1976). Reduction in the alkaloid content of lupins has resulted in increased susceptibility to disease and insect pests (Gladstones 1970).

Because seed size and seed coat colours are both positively related to commercial yield and are also desirable characters for human consumption, large seed and lighter seed coat colours are often the first choice in selection. For example, large-seeded lines of *L. angustifolius* had significantly higher seed yield than that of small-seeded lines (Delane *et al.* 1989). Normally, legume cultivars have larger seed than wild lines (Plitmann and Kislev 1989), but there are exceptions. For example, recently released cultivars of *L. angustifolius* such as Merrit have smaller seeds than earlier

released cultivars such as Yandee (Landers 1991 and Gladstones 1994). This may be because smaller seeds have less alkaloids (Watad 1980). Seed colours have also changed with breeding and selection. Many cultivars have seed coats with lighter colours due to reduction of phenolics in the seed coats (Plitmann and Kislev 1989). Wild lines of *L. angustifolius* have dark-coloured seed coats and higher alkaloid contents compared to domestic lines (Gladstones 1989).

Another obvious change occurring during legume domestication is reduction of seed coat thickness (Plitmann and Kislev 1989). The comparison of wild and domesticated accessions of *Vigna unguiculata, Lupinus*, *Pisum* and *Glycine* show that the testa of the domesticated lines was thinner and had lower specific weight than those of wild types, mainly because the palisade cells of domesticated ones are not as long or as thick-walled as in wild accessions. The specific weight of the coats declined. This reduction may be associated with the disappearance of the starch and cell contents from the mesophyll layer; and also in rough-seeded lines with loss of cell contents from the palisade layer as well (Lush and Evans 1980).

There have also been great changes both in physical and chemical characteristics of lupins with domestication. Domestic lupins contain very lower levels of alkaloids, and have a higher harvest index and their desirable characters for agronomic practice compared with wild lupins (Delane *et al.* 1986b, Hamblin *et al.* 1986, Seaton and Sedgley 1986, Delane and Gladstones 1988, Gladstones 1989, Landers 1991 Gladstones *et al.* 1992 and Gladstones 1994). These changes may be associated with the breeding objectives. The history of breeding and selection of *L. angustifolius* is outlined in Table 2.3.

Table 2.3 Cultivars of *L. angustifolius*

Cultivar	Released time	Characteristics
Uniwhite	1967	Uniwhite is tall, late maturing and prone to pod shattering.
Uniharvest	1971	A replacement for Uniwhite, late maturing with greatly reduced pod shattering
Unicrop	1973	The first early maturing variety, otherwise similar to Uniharvest
Marri	1976	A late maturing variety with resistance to Grey Leaf Spot and Anthracnose. It was never very successful due to low yield and susceptibility to lodging
Illyarrie	1979	A quick maturing variety with Grey Leaf Spot and Anthracnose resistance, and improved yield over Unicrop
Yandee	1980	A sister line of Illyarrie, very similar but higher yielding in some areas
Chittick	1982	A mid-season variety suitable for early sowing, maturity conditioned by artificially induced gene, efl
Danja	1987	An early variety with substantially improved pod set and yield over Yandee and Illyarrie
Wandoo	1987	A mid-season replacement for Chittick, released specifically for southern NSW. It yields well but is no longer grown much due to high CMV susceptibility
Geebung	1987	A late season replacement for Uniharvest and sister line of Wandoo, short with good pod set, improved yield over Uniharvest and good resistance to lodging. It is no longer in extensive use due to high CMV levels
Gungurru	1988	An early variety with moderate resistance to Phomopsis. Gungurru is short, highly resistant to lodging and has some tolerance to waterlogging. It has good pod set and yields equal to or higher than Danja in most areas. Gungurru is expected to become the dominant variety in most lupin growing areas
Yorrel	1988	A very early maturing variety with very low seed alkaloid levels and resistance to Phomopsis equal to or slightly better than that of Gungurru. It has vigorous early growth but is very prone to lodging. It is slightly better adapted to heavy textured soils than most varieties. It may find a place in low rainfall areas
Warrah	1988	Also resistant to Phomopsis, Warrah was released in SA only, for is adaptation to deep sandy soils there. It yields well with a long, cool spring, but is highly susceptible to spring drought. It is short with very good pod set but lodging resistance isn't as good as that of Gungurru

Source: Landers (1991).

Table 2.3 shows that the breeding aims focussed on improving the agronomic attributes of plants such as permeability of the seed coat, adaptation to specific environments, high-yield, drought tolerance, disease resistance, early maturity, early flowering and non-shattering pods. The changes also included reducing the branches and increasing the pod setting on main stem of lupins (Delane and Gladstones 1988). With these changes, the chemical composition of lupins may have also been changed, but such information is less readily available.

2.4.2 Environmental factors

Efficient crop production requires the tailoring of a crop type to a particular environment which includes soil fertility level, soil moisture-holding capacity, length of growing season, photoperiod, day and night time temperatures, relative humidity, solar radiation and numerous biological and management variables (Boyd *et al.* 1976, Adams 1982 and Gladstones 1982a). Environmental conditions cause large variations in chemical components and physical characteristics of the plants. For example, in cowpeas environmental effects accounted for the following proportion of variation observed in yield (93%), protein (71%), lipid (100%), threonine (96%), cystine (80%) and arginine (81%) (Oluwatosin 1997). Yield of cowpea was higher in one location than the others and yield of cowpea genotypes was from 924 to 2886 kg/ha at the same location, indicating the dependence of cowpea yield on the variety grown and the environment (Oluwatosin 1997). Protein content of an Australian collection of *L. angustifolius* varied from 31.5% to 42.3% (Gladstones and Crosbie 1979) and protein content of reduced branching lines of *L. angustifolius* varied from 35.6% to 44.4% (Petterson and Mackintosh 1994b).

2.4.2.1 Length of growing season

The length of the growing season affects plant growth, development and seed yield. Lupin (*L. angustifolius*) plants grown in a short season environment had poor yield because the plants did not produce enough branches. In contrast, when grown in a

long season environment, plants continued branching for a long time, producing tall and vegetative crops with low seed yield (Delane and Gladstones 1988). Boundy *et al.* (1982) also found that Unicrop grown in a very long season in Victoria had a HI of only 0.11 compared with a later sown crop that had a HI of 0.24.

The length of the growing season interacted with cultivars. In short growing season regions, early flowering cultivars are necessary due to late season high temperatures, low moisture and different length of photoperiod (Walton 1976 and Shanmugasundaram *et al.* 1980). The length of growing season also depends on when the plant is sown. Perry (1975) found late sowing decreased DM production and seed yield due to a foreshortened growing season, and hence reduced the lateral branch production. Perry and Poole (1975) found that late sowing reduced the length of all growth phases but drastically reduced the duration of flowering. However, early and late flowering types of lupins had different responses to late sowing. For example, later planting resulted in shortening the period to flower initiation of Uniharvest, while no such response occurred in Unicrop.

2.4.2.2 Within season effects

Temperature Temperatures play a very important role in plant growth and development as it affects all growth phases of plants. It was found that soil temperatures lower than 15°C resulted in poor germination of bean seeds (*Phaseolus vulgaris*), especially when the seeds contain less than 10% moisture at planting (Wallace 1979). Summerfield and Wien (1980) suggested that temperature affects the rate of emergence of soy beans. The lower temperatures presumably slow the rate of development to initiation (Perry and Poole 1975). However, plants are more sensitive to temperature during the flowering period. For example, once flowering begins, even three days of stress reduces the number of late flowers which produce pods and the numbers of seeds per pod (Downes and Gladstones 1984). Low temperatures (<11°C) during flowering will cause flower and seed losses, while high temperatures (>25°C) during flowering will cause flowers to abort in lupins (Walton 1982). Downes and Gladstones (1984) found that high day temperature stress at

flowering caused reduction in numbers of seed-containing pods, after flowering, and increase in ovule abortion of *L. angustifolius*. Similar results were found in beans (*Phaseolus vulgaris*), where pod set and the number of seeds per pod were negatively correlated with maximum air temperature on the days around first flowering (Wallace 1979).

In general, hot and dry weather terminated growth of lupins prematurely (Perry and Poole 1975). High temperature may reduce branching (Perry and Poole 1975), increase flower and pod abortion (Downes and Gladstones 1984) and cause poor seed filling (Delane and Gladstones 1988). Furthermore, Shibles (1980) found that plant size and leaf area at flowering with 21°C/16°C day/night temperatures were greater than in supposedly more favourable warmer regimes.

Temperatures also affected the seed quality of crops. Williams and McGibbon (1980) reported both seed weight and percentage seed oil of *L. albus* were reduced at higher temperatures, and day and night temperatures also highly affected oil content. High temperatures during the dark period resulted in low oil content only when combined with day temperatures of 20°C. Highly unsaturated fats were most commonly found in plants grown in temperate or cold regions.

Photoperiod Photoperiod is a very important factor in plant development and growth. Shanmugasundaram *et al.* (1980) reported that photoperiod influenced the phenological and physiological development of soy bean plants. Shibles (1980) reported similar results. For example, when daylength was extended from 13 h to 14 h 30 min in late development of soy bean, maturation was delayed by up to 8 days (Johnson *et al.* 1960 cited by Shibles 1980). Photoperiods longer than 8 hours promoted nodulation of both soy bean and cowpea due to receipt of greater energy in longer days (Summerfield and Wien 1980). There is evidence that lupins respond to photoperiod in different ways. For example, longer photoperiod can largely substitute for the modest vernalisation requirement of *L. luteus* (Summerfield and Wien 1980). However, *L. angustifolius* has a weak response to photoperiod (Gladstones and Hill 1969, Rahman and Gladstones 1972, 1974). A similar result

was reported by Perry and Poole (1975), who found that the photoperiod from 10 to 12 hours did not significantly influence development of *L. angustifolius*.

Soil type Soil types also play an important role for plant adaptation and seed quality. Lupins (*L. angustifolius*) performed well on coarse-textured, well-drained soils of acid-neutral reaction (Gladstones 1970 1982b). However, lupins did not perform well on heavy textured alkaline soils, becoming chlorotic, stunted and yielding poorly (Reeves 1974, Badawy *et al.* 1985 and Landers 1991). Heavy clay soils disrupt nodulation, hence reduce nitrogen fixation and lupin growth (White 1990). Several factors contribute to this poor performance. Tang and Robson (1993) found that nodulation as well as shoot and root growth of lupins was markedly reduced with increasing pH up to 7.5. The growth of shoots and roots of lupin plants was significantly decreased by an increase of the Ca^{2+} concentration or pH of the nutrient solution (Jessop *et al.* 1990 and Birchall *et al.* 1995). However, seasonal effects such as moisture and temperature significantly moderated soil type effects on plant growth. For example, in some seasons plants grown on clay soil produced a higher percentage of oil than those grown on loam, and *vice versa* (McNair 1945). Average protein content of *L. angustifolius* varied with growing states of Australia, indicated by 28.3% for Vic., 28.9% for NSW, 31.9% for WA and 32.4% for SA (Petterson and Mackintosh 1994b).

There is evidence that fertiliser application can improve seed quality. Phosphorus application increased protein content and decreased the oil content of soy beans (Kapoor and Gupta 1977). When lupins were grown with different levels of applied sulphur, the ratio of total nitrogen to total sulfur in the whole seed, total seed protein and in the extracted globulin fraction were different (Table 2.4).

Table 2.4 The ratio of N:S in the whole seed powder, total seed protein and in the extracted globulin fraction

	Uniharvest			CPI 47644		
	LS*	MS*	HS*	LS	MS	HS
Whole seed	80	22	16	80	18	13
Total protein	73	33	31	58	27	24
Globulin fraction	141	36	29	135	28	25

* LS (low sulphur), MS (medium sulphur), HS (high sulphur).

Source: Blagrove *et al.* (1976), and Gillespie *et al.* (1978).

Some wild lupin species, because of their adaptation to different soil types, have a high potential for use in Australian agriculture (Buirchell 1994, Cowling 1994, Egan and Hawthorne 1994). With adaptation to heavy-textured or alkaline soils and good tolerance to cold weather conditions, *L. atlanticus* and *L. pilosus* are potentially valuable 'rough seeded' species for these soil conditions.

Moisture Adequate soil moisture is important for plant growth and development, particularly at some stages of plant development. Yield was reduced by water stress throughout the last week of pod formation and during the rapid seed growth stage of soy beans, excess soil water reduced seed yield, seed germination and plant growth (Shanmugasundaram *et al.* 1980). For lupins, where the annual rainfall is less than 450 mm, high yield may not be achieved because of too little rainfall during the reproductive phase in September and October (Walton 1982). Research has shown that seed yield increases by 5 kg/ha for each 1mm of rain that falls during the crucial phase from August to November (Walton 1982). However, the yield of lupins declined when the rainfall was higher than 467mm, but is strongly correlated with growing season rainfall between 196 and 467 mm Walton (1986).

The responses to moisture stress at different growth stages varied. Seed yield of Unicrop decreased by 47%, produced small plants with few flowering nodes and increased flower abortion when moisture stress occurred during flowering. If stress

occurred post-flowering, seed yield was decreased by 20% (Biddiscombe 1975). Similar results were reported by Withers and Forde (1979). The effect of water stress or excess on the plant growth may be due to the impact on the physiological functions of plants and the functions of Rhizobium (Shanmugasundaram *et al.* 1980). Herbert (1978) investigated irrigation effects on plant performance, and found that irrigation during flowering and seed-filling, reduced seed yield because of an unidentified root pathogen.

2.4.3 Interaction between genetic and environmental factors

The growth, development and composition of plants largely depend on the combined action of genotype and the environment (McNair 1945 and Boyd *et al.* 1976). In soy beans, different varieties are not always influenced in the same way by the environment (McNair 1945). Wallace (1979) reported that the time from sowing to the first flower appearance was markedly affected by genotype, daylength, temperature and interactions between these factors. Yield was reduced by 161 kg/ha and 222 kg/ha for each week's delay in sowing for Uniharvest and Uniwhite respectively, whereas this effect was offset in the early flowering cultivars such as Unicrop by greater development of lateral branches (Boundy *et al.* 1982). A similar result was reported by Rahman and Gladstones (1974). Cultivar differences in methionine content of cowpeas were not significant for long days at one location, whereas they were highly significant under long days at the other locations (Bliss *et al.* 1973). Bacon *et al.* (1995) tested trypsin inhibitor activities in peas grown in different locations and suggested that some cultivars were more susceptible to environmental factors than others, and that the variance resulting from the genetic and environment interaction for trypsin inhibitor activities was up to 64% of total variance.

Regional variations in yield and nutritional components within years, although comparatively small, are more strongly correlated with climatic variables. For example, lupins (17 lines of *L. albus*) grown in one location had higher protein content (37.4% ± 2.00) than those grown in another location (32.4% ± 2.31).

Furthermore, locations clearly caused a difference in both protein and oil contents (Jimenez *et al.* 1991). Due to this interaction, it is suggested that new cultivars need to be tested under different environments before release, and also that good management systems are necessary for good yield.

2.5 Improvement of the nutritive value of lupins

2.5.1 Breeding and selection

As reviewed previously, domestication has had strong effects on physical characteristics of plants such as growth habit, plant size, seed size and seed coat thickness and colour, with these changes, the nutrient content may change as well. The major change in chemical composition of lupins is that the alkaloid level in domesticated lines is very low compared with wild lines. Early research also showed that breeding and selection could improve sulphur-containing amino acids. This is because, in lupin seeds, there are three kind of globulins, α, β and γ and only the γ-globulin contains a significant amount of methionine (Gillespie and Blagrove 1974, Blagrove and Gillespie 1975 and Hill 1986). After studying the inheritance of conglutin γ in *L. angustifolius* by crossing lines with low and high concentrations of conglutin γ, Oram *et al.* (1981) found that the concentration of conglutin γ was not controlled by a major gene. However, in the F3 conglutin γ levels of some genotypes were as high as that in the higher parental lines indicating that it was probably not controlled by a large number of loci (Oram *et al.* 1981). This suggests that it may be possible to select lupin seed for increased conglutin γ. When this selection is integrated with over 200,000 tonnes of lupins consumed by sheep annually, significant improvements in animal production would occur as proper S content in the diet of sheep could increase feed utilisation (Hegarty *et al.* 1994) and wool production (Peter *et al.* 1987). Another way for breeders to improve the quality of lupins is to draw upon the large pool of genetic diversity. For example, *L. pilosus*, *L. cosentinii* and *L. atlanticus* have shown promise in early trials, they contain high protein and may have some desirable features for animal production as well.

2.5.2 General management for livestock using lupins

2.5.2.1 Ruminants

The high solubility of protein in the rumen and the high ratio of nitrogen to sulphur may be major problems for ruminant production because of adverse effects on rumen bacteria. So pre-treatment may be useful to improve of the utilisation of lupins on farms.

Supplement strategies The efficiency of lupins as a feed supplement was affected by both the basal diet used and the levels supplied. Lupins mixed with cereals could result in high efficiency of utilisation of lupins in terms of animal production (Kenney 1980, 1986, Kenney and Smith 1985 and Murray 1994). Kenney (1986) reported that inclusion of lupins in wheat-based diets had no effect on the digestibility or intake of feed or weight gain of lambs, but these parameters were increased with the inclusion of lupins in diets based on oaten seed. Lupin inclusion level in the diets also affected the efficiency of utilisation . There was evidence that the most pronounced response to lupins occurred at inclusion levels of 0 to 30% (Kenney 1986) and 0 to 15% (Kenney and Smith 1985). This suggests that there might be different maximum inclusion levels, depending on the basal diet.

Sulfur supplementation Due to the high ratio of N to sulphur in lupins, the rumen bacteria use crude protein inefficiently. The addition of sulphur to lupin diets has been tested in numerous experiments. However, the responses to sulphur addition were not consistent. The inclusion of sulphur in a mineral lick for young Merino sheep increased clean fleece weight, and the addition of 1.2% methionine increased liveweight of sheep fed lupin diets (Peter *et al.* 1987). In contrast, Doyle *et al.* (1992) reported that neither the addition of sulphur nor the mineral lick had any significant effects on fleece weight and liveweight. This difference may be due to differences in the basal diets fed in these experiments. These effects of the basal diet

may overshadow some of the beneficial effects on production parameters with sulfur supplementation of lupin diets.

Different sulphur sources have also been compared by Murray *et al.* (1990 and 1991), who found that wool growth was decreased by supplementation with gypsum and Mepron, and increased by addition of fishmeal. Liveweight gain was increased by the addition of Mepron, methionine and fishmeal.

Mechanical processing of lupin seeds Coarsely crushing lupin seeds could improve their utilisation by animals. This is because the lupin coat is considerably thicker than that of soy bean and may be digested more slowly in the rumen. It seems that coarsely ground lupins probably supply more available energy than whole lupins. May *et al.* (1993) reported that cows produced 2.0 kg/day more milk by consuming ground lupins compared to whole lupins. The digestibility of hammer milled lupin seed was higher than that of whole seed due to less whole seed excreted in faeces (Valentine and Bartsch 1986). May and Barker (1984) reported that milling seed had positive effects on cattle production, with an increase in growth rate and a reduction in the conversion ratio of DM/kg live weight gain. There was evidence that milling lupin seed has a greater positive influence on rumen pH and fibre digestion than milling barley seed (Valentine and Bartsch 1987).

Roasting and formaldehyde treatments of lupins Due to the considerable net loss in crude protein between the mouth and the abomasum of sheep fed lupins (Lindsay *et al.* 1980 and Robinson and McNiven 1993), a number of experiments were conducted to test the effects of roasting and formaldehyde treatments on lupin utilisation. The results of these experiments were variable. Robinson and McNiven (1993) compared soy bean meal, raw lupin and roasted lupin and found that roasting reduced *in situ* nitrogen disappearance of lupins due to a reduction in the soluble crude protein fraction, and increased the retention of nitrogen compared with soy bean meal. However, there was no difference in the growth rate and feed efficiency among these three protein supplements. Kung *et al.* (1991), Kibelolaud *et al.* (1993) and Singh *et al.* (1995) reported similar results. In contrast, Moate *et al.* (1984) found that heating lupins at 130° C for 3 hours did not significantly protect lupin

protein from ruminal digestion. There were no significant positive responses to formaldehyde treatment of lupin seed fed to young sheep, cattle and cows (Fortune *et al.* 1980, Hynd and Allden 1986, Davis *et al.* 1987 and Hough 1991). The poor responses to roasting and formaldehyde treatment may be because these treatments failed to increase the level and availability of essential amino acids in the small intestine of animals (Fortune *et al.* 1980, Ashes *et al.* 1984 and Gatel 1994).

2.5.2.2 Non-ruminant utilisation

Deficiency of some essential amino acids such as methionine, the high levels of NSP, oligosaccharides and alkaloids in lupin seeds may contribute to the poor performance of monogastrics fed relatively high levels of lupins. To overcome the limitations of lupins as a monogastric feed, physical treatment (dehulling, crushing) and supplementation (sulfur-containing amino acids, enzymes) has been used.

Mechanical processing Crushing is necessary for lupins to be included in monogastric diets (Gladstones 1970 and King 1990). This is because most lupins have large amounts of fibrous testa that consists mainly of NSP and oligosaccharides (Brenes *et al.* 1993, Hove 1974 and Withers *et al.* 1975). In trials (pig) by Mangold and Columbus (1938), grinding improved the digestibility of organic matter (OM) and CP of *L. luteus* by 26% and 32%, respectively.

Dehulling may be another effective method to improve the nutritive value of lupins for monogastrics (Godfrey and Payne 1987, Rowe and Hargreave 1988, Wigan *et al.* 1994 and Edwards and van Barneveld 1998). Removal of seed coats which contain large amounts of complex carbohydrates increased the apparent metabolisable energy by 11%, the apparent protein digestibility by 7%, and reduced feed consumption by 3% and feed to gain ratio by 5% (Brenes *et al.* 1993).

Addition of amino acids Addition of synthetic sulfur-containing amino acids in monogastric diets containing lupins could improve the efficiency of utilisation of lupins (Liebholz 1984 and van Barneveld and Hughes 1994).

Supplementation of enzymes High NSP and oligosaccharides, which limit nutrient utilisation by monogastrics, have been tested in a number of experiments with the addition of enzymes. It has been found that addition of enzymes to lupin diets facilitated the break down some of these complex carbohydrates, hence improved the efficiency of lupins for non-ruminants, especially for chickens. This is because chickens have a simple gut and small fermentation ability, hence cannot efficiently digest the more complex carbohydrates of lupins (Khan 1992). Pigs were able to ferment these carbohydrates, but the efficiency was low (Edwards and van Barneveld 1998). Brenes *et al.* (1993) reported that enzyme addition (combination of Energex® carbohydrase, Bio-Feed pro®-protease, and Novozyme ®-α-galactosidase) to a diet containing 70% raw lupins improved the weight gain and feed to gain ratio of broiler chicks by 18 and 10% respectively. The possible reason for this result is that the hydrolysis of these carbohydrates by enzymes may increase the energy value of lupin meal and also improve the nutritive value of other dietary components (Brenes *et al.* 1993).

2.6 Objectives of this research

It is evident that the process of selecting and breeding plant species often results in a number of simultaneous changes in the plant and its harvested components. In this work, it is evident that rapid changes in both growth habit and alkaloid content have occurred for *L. angustifolius* over a comparatively short period (30-40 years). However, with these changes, adaptation to environment and chemical composition (protein, fibre and minerals) may be changed as well. In beginning the path to domestication of *L. atlanticus* and *L. pilosus*, we may learn from the process of breeding and selection of *L. angustifolius*.

Therefore, the focus of this work was to:

1. study the changes in seed structure, yield and chemical composition of *L. angustifolius* with breeding and selection,
2. study the influence of the environment on nutrient content of lupins, and

3. compare the nutritive value of wild lupins with domesticated lupins.

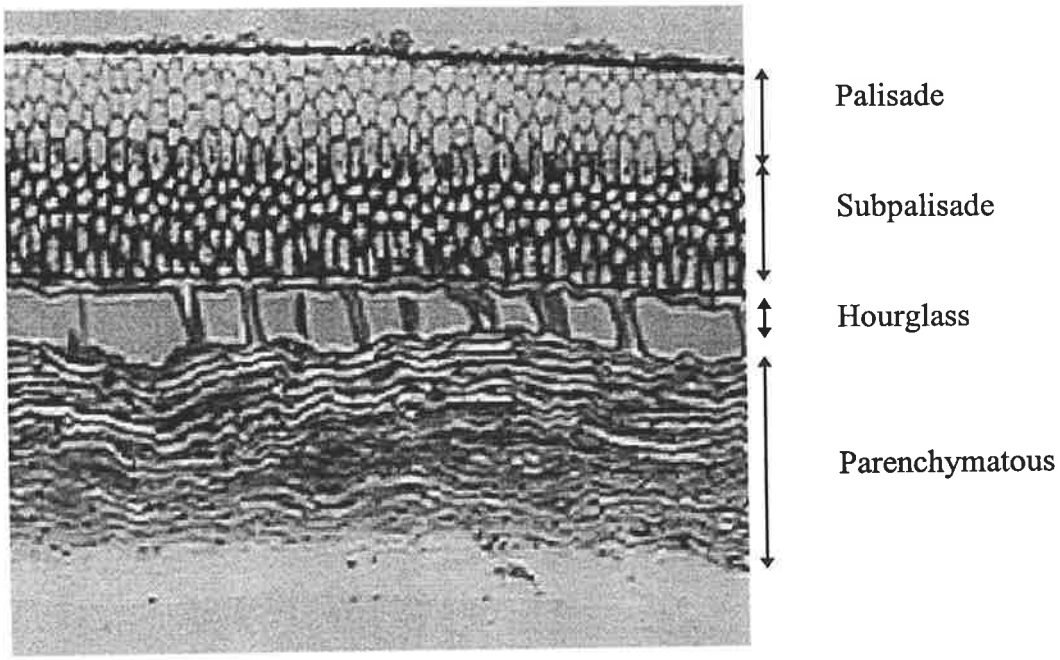
CHAPTER 3

ANATOMICAL STRUCTURE AND NUTRITIVE VALUE OF LUPIN SEED COATS

3.1 Introduction

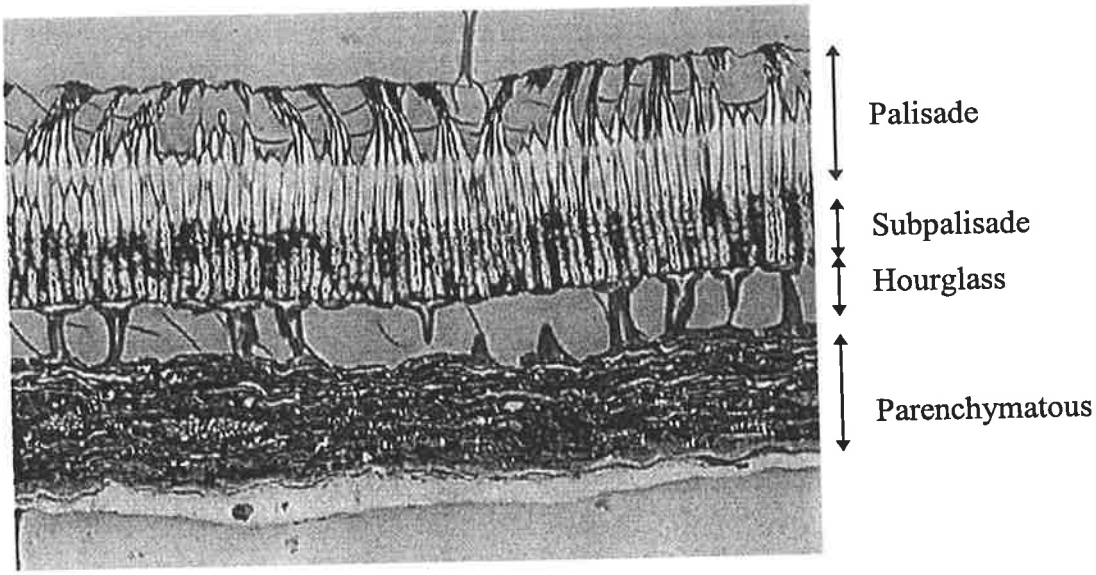
The seed coat is an important structure because it is the protective barrier between the embryo and the external environment. Seed coats affect seed performance through factors such as interference with water uptake and gas exchange, mechanical restraint, prevention of the exit of inhibitors from the embryo and supply of inhibitors to the embryo (Esau 1977, and Bewley and Black 1994a). These functions play an important role in the adaptation of legumes. For example, the wild legume seed coat is thicker and less permeable to water, which is important for wild plants as it delays germination until environmental conditions are suitable. On the other hand, the seed coat of domesticated legumes is thinner and more permeable to water, making it easier to germinate seeds simultaneously (Arrieta *et al.* 1994, and Lush and Evans 1980).

The legume seed coat basically has three layers. The outermost layer is the palisade layer, which is a single or in some places a double layer of palisade cells (Lush and Evans 1980). Kadam *et al.* (1989b) found that the palisade cells did not seem to have uniform thickness. Between the palisade and the parenchymatous layer are hourglass cells, mostly in a single layer. The shape of the hourglass cells varies from species to species, and even within species (Kadam *et al.* 1989b and Chowdhury 1970). The last layer is the parenchymatous layer, which is composed of several layers of deposited mesophyll cells (Kadam *et al.* 1989b). The seed coat structure of lupins is shown in plate 1.



L. angustifolius (cv. Gungurru)

(45×32 micron)



L. atlanticus (P22927)

(90×64 micron)

Plate 1 The seed coat of structure of lupins.

The most important layer for hardseedness is the palisade layer. Corner (1951) reported that the hardness and impermeability of the dried seed coat are mainly caused by the contraction of the walls of the palisade cells as the seeds mature. Lush and Evans (1980) studied the relation between seed coat structure and its function using cowpeas, and found that while there was no general relation between seed coat structure or thickness and seed hardness, the palisade layer was involved. They also suggested that the palisade cells of domesticated legumes were shorter and thinner than wild ones. This finding was supported by Plitman and Kislev (1989), who reported that the most obvious changes during legume domestication were related to a decrease in seed coat thickness, and that this decrease was due to a reduction of the palisade layer.

The nutrient content of the seed coat has been widely investigated by Hill (1977), Withers *et al.* (1975), Brillouet and Riochet (1983), and Gil and Cubrero (1993). The main constituent of the seed coat is crude fibre, but it also contains low quantities of crude protein, starch and lignin. However, fibre in the lupin seed coat was highly digested by sheep (Rowe and Hargreave 1988, and Kadam *et al.* 1989b). Also after 48 hours incubation *in vitro* using an enzyme preparation, 75% of sweet lupin and 85% of bitter lupin seed coats were digested (Bailey *et al.* 1974).

The seed coat proportion and nutrient content may change with domestication and the environmental conditions in areas where the crop is grown. This is because during the breeding and selection process, the modification of one or more characteristics can cause a chain of events where each change is accompanied by or associated with other changes (Lush and Evans 1980 and Plitman and Kislev 1989). For example, reduction in branching of lupins (*L. angustifolius*) was associated with a decrease in seed size (Landers 1991). Reduction of toxins of legumes was associated with changes in seed size and seed number (Janzen, 1969 cited by Plitman and Kislev 1989). Seed coat thickness and colour were affected by photoperiodic conditions, and these conditions at maturation (Bewley and Black 1994b). For example, seeds maturing under long days were smaller and thicker coated than those maturing under short days (Bewley and Black 1994b). While examples of such linkages are numerous, they are not always easy to predict for different species.

The seed coat has significant effects on seed biochemistry and physiological functions. Its structure, thickness and chemical composition are affected by domestication and environment. There is little known about differences in seed coat anatomical structure between lupins at different stages of domestication or between seeds grown under different environmental conditions. Both of these factors may influence the relationship between the structure and the nutritive value of lupin seed coats. Therefore, the aim of the experiment reported in this chapter was to investigate:

1. the impact of domestication on seed coat structure and seed coat thickness of *L. angustifolius*,
2. the difference in seed coat structure and thickness between hard and soft lupin seeds, smooth and rough lupin seeds and large and small lupin seeds,
3. the impact of sowing time and growing season length on the seed coat structure and thickness, and
4. the nutritive value of seed coats from different lines of lupins, and the relation between the nutritive value and the seed coat structure or thickness.

3.2 Materials and methods

3.2.1 Materials

Part 1 The seed coat structure of *L. angustifolius* cultivars released in different years

The cultivars A total of 12 cultivars of *L. angustifolius* (Table 3.1) from Western Australia were used to determine the seed coat structure and thickness. This seed had been grown in small plots to maintain seed lines.

Part 2 The seed coat structure of lupin seeds with different agronomic characteristics

The lupin lines A total of 11 lines of lupins (Table 3.2) including 2 lines of *Lupinus cosentinii,* 3 lines of *L. pilosus* and 6 lines of *L. atlanticus* from Western Australia were used to determine the seed coat structure and thickness of hard, soft, smooth and rough seeds.

Table 3.1 Cultivars* of *L. angustifolius* and their year of release (refer Table 2.3 for detail)

Year released	Genotype
1967	Uniwhite
1971	Uniharvest
1976	Marri
1979	Illyarrie
1980	Yandee
1982	Chittick
1987	Geebung, Danja, Wandoo
1988	Gungurru, Warrah, Yorrel

* Cultivars were from WA.

Table 3.2 Seed characteristics of lupin lines*

Species	Line	Seed characteristics
L. cosentinii	Erregulla	hardseeded
	Erregulla-so	softseeded
L. pilosus	P27445	smoothseeded
	P1993	roughseeded
	P23030	roughseeded
L. atlanticus	AM2.9.2	hardseeded
	P22927	hardseeded
	MD92(96)	hardseeded
	AM3.18	hardseeded
	AM3.18M	softseeded
	85E10	softseeded

* Lines were from WA.

Part 3 Effect of sowing time on seed coat structure of lupins

Six lines of lupins including 2 lines of *L. angustifolius* (cvv. Chittick and Gungurru SA), 2 lines of *L. pilosus* (P1993 and P23030) and 2 lines of *L. atlanticus* (P22927 and MD92(96)) were sown at Turretfield Research Centre on May 12 and June 5 in 1995 (for details see Chapter 4), and were used to measure the impact of sowing time on the seed coat structure and thickness of lupins.

Part 4 Effects of growing year on seed coat structures of lupin seeds

Seven lines of lupins were grown in 1993 and 1994 at MRC, SA. One line of *L. cosentinii* (cv. Erregulla), 3 lines of *L. atlanticus* (P22924, MAR5300 and MAR3064) and 3 lines of *L. pilosus* (P20954, P23341 and P23342) were used to analyse the impact of growing year on seed coat structure and seed coat thickness.

Part 5 The effect of seed size on the seed coat structure of lupin seeds

One hundred seeds of each line from each sowing time (Part 3) were weighed. The 5 biggest and 5 smallest seeds were selected to measure the seed coat structure and thickness of large and small seeds.

Part 6 The relationship between seed coat chemical composition, structure and degradability

Several hundred seeds of each line (Table 3.3) were separated by hand into coat and kernel components, and the seed coat structure and thickness, seed coat percentage of whole seed weight, the chemical components and degradability in the rumen of sheep of the seed coat were determined.

Table 3.3 Lines of lupins used for studies of coat structure and degradability

Species	Line	Place
L. angustifolius	Illyarrie	WA
	Warrah	WA
	Yorrel	WA
	Chittick	SA
	Gungurru	SA
L. atlanticus	AM2.9.2	WA
	P22927	WA
	P22927	SA
	MD92(96)	SA
L. pilosus	P1993	SA
	P23030	WA
	P23030	SA

3.2.2 Methods

Coat thickness analysis Three seed coat pieces (5 mm in any dimension) of each line were embedded in GMA monomer (GMA, polyethylene glycol and benzoyl perioxide) for 2 days. Slides were prepared for the analysis of thickness using a Sorvall JB4 microtome with glass knives. The 2.0 μm thick tissue sections were observed with a x10 objective lens. The thickness of four tissue layers (palisade, sub-palisade, hourglass and parenchymatous) was measured from images collected with a Bio-Rad computer imaging system MRC1000 connected to a Nikon Diaphot 300 microscope and using CoMOS image analysis software.

Chemical component analysis NDF and ADF in 12 samples were determined with 2 replicates using the method of Harris (1984).

In sacco digestibility

Animal, feeding and management Four mature Merino wethers with rumen cannula were kept in individual pens and fed twice daily at 8:30 and 16:00 with a ration consisting of 65% oaten chaff, 15% lucerne chaff, 15% lupin seed (cv. Gungurru) and 5% mineral mix. The rumen cannula of each animal was routinely cleared to ensure it remained secure and stable. The sheep were fed for 7 days to adapt to the diet. Water was available at all times.

Procedure For each of the incubation runs, 1 g of seed coats from each sample were weighed into previously dried and weighed nylon bags (10.4×10.4 cm, pore size of 44 μm, Swiss Screens). There were 4 replicates for each sample. The bags containing the sample and glass marbles were fastened with nylon fishing line and wetted before inserting into the rumen. Nine bags were inserted into the rumen via the cannula at 8:00 am on the first day of any given incubation period. Samples were incubated in the rumen for periods of 2, 4, 8, 12, 24, 36, 48 and 72 hours. Ground lucerne chaff (1 g samples) were used as a uniform standard and incubated for 48 hours in all animals and runs. This provided some control both within time periods and between animals, and for longer term variation over the experimental period.

After removal from the rumen, the bags were immediately washed under running water until the rinse water was clear. On average, this took about 3 minutes per bag. The bags were dried in an oven at 65 °C for 48 hours, then weighed.

3.2.3 Statistics

Year of releases in part 1, seed agronomic characteristics in part 2, sowing time in part 3, growing year in part 4, seed size in part 5 and lines in part 6 were the main factors in these experiments. Data were analysed by using a general linear model and linear regression from Systat (Wilkinson 1996).

3.3 Results

3.3.1 Seed coat structure of *L. angustifolius* genotypes released in different years

There was no significant difference in total seed coat thickness with year of release (Table 3.4), but the depth of palisade and sub-palisade of cultivars released in 1971 was 25 μm and 29 μm thinner respectively than those released in 1987. There were no significant differences in depth of hourglass and parenchymatous layers.

The one hundred seed weight was significantly less by an average of 23% for cultivars released in 1987 and 1988 compared to cultivars released in 1971. The correlation between 100 seed weight and year of release was significant and negative (R=0.595, n=12, SD=1.63) (Figure 3.1). The correlation between 100 seed weight and the seed coat thickness was significant and negative (R=0.849, n=12, SD=14.47).

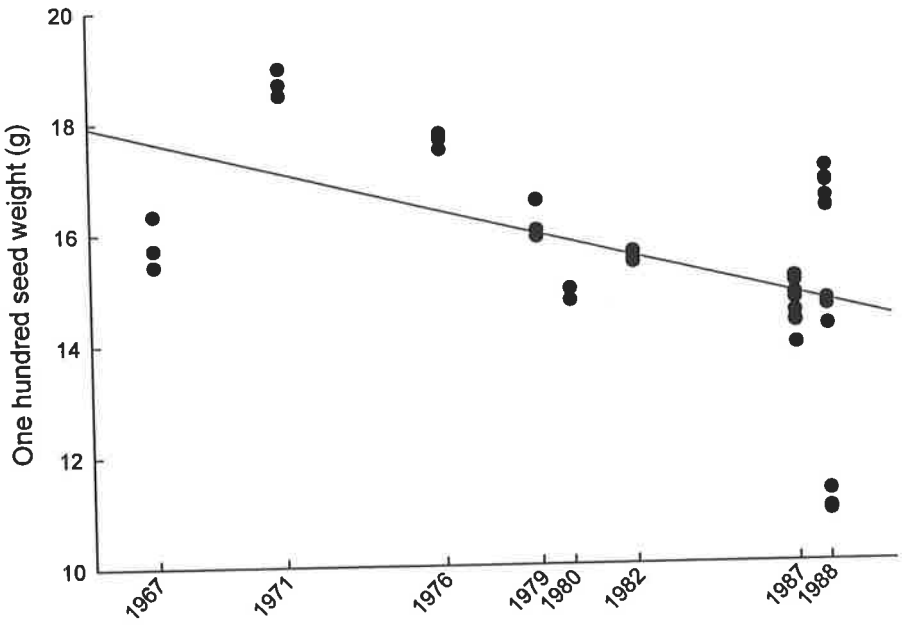


Figure 3.1 The relationship between seed weight and year of release of cultivars of *L. angustifolius*.

Table 3.4 The seed weight and depth (μm) ** of the 4 cell types that form distinctive layers in the seed coat of different genotypes of *L. angustifolius* (means ± s.e.)

Year released	n*	Weight /100seed(g)	Palisade	Sub-palisade	Hour-glass	Parenchy-matous	Seed coat thickness
1967	6	15.8ab	67±2.2ab	77±2.7ab	34±3.5	115±4.9	293±8.6
1971	6	18.7a	55±2.5b	61±1.8b	36±2.7	105±2.2	257±3.4
1976	6	17.6ab	67±2.6ab	73±3.7ab	30±4.2	106±4.7	275±12.4
1979	6	16.2ab	66±2.7ab	75±2.3ab	36±1.7	111±3.8	288±5.8
1980	4	14.8ab	74±4.7ab	104±7.9a	35±1.4	99±3.0	313±4.7
1982	6	15.5ab	70±4.9ab	94±8.7a	31±1.3	109±4.0	304±13.8
1987	18	14.6b	80±4.6a	90±4.4a	40±2.7	105±4.9	316±13.1
1988	17	14.1b	77±4.4ab	84±4.2ab	36±4.3	112±5.8	309±14.5

* number of samples tested for each year.

** Figures in the same column with different letters were significantly different ($p<0.05$).

3.3.2 The structure and thickness of the seed coat of hardseeded, softseeded, smoothseeded and roughseeded lupins

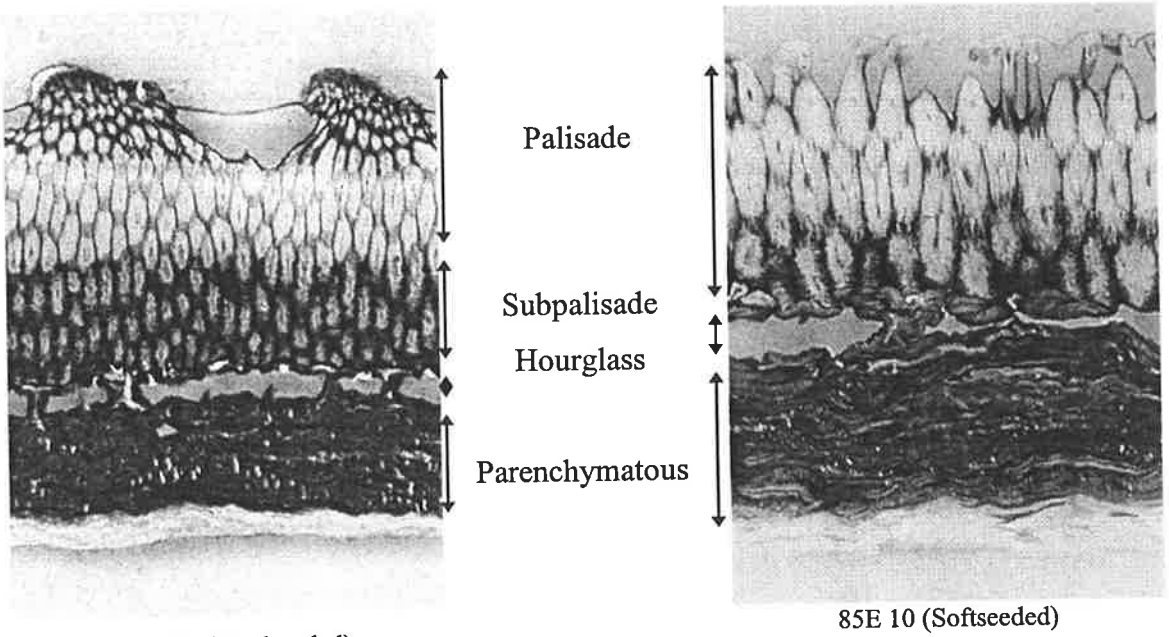
The structure of softseeded, hardseeded, smoothseeded and roughseeded lupins varied. Generally, hardseeded and roughseeded lines had thicker coats than softseeded and smoothseeded, but the layers contributing to these thick seed coats were different. The main contributor to the thick seed coat of hardseeded lines was the hourglass layer, which was almost twice as thick as that of the softseeded line ($p<0.01$). Also the palisade layer in hardseeded lines tended to be thicker than for the softseeded line ($p>0.05$). Roughseeded lines had seed coats with a much thicker palisade layer than the smoothseeded line ($p<0.01$). However, the smoothseeded line had a thicker parenchymatous layer than the roughseeded lines ($p<0.05$) (Table 3.5,

Plate 2 and 3). Plate 2 and Plate 3 show that the cell shape and cell sizes are different between hardseeded and softseeded seeds and between smoothseeded and roughseeded seeds. Softseeded seed coats tended to have short and round cells for the palisade layer while hardseeded seed tended to have long cells for the palisade layer. Smoothseeded seed had round cells for sub-palisade, but roughseeded had long cells for this layer.

Table 3.5 The depth of 4 layers (μm) of the seed coats of soft seeded, hard seeded, smooth seeded and rough seeded lupins

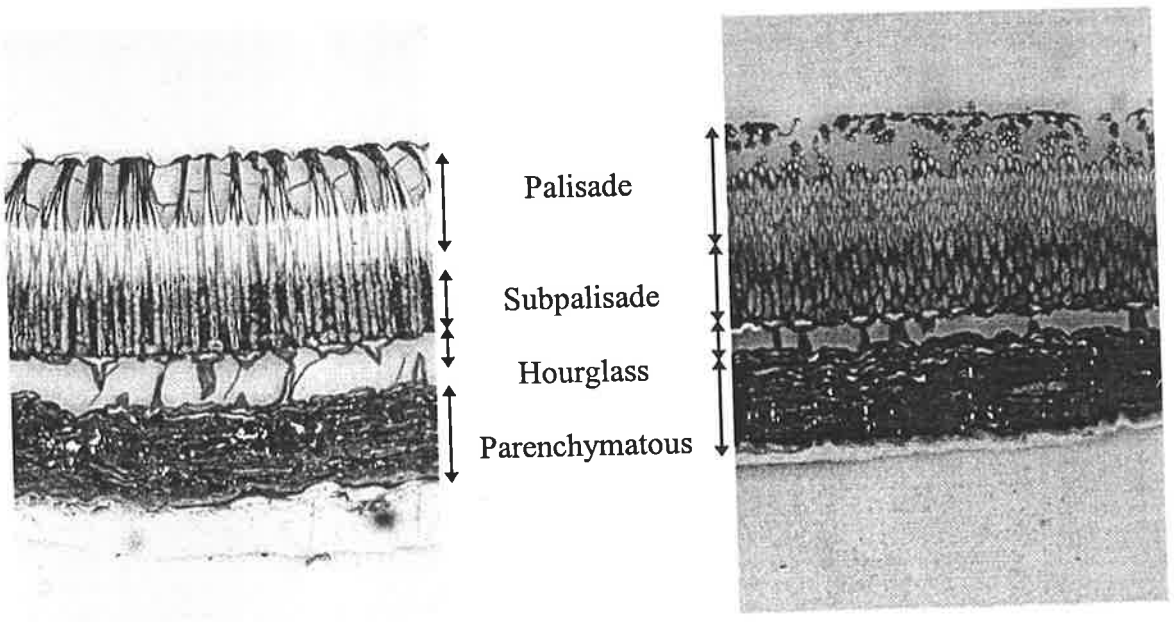
Line	n#	Palisade	Sub-palisade	Hourglass	Parenchy-matous	Coat thickness
Soft	3	123.7	134.1	35.2**	136.3	388.1*
Hard	5	149.6	115.0	62.3**	132.5	459.5*
Smooth	1	131.3**	86.8	49.8	164.0*	432.0*
Rough	2	337.4**	84.9	59.4	129.8*	611.5*

line numbers, figures in the same column within soft and hard or smooth and rough seed category were significantly different (* $p<0.05$, ** $p<0.01$).



Erregulla (Hardseeded)
(45×32 micron)

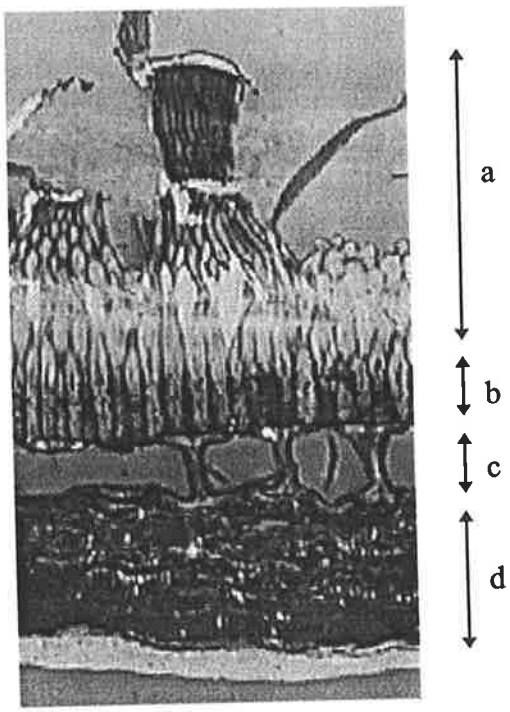
85E 10 (Softseeded)
(90×64 micron)



AM3.18 (Rough-seeded)
(90×64 micron)

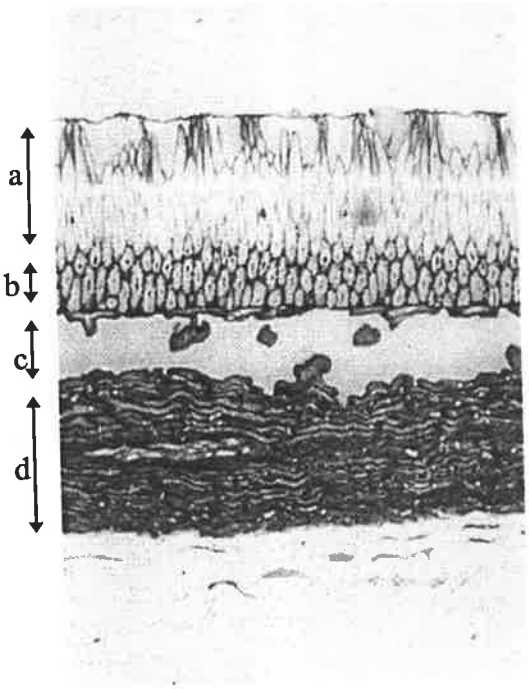
AM3.18m (Smooth-seeded)
(90×64 micron)

Plate 2 The structure of the seed coat of lupin seeds with different agronomic characteristics.



P23030 (roughseeded seed coat)

(90×64 micron)



P27445 (smoothseeded seed coat)

(45×32 micron)

Plate 3 The structure of the seed coat of roughseeded seed and smoothseeded seed of *L. pilosus* (a=Palisade, b=Subpalisade, c=Hourglass and d=Parenchymatous).

3.3.3 The effects of sowing time on seed coat structure and thickness

There was no significant difference between the two sowing times in seed coat thickness of lupins ($p>0.05$) (Table 3.6). There was no interaction between line and sowing time ($p>0.05$).

Table 3.6 The depth (μm) of 4 layers and total thickness of the seed coat of lupins from plants sown at different times

ST	n	Palisade	Sub-palisade	Hourglass	Parenchy-matous	Coat thickness
Early	6	164.4	107.3	41.6	130.2	443.4
Late	6	156.3	100.8	41.7	130.7	429.5

3.3.4 The effects of growing year on seed coat structure and thickness

Seeds grown in 1994 had a significantly thinner seed coat (365.9 vs 439.5 μm) for all lines than seeds grown in 1993. The thickness of sub-palisade and parenchymatous layers was reduced by 15% and 11% respectively for seeds grown in 1994. Growing year had no influence on the thickness of the hourglass layer. There were significant interactions between growing year and line in palisade and seed coat thickness ($p<0.01$). The palisade layers of P23342 ($p<0.01$), P20954 ($p<0.01$) and MAR5300 ($p<0.05$) were much thinner in 1994 than in 1993, while slightly decreased for P23341, MAR6034 and Erregulla in 1994, with almost no change for P22924. The total coat thickness of 6 lines reduced in 1994, but the line P22924 was rather consistent between the two years (375.8 μm for 1993, 378.8 μm for 1994) (Table 3.7).

Table 3.7 The depth of 4 layers (μm) and total thickness of the seed coat of lupins grown in 1993 and 1994

	Palisade		Sub-palisade		Hourglass		Parenchymatous		Coat thickness	
Gyear* / Line	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
P20954	249.5	188.4	79.1	61.7	27.2	15.5	140.5	106.0	496.1	366.0
P23341	247.2	161.8	81.7	62.6	31.4	28.4	124.6	114.2	485.0	367.0
P23342	265.2	152.4	86.2	67.1	36.2	26.0	154.6	117.3	541.9	362.7
MAR5300	155.2	114.2	93.0	74.8	28.8	38.2	141.8	144.6	418.9	371.9
MAR6034	126.9	118.6	105.6	86.5	41.6	35.3	154.0	153.5	428.2	393.7
P22924	121.5	122.0	96.4	92.0	34.5	42.0	137.0	122.9	375.8	378.8
Erregulla	106.5	101.4	97.3	96.9	20.5	25.4	106.5	94.5	330.7	321.2
Mean	181.7	137.0	91.3	77.4	31.5	30.1	136.9	122.3	439.5	365.9
Year		**		**		NS		**		**
Line×year		**		NS		NS		NS		**

* Gyear: growing year ** $p<0.01$, NS: no significance, The residual degrees of freedom was 27.

3.3.5 The structure and thickness of the seed coat for seeds of different sizes

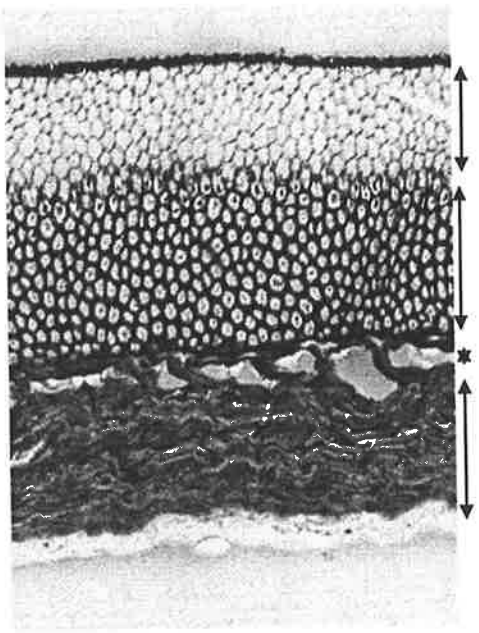
As there were no interactions between sowing time and line, or sowing time and seed size, data were pooled to analyse the effects of seed size on seed coat thickness. The depth of seed coat layer and the total seed coat thickness of small and large seeds varied except for the hourglass layer, which averaged 42.5 μm for large seeds and 40.8 μm for small seeds. This variation was significantly affected by the interaction between seed size and line (Table 3.8). Generally, larger seeds of wild lines had a thicker palisade, sub-palisade and parenchymatous layer, hence a thicker seed coat compared to small seeds. Larger seeds of domesticated lines had a thinner palisade and sub-palisade layer, hence a thinner seed coat than small seeds (Plate 4).

Because of the interaction between seed size and lines, only wild lines were used for regression analysis of seed weight and seed thickness. Seed weight was significantly and positively correlated to palisade, parenchymatous layer and the total seed coat thickness ($p<0.01$) ($R=0.84$, $SD=31.33$, $R=0.64$, $SD=14.23$ and $R=0.82$, $SD=41.55$, $n=16$ respectively) (Figure 3.2).

Table 3.8 The depth of 4 layers (μm) and total thickness of the seed coat in large or small seeds of the same lines of lupins

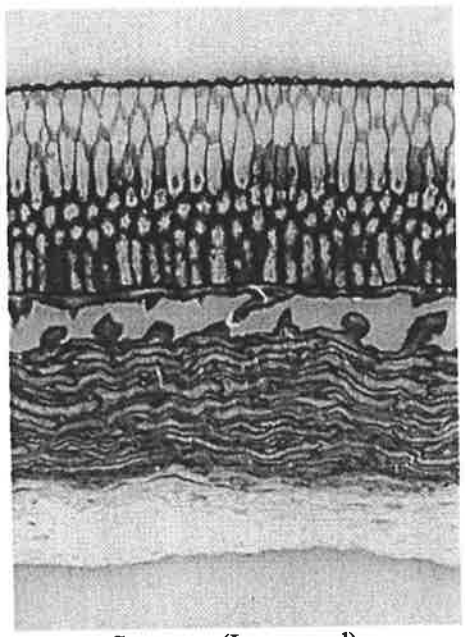
	Weight/seed (mg)		Palisade		Sub-palisade		Hourglass		Parenchymatous		Coat thickness	
Seed size / Line	l	s	l	s	l	s	l	s	l	s	l	s
MD92(96)	392	195	189.5	161.2	120.8	115.5	41.7	39.9	147.9	124.7	499.9	441.3
P22927	315	162	161.8	145.9	108.3	111.4	41.1	36.7	151.2	112.2	462.4	406.3
Chittick	187	98	72.9	83.4	85.2	106.1	35.1	30.3	132.4	114.3	325.6	334.1
Gungurru	163	77	75.0	103.4	99.8	143.2	35.2	37.9	128.7	139.2	338.7	423.6
P1993	627	308	327.3	201.6	102.6	87.6	50.7	51.9	151.7	115.2	632.3	456.3
P23030	482	290	211.6	190.4	87.3	80.3	51.0	48.2	135.7	112.4	485.6	431.2
Mean	361	188	173.0	147.6	100.7	107.4	42.5	40.8	141.3	119.7	457.4	415.5
Seed size				**		NS		NS		**		**
Line×seed size				**		**		NS		**		**

l: large seed s: small seed ** $p<0.01$, NS: no significance.



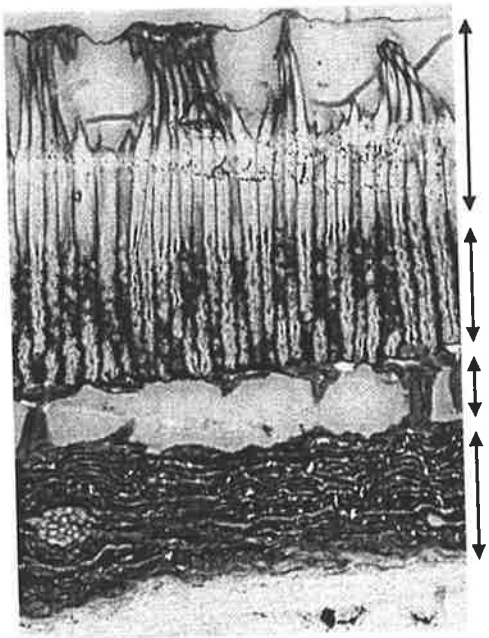
Gungurru (small seed)

(45×32 micron)



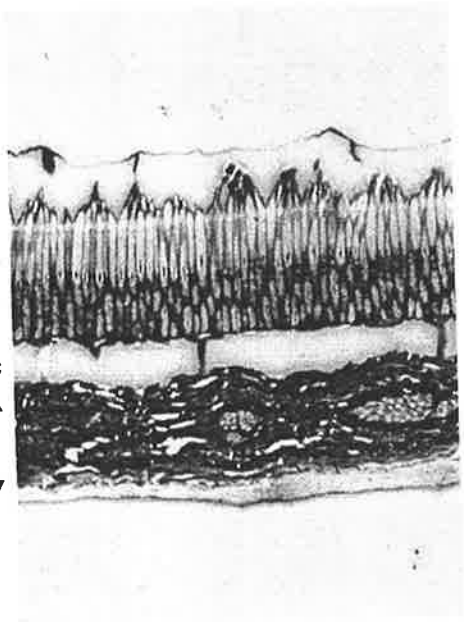
Gungurru (Large seed)

(45×32 micron)



P22927 (Small seed)

(45×32 micron)



P22927 (Large seed)

(90×64 micron)

Plate 4 The seed coat structure of small or large seeds from the same line of lupins.

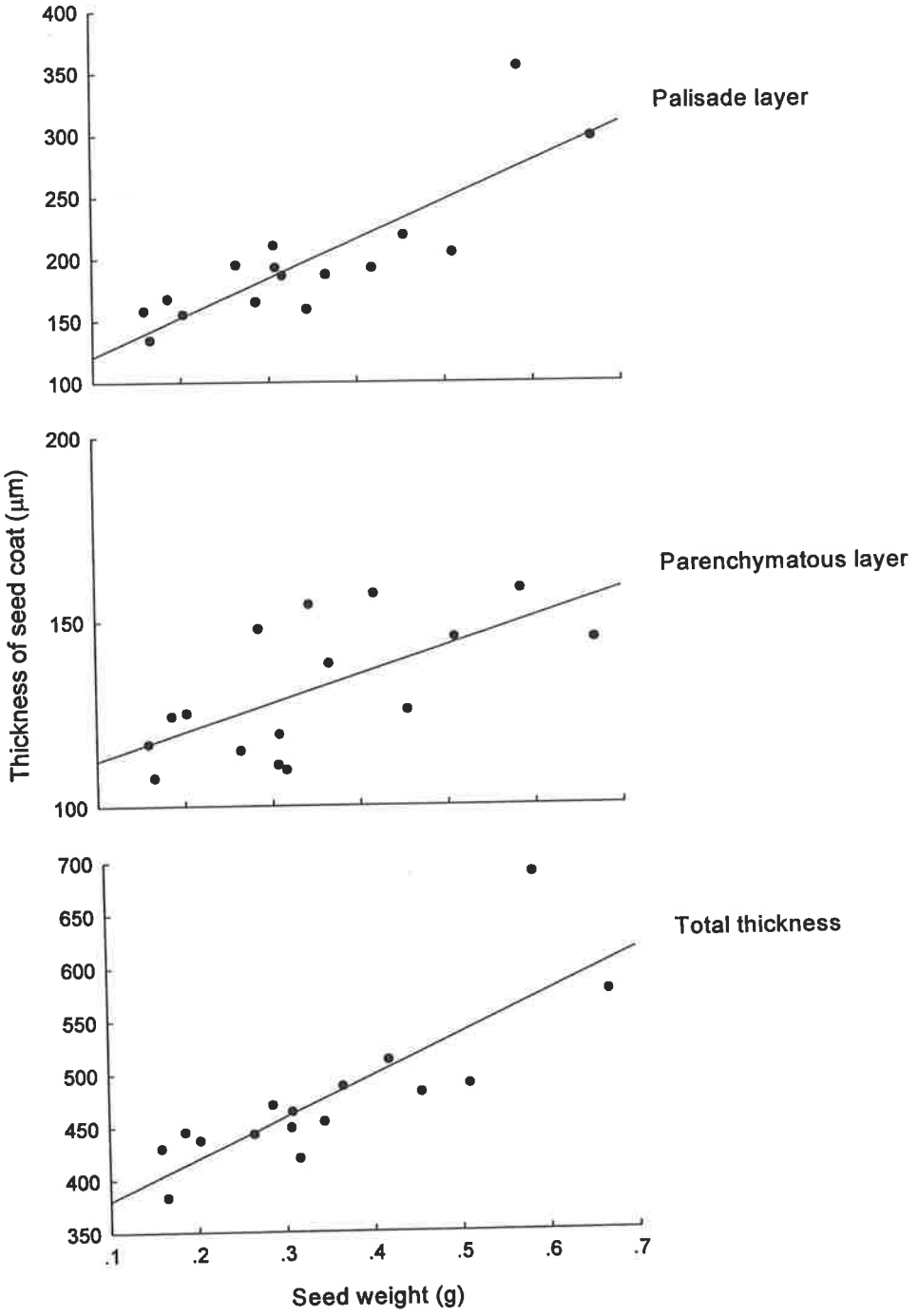


Figure 3.2 The relationship between individual seed weight and the depth of layers of the seed coat of wild lupins.

3.3.6 The chemical composition and degradability of the seed coat of lupins

Seed structure, seed coat thickness and chemical composition of lupins One hundred seed weight and seed coat percentage varied considerably among species. *L. pilosus* had the heaviest seed and *L. angustifolius* had the lightest with a lower proportion of seed coat compared with the other species.

The seed coats of lupins contained about 80% crude fibre. With large variation between species, wild species (*L. pilosus* and *L. atlanticus*) had nearly 10% more ADF and NDF than *L. angustifolius* (Table 3.9).

The depth of layers and the total thickness also varied. *L. pilosus* had the thickest seed coat, while *L. angustifolius* had the thinnest, mainly resulting from the thick palisade layer of *L. pilosus*.

Both ADF and NDF content were significantly related to the depth of the palisade layer and the total seed coat thickness ($p<0.01$) ($R=0.84$, $SD=43.27$ and $R=0.82$, $SD=60.29$ for ADF, and $R=0.83$, $SD=45.22$ and $R=0.90$, $SD=45.13$, $n=12$ for NDF respectively). In addition, NDF content was correlated with the thickness of the hourglass layer ($P<0.05$ and $R=0.613$, $SD=12.81$, $n=12$).

Table 3.9 Seed structure, seed coat thickness (μm) and seed coat chemical composition of 12 lines of lupins

Species	Line	100seed weight(g)	Seed coat(g/kg)	ADF (g/kg)	NDF (g/kg)	Palisade	Sub-palisade	Hour-glass	Parenchy-matous	Total seed coat thickness
L. pilosus	P1993	42.7	288	731	821	283.0	99.2	51.5	134.8	568.6
	P23030	41.7	272	745	825	275.5	72.1	46.8	124.9	519.3
	P23030s*	37.3	278	736	829	206.6	81.8	53.4	120.4	462.2
	SEM**	1.66	4.7	2.7	2.3	18.00	4.75	5.05	2.95	23.03
L. atlanticus	AM2.9.2	20.2	324	651	767	153.7	92.4	63.2	138.7	448.0
	P22927	24.4	314	690	808	99.5	200.0	77.2	154.3	530.9
	P22927s*	23.1	315	732	808	149.5	112.8	36.4	127.8	426.6
	MD92(96)	29.5	300	715	802	173.7	115.2	44.5	141.2	474.7
	SEM	1.92	5.0	9.0	5.7	8.28	12.70	6.39	6.06	16.67
L. angustifolius	Chittick	14.1	249	615	721	82.4	102.1	30.0	120.3	334.8
	Gungurru	12.3	261	606	726	90.9	132.4	33.6	136.7	393.7
	Illyarrie	16.2	238	616	698	66.2	74.9	35.9	111.3	288.3
	Warrah	11.0	265	608	732	92.7	82.3	42.6	103.1	320.8
	Yorrel	16.8	219	616	711	66.2	80.2	19.9	99.2	265.5
	SEM	1.11	8.3	2.8	3.3	3.85	7.10	2.39	5.20	15.75

* seeds from Turretfield in 1995.

** SEM=standard error of mean within each species.

The degradability of dry matter of 12 samples of lupin seed coats The results (Table 3.10) of degradability of lupin seed coats showed that after 24 hours incubation in the rumen, about 80% of seed coats disappeared for *L. angustifolius*, and only about 65% for *L. pilosus* and *L. atlanticus*. After 48 hours incubation, more than 90% of seed coat DM was degraded for all species. Generally, domesticated lines had a higher rate of degradation of seed coats in the rumen than wild lines at any incubation time. For example, domesticated lines had almost 10% more seed coat degraded than wild lines at incubation times of up to 48 hours. However, the degradation trends were similar for the three species (Figure 3.3).

Table 3.10 The disappearance of DM of seed coats (%) for 3 species of lupins incubated in nylon bags in the rumen of sheep

	Incubation time (hours)							
Species	2	4	8	12	24	36	48	72
L. pilosus	14.0	18.6	29.2	38.6	67.4	86.8	91.9	93.0
SEM*	0.43	0.51	0.78	1.62	2.15	1.79	0.62	0.41
L. atlanticus	14.4	19.5	29.2	38.0	62.8	88.5	93.6	94.9
SEM	0.32	0.73	0.77	1.32	2.31	1.78	0.56	0.12
L. angustifolius	20.8	26.9	39.0	51.0	82.5	93.5	94.7	94.5
SEM	1.36	1.71	1.90	2.04	2.01	0.55	0.23	0.21

* SEM=standard error of mean within each species.

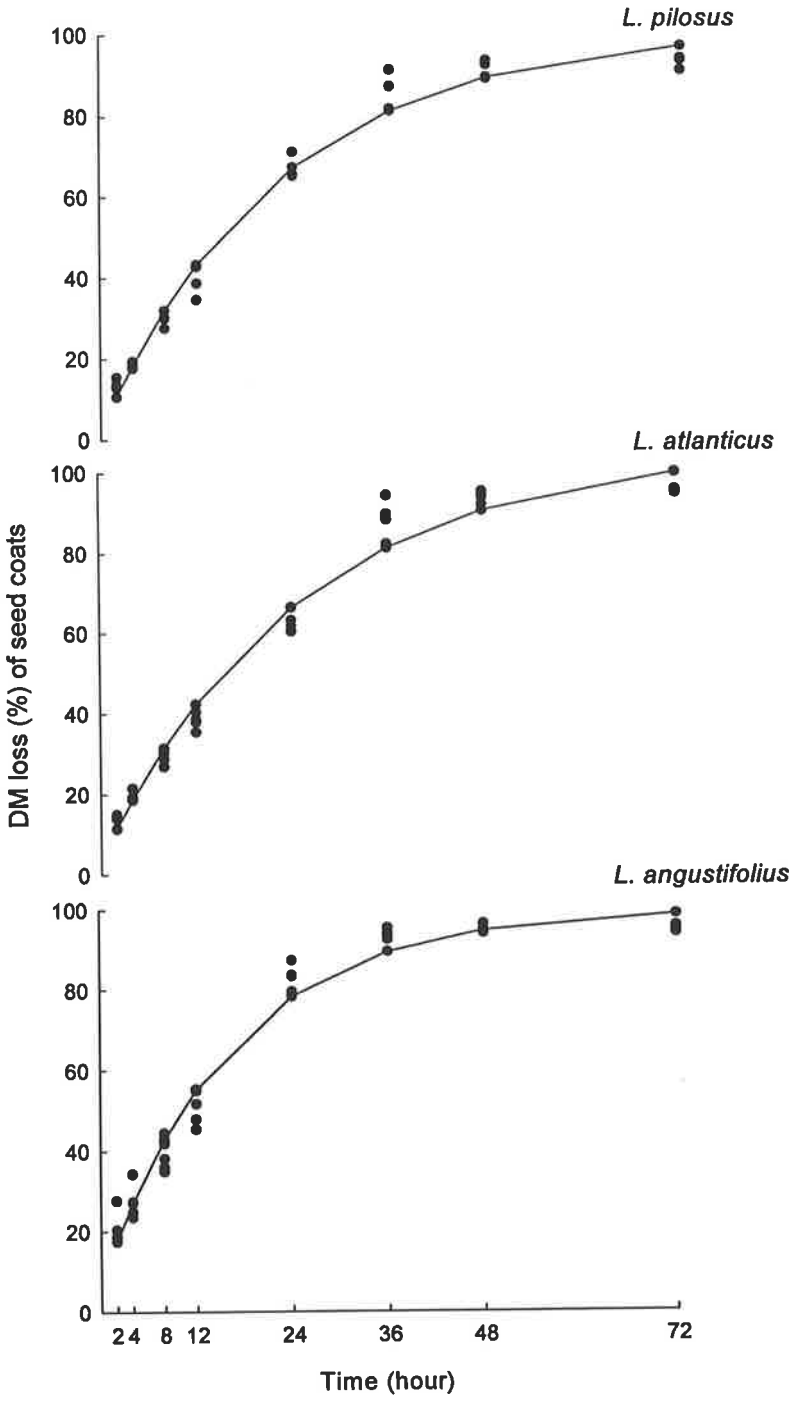


Figure 3.3 The disappearance of DM of the seed coat of 3 species of lupins incubated in nylon bags in the rumen of sheep.

The correlation between the degradability and seed coat fibre content or between the degradability and the seed coat thickness showed that degradability was negatively correlated with ADF and NDF content and seed coat thickness (Table 3.11).

Table 3.11 The correlation coefficient (R) of degradability of lupin seed coats with seed coat thickness and a seed coat fibre content

Time (hour)	ADF	SD*	NDF	SD	Seed coat thickness	SD
2	0.77**	2.80	0.72**	3.01	0.76**	2.86
4	0.81**	3.66	0.83**	3.52	0.89**	2.90
8	0.84**	3.25	0.83**	3.31	0.81**	3.55
12	0.82**	4.42	0.83**	4.29	0.83**	4.30
24	0.77**	6.33	0.79**	6.21	0.82**	5.86

* SD: standard deviation.

** $p<0.01$, $n=12$.

3.4 Discussion

The important finding in this experiment was that a reduction in seed size due to selection for other agronomic characters might increase the seed coat thickness. A reduction in seed coat thickness in soft seeded or smooth seeded seeds may reduce the seed size of wild lupins, hence the proportion of seed coat. The seed coat thickness was significantly influenced by environmental factors. Dry conditions in 1994 significantly reduced the seed coat thickness of lupins. A change in the proportion of seed coat didn't influence the nutritive value of the seed coat for ruminants because the seed coat of lupins is highly degraded in the rumen. However, this is not the case for non-ruminants due to the high fibre content of the seed coat resulting in a lowered efficiency of utilisation of the nutrients in a diet.

Domestication Recently released cultivars (1987 and 1988) of *L. angustifolius* had smaller seeds than those released in 1971. This may be part of the reason for a thicker seed coat for currently released cultivars (1987) as there is the negative correlation between seed weight and thickness of seed coats of genotypes of *L. angustifolius*. This relationship only existed in some species following domestication (Lush and Evans 1980). For example, in the current study, large seeds of *L. pilosus* and *L. atlanticus,* which are essentially wild types, had a thicker seed coat than small seeds due to much thicker palisade and parenchymatous layers. However, large seeds of cultivars of *L. angustifolius* had a thinner seed coat because of thinner palisade and sub-palisade layers. Among wild lines, the depth of the palisade, the hourglass and the parenchymatous layer was significantly and positively related to seed weight. This result contrasts with that reported by Gil and Cubrero (1993), who found that there was little correlation between seed size and seed coat thickness in chickpea. Lush and Evans (1980) more specifically suggested that within domesticates of particular species, there was no consistent association between small seeds and thick seed coat; while Plitman and Kislev (1989) reported that the seed weight and seed size were significantly and positively correlated with the thickness of seed coats of legumes. Such differences may be the result of species variation and the stage of domestication or breeding.

Seeds with different agronomic characteristics Selecting for different agronomic characters induced variation in related characters. For example, soft lupin seed tended to be lighter in colour than hard seeds, and to have a dent in the central portion of the seed coat (Buirchell and Cowling 1992). When seeds were categorised in this way, the structure and thickness of the seed coat also varied considerably. Softseeded seeds had thinner coats than hardseeded ones, mainly because of the thinner palisade and hourglass layers. This was supported by Lush and Evans (1980), who found that seeds of cowpea which were permeable to water had thinner palisade cell walls. Domesticated accessions of pulses have thinner seed coats compared to wild ones due to shorter and thin-walled palisade cells (Plitman and Kislev 1989). The current result provides further evidence that hard or rough seeded seeds had thick seed coats (Lush and Evans 1980).

The palisade layer is also associated with seed germination. Corner (1951) suggested that seeds germinate when palisade cells swell because palisade cells do not readily take up water unless the coat is cracked. A similar result was reported by Buirchell and Cowling (1992), who found that wild lupins had hard seeds that did not absorb water until the outside layer on the seed coat had degenerated. In the present experiment, the hourglass layer was much thinner ($p<0.01$) in soft seeds than in hard seeds. Previous literature does not refer to this difference.

Smooth coated seeds had thinner seed coats than rough coated seeds due to a much thinner palisade layer in smooth coated seeds. This result differed from that reported by Lush and Evans (1980). They found that rough-seeded cowpeas had relatively thinner palisade cell walls than smooth-seeded ones. This may be a species difference.

Sowing time Sowing time had no significant effects on seed coat thickness of lupins, but late sowing tended to reduce the depth of layers and hence, the whole seed coat thickness. No significant interaction was found between sowing time and line in seed coat thickness. This result differed from that reported by Bewley and Black (1994b), who found day-length affected the structure of seeds. One example was that seeds of onions grown in longer days (the last 8 days of maturation are most important) were relatively large with thicker coats. In contrast, seeds grown in shorter days were smaller and had thinner coats. This difference may be because the late sown treatment in the current experiments did not exhibit large differences in temperature, moisture and day length at the period of seed maturation compared with early sowing.

Year of production Seeds grown in 1994 had much thinner coats than seeds grown in 1993, probably due to the extremely dry season in 1994. Plitman and Kislev (1989) also found that temperature and moisture affected the longevity of each phase during seed development. This suggests that each part of the seed, including the seed coat, may be poorly developed under moisture stress. The consequence of poor seed development was poor yield. This was supported by Donald and Hamblin (1976), who found that moisture stress in the late stage of plant growth resulted in decreased

grain yield. A significant interaction between year of growth and line was found in the depth of the palisade layer in the current experiment. This was indicated by no change for P22924 (*L. atlanticus*) seed coats and a decrease for others under dry conditions.

The digestibility of lupin seed coats Wild lupins (*L. pilosus* and *L. atlanticus*) had thicker and larger seed coats, hence more fibre than domesticated types (*L. angustifolius*). However, the thick seed coat and higher fibre content of wild lupin only reduced the degradation rate up until 48 hours incubation in the rumen, but not the degradability over all as over 90% of the seed coat disappeared after 48 hours incubation for all lines. This indicated that fibre in lupin seed coats was highly digested by sheep (Rowe and Hargreave 1988, Nehring and Schramm cited by Bailey *et al.* 1974). On the other hand, lupin seed can easily be dehulled. The kernels could be used in diets of pigs and poultry and the coats could be efficiently used as feed for ruminants. As Gladstones (1994) indicated, "lupin seed coat is a readily digested and valuable energy source for ruminants, and is very significantly digested by pigs. It is also worth mentioning that thick seed coats, provided that they are unbroken, are responsible for the unusually good resistance of lupins to storage insects".

In conclusion, domestication and environment had strong effects on seed coat structure. Wild lupins had thicker seed coats than domesticated lupins. Dry conditions in 1994 significantly reduced the thickness of the seed coat. This provided clear indications that the domestication and environment can affect the yield and chemical composition of lupins. Subsequent Chapters (4, 5 and 6) will explore these effects on yield, seed structure and chemical composition of domesticated and wild lupins.

CHAPTER 4
EFFECTS OF SOWING TIME ON YIELD AND YIELD COMPONENTS OF LUPINS

4.1 Introduction

Lupins have good potential in Australian agriculture due to their high protein concentration and their adaptation to a range of soil types (Gladstones 1970, Hill 1986, Buirchell 1994, Cowling 1994 and Gladstones 1994). For example, *L. angustifolius* prefers slightly acidic soils, and cannot tolerate free lime (Landers 1991 and Cowling 1994), while rough seeded *L. atlanticus* and *L. pilosus* can grow on fine-textured neutral to alkaline soils and are tolerant of free lime (Buirchell and Cowling 1992 and Buirchell 1994). However, the performance of lupins may differ even when grown in suitable soils because of climatic interactions. For example, under similar soil conditions, the various growth stages of *L. angustifolius* cv. Uniharvest were almost entirely dependent on temperature (Reeves 1974). Perry (1975) used Unicrop and Uniharvest to test the effect of sowing time on yield and yield components and found that seed yield declined with late sowing, with similar results being reported by Heenan (1994). Early research (Reeves 1974, Biddiscombe 1975, Perry 1975, Perry and Poole 1975, Goulden 1976, Walton 1976, Withers 1979, Downes and Gladstones 1984) with cultivars of *L. angustifolius* showed that early sowing was the most critical management factor for this species because of:

1. reduced lateral branch growth and pod setting with a short growing seasons,
2. increased flower abortion, hence reduced pod numbers per plant due to high temperature and moisture stress in late spring, and
3. increased competition from weeds due to slow growth of lupins in early winter.

L. atlanticus and *L. pilosus* have potential to grow on calcareous soils (Egan and Hawthorne 1994, and Buirchell and Cowling 1992). However, no information is available about the impact of sowing time on yield and yield components of these lupins. Thus this experiment was designed to evaluate the effect of sowing time on yield and yield components of these lupins compared with those of cultivars of *L. angustifolius* that are commonly grown in Australia.

4.2 Materials and methods

4.2.1 Materials

Nineteen lines of lupins from four species (Table 4.1) were sown evenly by hand on May 12 (early) and June 5 (late) 1995 at Turretfield Research Centre, South Australia. Plot size was 2×1 m^2 with a 50 cm border between plots. The experiment was a factorial design with sowing time as the main factor. All 19 lines were sown randomly in the block at each sowing time, with 2 replicates. Seed coats of wild lupins were cut about 1 mm^2 with a scalpel before planting to enable seeds to imbibe. The sowing rate was 40 seeds/m^2. The soil was a clay loam with a pH of 7.1, and the concentrations of P, S, K and nitrate-N at 10 cm depth were 41, 7.6, 665 and 11.3 mg/kg respectively. Turretfield Research Centre recorded the temperature and rainfall during the experimental period. No fertiliser was applied.

Table 4.1 Characteristics of lines of lupins sown at Turretfield, SA in 1995 in the yield and yield components experiment

Line	Year of release	Flowering (WA)
Domesticated		
L. angustifolius		
Illyarrie	1979	early
Yandee	1980	early
Danja	1986	early
Gungurru (SA, WA)	1988	early
Warrah	1988	early
Yorrel	1988	very early
Chittick	1982	Mid
Wandoo	1987	Mid
Uniwhite	1967	late
Marri	1976	late
Uniharvest	1971	late
Geebung	1987	late
Wild		
L. atlanticus		
AM2.9.2	-	-
MD92(96)	-	-
P22927	-	-
L. pilosus		
P1993	-	-
P23030	-	-
L. cosentinii		
Blue lupin	-	-

* *source* : Landers (1991), Lamb and Poddar (1994).

4.2.2 Methods of determining yield and yield components

After maturity, plants were harvested by hand, and 10 plants were taken randomly from each plot to measure the numbers of seeds, pods and branches per plant, seed

number per pod, pod number on the main stem, 100 seed weight and the yield per plant.

4.2.3 Statistics

The experiment was a factorial design. Sowing time was the main factor and lines were the sub-factor. The treatment effect was estimated using a general linear model from Systat (Wilkinson 1996).

4.3 Results

4.3.1 Climate

There was large variation in mean monthly minimum and maximum air temperatures from May to November, with a range of 5.1-19.5 °C for minimum temperature and 13.5-25.5 °C for maximum temperature. The minimum temperature dropped slightly from May to August followed by a consistent increase from August to October, and a decline from October to November. The maximum temperature decreased from May to July and then increased until November. Thus May sown plants in this experiment had the advantage of growing in relatively warm soil in the early growing stage. The total rainfall from May to November was 332.9 mm. Around 68% of the total rainfall in this period fell in May, June and July (Figure 4.1).

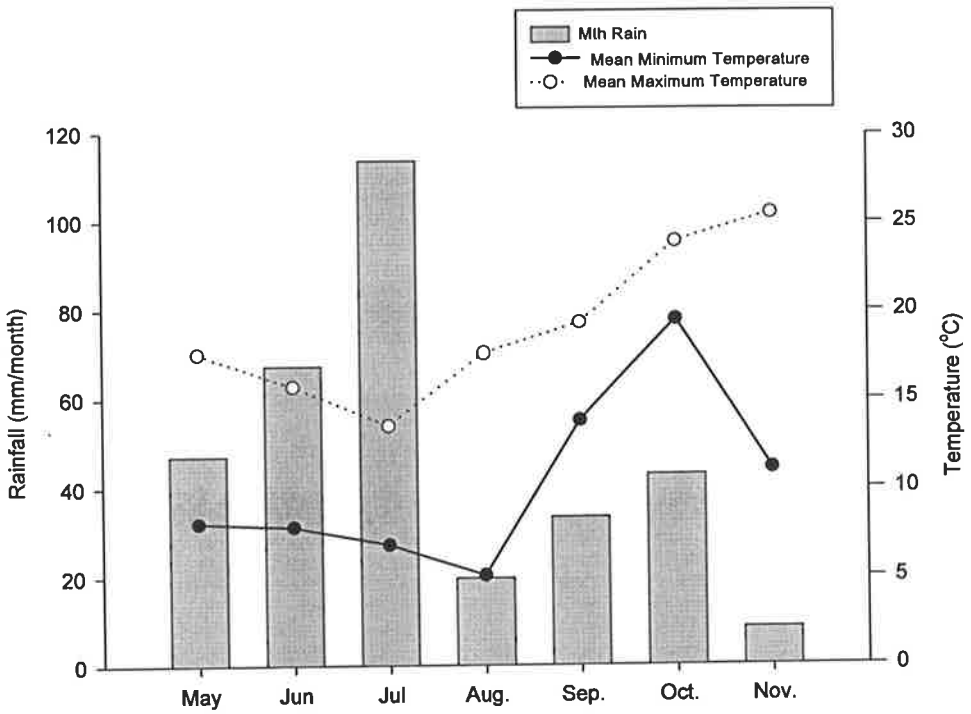


Figure 4.1 The mean monthly minimum and maximum air temperatures (°C) and monthly rainfall (mm) during the experimental period.

4.3.2 Effects of sowing time on yield and yield components of lupins

Nine lines selected according to plant density were used to analyse sowing time effects on yield and yield components of lupins. Early sowing significantly increased grain yield per unit area by 41%, per plant yield by 38% and numbers of pods and seeds per plant by 36% and 40% respectively compared with late sowing. However, sowing time had no significant impact on numbers of branches per plant and individual seed weight for all lines (Table 4.2).

Table 4.2 The yield and yield of components for lupins sown at two different dates at Turretfield, SA

	Grain yield (g/m^2)		Yield/plant (g)		Branches /plant		Pods /plant		Seeds /plant		Seed weight (mg)		Pods /main stem		Seeds /main stem	
ST / Line	1#	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
AM2.9.2	150.7	92.8	4.9	3.6	3.2	2.7	8.2	6.8	23.4	18.9	215	202	8.2	6.8	23.4	18.9
MD92(96)	300.9	206.4	10.2	7.4	3.1	3.2	9.2	8.6	34.9	28.7	314	261	9.0	8.3	34.4	28.0
Chittick	239.6	190.0	7.5	6.4	5.0	4.2	17.0	13.8	58.6	48.1	133	136	6.8	8.8	27.1	32.6
Blue lupin	345.9	165.1	11.7	6.8	4.1	3.8	18.5	10.2	62.8	32.3	193	211	15.3	10.1	54.7	32.1
Danja	350.5	259.6	11.7	8.6	5.1	4.4	25.7	16.5	98.0	63.7	132	139	7.7	8.3	29.6	33.4
Geebung	298.2	175.1	8.9	7.4	6.6	6.6	17.9	16.2	70.6	60.4	133	124	7.4	10.7	32.2	43.2
Gungurru(SA)	308.6	256.5	9.6	7.5	4.8	4.3	22.3	15.3	77.9	56.1	127	136	7.4	9.2	27.0	34.6
P1993	329.6	295.6	11.4	7.8	3.7	3.6	7.4	5.7	24.8	17.3	476	474	6.8	5.5	22.6	17.0
P23030	394.8	280.6	12.1	8.0	3.8	3.8	8.7	5.9	31.3	19.4	382	408	8.3	5.9	30.3	19.3
Mean	302.1	213.5	9.8	7.1	4.4	4.1	15.0	11.0	53.6	38.3	234	232	8.6	8.2	31.3	28.8
ST	**		**		NS		**		**		NS		NS		*	
Line×ST	NS		NS		NS		NS		NS		NS		**		**	

1=May sown, 2=June sown

*p<0.05, **p<0.01, NS: no significance.

There were no interactions between sowing time and line in yield per unit area, single plant yield, numbers of branches, pods and seeds per plant and individual seed weight, but there were significant interactions between sowing time and line in numbers of seeds and pods on the main stem. June sowing reduced the numbers of pods and seeds on the main stem of wild lines (AM2.9.2, MD92(96), Blue lupin, P1993 and P23030), and increased those on the main stem of *L. angustifolius* (cvv. Chittick, Geebung, Danja and Gungurru (SA)).

4.3.3 Genetic variation

Due to some interactions between sowing time and line, only the data from the early sowing were used for analyses of species and genotype variation.

Species variation The seed yields of the four species were different under the same environmental conditions. *L. pilosus* had the highest, while *L. atlanticus* had the lowest seed yield. However, there were no significant differences among the other species in yield (Table 4.3).

Table 4.3 The yield and yield components of lupin species sown on May 12, 1995 at Turretfield, SA

Species	Grain yield (g/m^2)	SWt. /plant (g)	Bs /plant	Pods /plant	Seeds /plant	Seeds /pod	Pods /main	Seeds /main	Wt. /seed (g)
L .angustifolius	320.9ab	10.2ab	5.3a	22.1a	80.9a	3.7	7.5b	29.3b	0.13c
L. atlanticus	222.7a	7.9b	3.2b	9.1b	32.0bc	3.5	8.9b	31.5b	0.25b
L. cosentinii	345.9ab	11.7ab	4.1ab	18.5a	62.8ac	3.4	15.3a	54.7a	0.19b
L. pilosus	362.2b	11.8a	3.8b	8.0b	28.0b	3.5	7.5b	26.5b	0.43a

SWt.: seed weight; Bs: branch number; Pods and Seeds: the numbers of pods and seeds, main: on main stem; Wt. weight, * Figures in the same column with different letters were significantly different ($p<0.05$).

The yield components also varied between species. *L. angustifolius* had more branches, pods and seeds per plant than *L. pilosus* and *L. atlanticus* ($p<0.05$). But there was no significant difference between *L. angustifolius* and *L. cosentinii* in these parameters ($p>0.05$). *L. cosentinii* had the most seeds and pods on the main stem, while *L. pilosus* had the heaviest individual seed weight and individual plant yield among the four species. There was no significant difference in the number of seeds per pod among the four species, averaging 3.5 seeds/pod ($p>0.05$).

Cultivar variation Differences occurred only in seed yield, seed number per pod and individual seed weight among the cultivars of *L. angustifolius* ($p<0.05$). Yorrel had a significantly higher seed yield (492 g/m^2) than Uniharvest (205 g/m^2), Warrah (261 g/m^2) and Yandee (251 g/m^2), resulting from large individual seeds (0.15 g) and relatively more seeds per pod (3.8 seeds/pod). Geebung, Illyarrie and Uniharvest had more seeds per pod ($p<0.01$) than Yandee. Individual seed weight also varied between cultivars of *L. angustifolius* ($p<0.01$), with Yorrel having the heaviest individual seeds and Warrah having the smallest in the current experiment (Figure 4.2 and Figure 4.3).

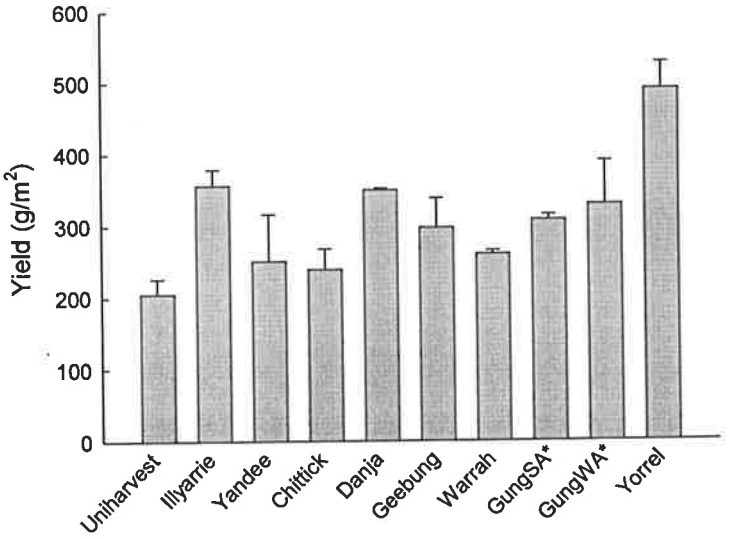


Figure 4.2 The yield of cultivars of *L. angustifolius* (x-axis arranged in order of year of release from 1971 to 1988) (bar = s.e). *GungSA: Gungurru (SA), GungWA: Gungurru (WA).

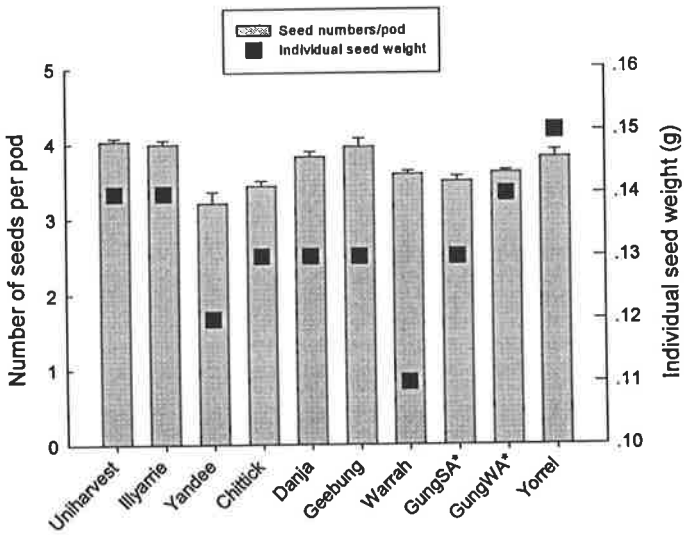


Figure 4.3 The number of seeds per pod and individual seed weight of cultivars of *L. angustifolius* (x-axis arranged in order of year of release from 1971 to 1988) (bar = s.e). * GungSA: Gungurru (SA), Gungurru (WA).

There were no significant differences in yield, individual plant yield, numbers of branches, seeds and pods per plant, numbers of seeds and pods on main stem and individual seed weight with year of release. However, there was a significant interaction between year of release and seeds per pod. Cultivars released in 1976 (4.2 seeds/pod) had significantly more seeds per pod than cultivars released in 1980 (3.2 seeds per pod), 1982 (3.4 seeds/pod) and 1988 (3.6 seeds/pod). There was a significant correlation between seed weight and yield ($R=0.435$, $p<0.05$, $n=21$, $SD=82.7$). Although yield of Yorrel (1988) was significantly higher than Uniharvest (1971), average yield of cultivars released in 1988 was not significantly higher than that of the cultivars released in earlier years.

4.4 Discussion

The important finding in this experiment was that June sowing significantly decreased grain yield due to a reduction in numbers of seeds and pods per plant. Under current experimental conditions, *L. pilosus* and *L. cosentinii* gave higher seed yield than *L. angustifolius*. May sowing of Yorrel gave the highest yield of the *L. angustifolius* genotypes due to a heavier individual seed weight and laterals that were more productive. No significant relationship was found in yield and yield components between the early released cultivars and the recent released cultivars of *L. angustifolius*.

Sowing time Early sowing significantly increased the seed yield of all lupin lines by 41%. This result was supported by Withers (1973), Withers *et al.* (1974), Perry (1975), Walton (1976), Boundy *et al.* (1982), Heenan (1994) and Noffsinger and van Santen (1995). Noffsinger and van Santen (1995) found that yield was reduced by about 50% for Ultra due to 4 weeks delay in sowing in Southern Alabama. In the current experiment, early sown lupins grew in relatively warm soil (min. 8.0 °C, max. 17.5 °C in May, min. 7.8 °C and max. 15.7 °C in June), which assisted rapid seed emergence and early growth (Farrington 1974 and Perry and Poole 1975). Rainfall in June (67.4 mm) and July (113.8 mm) during early growth, allowed plants to produce good vegetative and reproductive growth on the branches. Early sowing also gave plants more time to fill seed; while late sown plants did not have enough

time for grain filling and pod formation due to moisture stress late in the growing season (Perry 1975, Perry and Poole 1975 and Noffsinger and van Santen 1995).

Withers (1973) and Withers *et al.* (1974) found that there was a marked reduction in yield per plant with late sowing due to a decrease in pod number per plant and the shortened flowering period. Boundy *et al.* (1982) also found that late sowing significantly decreased total seed numbers per plant of Uniwhite and Uniharvest. However, numbers of seeds per pod were not significantly different, averaging 3.5 seeds/pod in the current experiment. Similar results were reported by Goulden (1976) and Rahman and Gladstones (1974), who found that numbers of seed per pod were not affected by sowing time. Sowing time had no significant effects on numbers of branches per plant. However, June sowing gave fewer seeds per branch (9.3 seeds/branch) than May sowing (12.2 seeds/branch) which showed a reduced conversion of vegetative potential. For example, seed numbers in higher order laterals of *L. angustifolius* had decreased by 5.3 seeds per lateral branch with June sowing compared to May sowing. This was supported by Perry (1975) who found that late sowing reduced the grain production from laterals.

There was a significant interaction between sowing time and line in numbers of pods and seeds on the main stem. June sowing increased the number of seeds and pods on the main stem of cultivars of *L. angustifolius*, and decreased these parameters for wild lines. Goulden (1976), and Fuentes and Bellido (1986) reported similar results. The former found that the primary inflorescence contributed 14 to 19% of the yield by early sown Uniharvest, but this contribution was up to 44-77% of the yield in late sowing. However, numbers of seeds per pod on the main stem remained consistent between the two sowing times (3.6 vs 3.5 for May and June sowing respectively) in this experiment.

The individual seed weight was not affected by sowing time. This differed from that reported by Perry (1975), who found early sown plants had heavier individual seed weights compared with later sowing, but Rahman and Gladstones (1974) and Goulden (1976) found seed weight of *L. angustifolius* was not generally affected by sowing time.

Species There was great variation in seed yield of lupin species. *L. pilosus* and *L. cosentinii* had 13% and 8% more seed yield than *L. angustifolius* in the May sowing. This difference among species may be due to different responses to environment (Rahman and Gladstones 1973). For example, *L. cosentinii* has a lower vernalisation requirement than *L. angustifolius*. In the current experiment, numbers of pods and seeds on main stems contributed to the high yield of *L. cosentinii* while individual seed weight was important for *L. pilosus*. This result contrasted with that reported by Hamblin *et al.* (1986), who found that *L. angustifolius* was higher yielding than *L. cosentinii* genotypes with similar maturity. This difference may be due to different varieties, soil types and different seasonal conditions.

There were also differences in growth patterns between wild lupins and *L. angustifolius*. Generally, wild lupins had less lateral branches, less pods and less seeds on these branches compared with *L. angustifolius*. Yield of *L. pilosus*, *L. atlanticus* came almost entirely from the main stem, while lateral branches made little contribution to the seed yield and seeds on the lateral branches matured later than those on the main stems (Miao, unpublished data). However, only 38.8% of total seed yield was produced from the main stem for *L. angustifolius*. This result was similar to findings by Delane *et al.* (1986a), who found that 42% of the yield in the branched type of *L. angustifolius* came from the main stem.

Cultivars Cultivars of *L. angustifolius* showed variation in yield and yield components. Very early flowering cultivars such as Yorrel sown in May gave higher seed yields by 59%, 105% and 106% than early , mid. and late flowering types. This was supported by Walton (1976), who found than Unicrop had a higher yield than Uniwhite and Uniharvest due to flowering early. Main contributors were the numbers of seeds on lateral branches and heavier individual seed weight. For example, the seed numbers on lateral branches and the seed weight were 24.4 and 0.15 for very early flowering types, and 12.2 and 0.13, 7.7 and 0.13, and 8.6 and 0.13 for early, mid. and late types. In contrast, very early flowering types had less lateral branches (3.2) compared with early (4.3), mid (4.1) and late types (5.3). This result differed from that reported by Green *et al.* (1977), who found little correlation

between yield and seed weight of *L. albus* genotypes. This indicated that May sowing was more suitable for very early flowering types. Seed numbers on the main stem also differed between genotypes with only 22.6% of total seeds per plant from the main stem of very early types, with 36.6%, 46.2% and 42.0% for early, mid and late flowering types.

There were no marked relationships in yield and yield components for *L. angustifolius* genotypes with year of release except for seeds per pod. This result differed from that reported by Cowling and Speijers (1994), who reported that the yield of *L. angustifolius* was increased by 2.4% per year by breeding from Unicrop (1973) to Merrit (1991). This may be due to the relatively lower density (average 30 plants/m^2 SEM=2.01) in this experiment. Another reason may be that environmental conditions had a strong effect on yield as yield heritability was only 57% of *L. albus* (Green *et al.* 1977). It is reasonable to expect that different growing conditions could create great differences in grain yield.

In conclusion, it was evident that under the conditions of the experiment, the recently released cultivars which have improved agronomic characteristics did not give significantly higher yields than earlier released cultivars. Wild lupins (*L. atlanticus*, *L. cosentinii* and *L. pilosus*) had similar grain yield to domesticated lupins (*L. angustifolius*) with May sowing in 1995 at Turretfield. However, environmental conditions strongly affected grain yield of lupins. Late sowing significantly decreased the lupin grain yield. These findings give reason for some optimism as the earliest lines of wild lupins were capable of producing grain yields of a similar magnitude to *L. angustifolius* which has had considerable breeding efforts. It is recognised that some of the comparable performance found in this work might be due to the soil type being below optimal for *L. angustifolius*. Subsequent work will focus on nutrient variability of these lupins when grown under a variety of conditions to determine impacts other than on simple yield.

CHAPTER 5
THE EFFECTS OF SOWING TIME ON THE NUTRIENT COMPOSITION OF LUPIN SEED

5.1 Introduction

Lupins are widely used as an animal feed in Australia and their value as a feed is largely dependent on nutrient composition. This is because the components are vitally important to formulate a high quality ration for animals to achieve production targets (Mackintosh *et al.* 1994). However, the nutrient content of lupins is influenced by both genetic and environmental factors. Jimenez *et al.* (1991) found that protein and oil content of *L. albus* varied due to the growing locations. Seed oil of *L. albus* was reduced and its degree of saturation increased at higher temperatures (Williams and McGibbon 1980). The chemical composition of *L. angustifolius* was found to vary considerably throughout Australia (Petterson and Mackintosh 1994a, b). However, this variation may be a result of cultivar, location, growing season or analytical method differences.

There is evidence that different sowing dates result in different photoperiod and temperature regimes which affect both phenology and various growth parameters in legumes such as *Vigna* spp., hence seed yield (Lawn 1979a, b). For example, late sowing decreased grain yields of *L. angustifolius* (Perry 1975, Boundy *et al.* 1982, Heenan 1994 and Chapter 4). Similar results for navy beans were reported by Redden *et al.* (1997). However, there is little information available for the changes in chemical composition (eg crude protein, fibre and mineral content) of lupins from different sowing times. This work determined the effects of sowing time on the nutrient composition of *L. cosentinii*, *L. pilosus* and *L. atlanticus* because these lupins have shown some promise under Australian environmental conditions, and compared with cultivars of *L. angustifolius* which are commonly used in Australia.

5.2 Materials and methods

5.2.1 Materials

Nineteen lines of lupins from four species were sown evenly by hand with 2 replicates on May 12 and June 5, 1995 at Turretfield Research Centre, South Australia. At maturity, plants were harvested by hand, and seeds were collected for chemical analyses. The details of growing conditions and plant management are described in Chapter 4.

5.2.2 Methods

Six hundred seeds from each line at each sowing time were dehulled by hand. Coat percentage was measured, and NDF, ADF of both seed coats and kernels were determined using Harris's method (1984). N content was determined using a Leco 2000 combustion system (Anon. 1994) and mineral content was determined using an inductively coupled plasma (ICP) analyser digested by nitric\perchloric acid.

5.2.3 Statistics

The experiment was a factorial design with sowing time as the main factor. The data were analysed by using ANOVA from Systat (Wilkinson 1996).

5.3 Results

5.3.1 Sowing time effects on seed coat percentage and chemical composition of seed coats and kernels

There was no interaction between sowing time and line in seed coat proportion and chemical composition (Table 5.1). Seeds from the later sowing had significant reductions in the proportion of seed coat (8 g/kg, $p<0.05$) and seed kernel nitrogen content (3 g/kg, $p<0.01$).

Table 5.1 Seed coat and kernel N and fibre content (g/kg) of lupins sown at different dates at Turretfield, SA

			Coat						Kernel					
	Coat proportion		N		ADF		NDF		N		ADF		NDF	
ST / Line	1#	2	1	2	1	2	1	2	1	2	1	2	1	2
AM2.9.2	314	314	4.8	5.6	708	716	787	795	67.9	64.0	33.2	40.6	56.7	61.9
Chittick	251	248	6.8	6.5	615	619	720	727	67.4	62.4	40.3	40.4	64.1	67.3
Blue lupin	310	292	5.2	5.0	704	713	796	799	70.7	70.4	43.4	37.2	43.1	42.9
Danja	252	238	7.7	7.8	597	593	725	722	64.1	60.6	36.8	32.0	67.3	67.5
Geebung	259	247	7.9	8.0	620	614	731	728	63.8	61.7	31.1	31.4	70.6	66.0
Gungurru (SA)	262	256	7.5	6.6	606	616	726	726	68.0	62.5	27.3	30.4	65.0	72.4
MD92(96)	297	307	4.9	5.3	715	712	805	794	66.4	62.7	39.5	43.6	52.2	56.2
P1993	289	279	4.9	5.2	731	737	819	814	59.2	57.3	40.8	35.8	49.7	48.9
P23030	276	259	5.3	5.7	736	703	832	811	60.7	60.4	33.4	44.5	51.3	43.9
Mean	279	271	6.1	6.2	670	669	771	768	65.4	62.4	36.2	37.3	57.8	58.6
ST	*		NS		NS		NS		**		NS		NS	
Line × ST	NS		NS		NS		NS		NS		NS		NS	

1=May sown, 2=June sown.

* P<0.05, ** P<0.01, NS: no significance.

5.3.2 Genetic variation

Species Due to relatively poor establishment of some of the lines sown in June (<20 plants/m^2), data from the May sowing were used for species and genotype variance analysis. There were significant differences in chemical composition between species except for ADF content in seed kernels (Table 5.2). Generally, ADF and NDF were concentrated in the seed coat rather than in the kernel, while nitrogen was concentrated in the kernel for all species.

Table 5.2 The chemical composition of the 4 lupin species (g/kg)

Species	n*	Coat proportion	Coat			Kernel		
			N	ADF	NDF	N	ADF	NDF
L. cosentinii	1	310a**	5.2b	704a	796b	70.7a	43.4	43.1b
L. pilosus	2	283b	5.1b	733a	826a	59.9c	37.1	50.5b
L. atlanticus	3	310a	4.7b	718a	800b	64.4ac	38.6	54.6b
L. angustifolius	11	257b	7.3a	614b	726c	65.9a	34.1	69.7a

* line numbers, ** Figures in the same column with different letters were significantly different ($p<0.01$).

L. cosentinii, *L. pilosus* and *L. atlanticus* had a larger proportion of seed coat, and greater ADF and NDF content in seed coat than *L. angustifolius*. *L. cosentinii* had the highest nitrogen content in the kernel, while *L. pilosus* had the lowest. *L. angustifolius* had the highest nitrogen content in the seed coat and the highest NDF content in the kernel.

Cultivar variation Significant differences in seed coat proportion, seed weight and coat nitrogen content were found between cultivars of *L. angustifolius*. There were no significant differences in other parameters (Figure 5.1). Warrah had the highest seed coat proportion (281 g/kg) with Yorrel having the lowest (237 g/kg). Yandee had the highest nitrogen content (70.7 g/kg) in kernels, and Uniharvest had the lowest (63.3 g/kg).

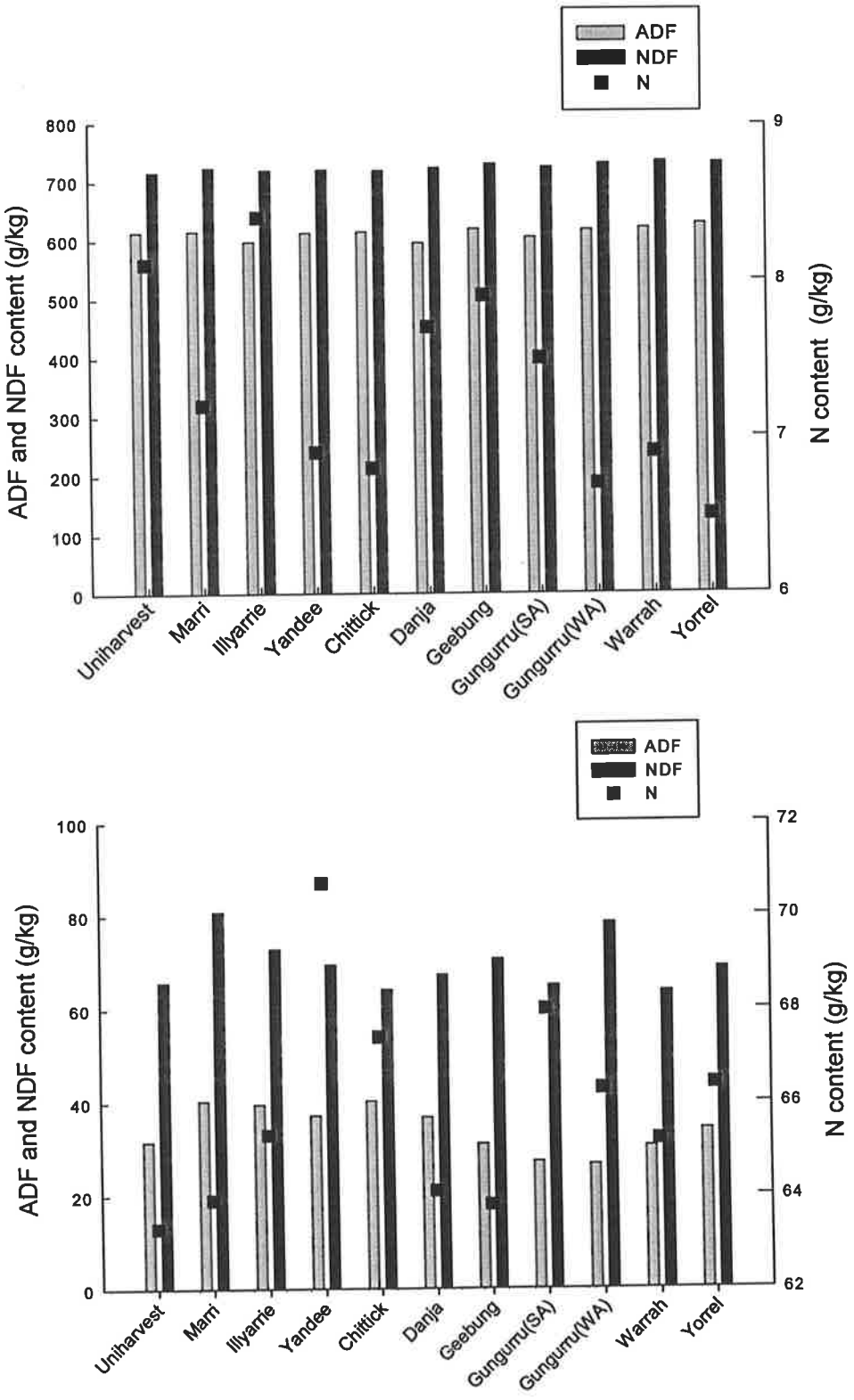


Figure 5.1 The chemical composition of the seed coat (a) and the kernel (b) of cultivars of *L. angustifolius* (x-axis arranged in order of year of release from 1971 to 1988).

There were no significant differences in coat proportion, nitrogen, ADF and NDF content in whole seeds of *L. angustifolius* cultivars based on year released (from 1971 to 1988). Values ranged from 49.1 g/kg to 54.9 g/kg for grain nitrogen content, 177.6 to 190.6 g/kg for grain ADF content, and 229.1 to 248.6 g/kg for grain NDF content.

5.3.3 Effect of sowing time on the mineral content of lupin seeds

There were significant interactions between sowing time and line in the Mn and S content of the kernels. June sowing significantly decreased the Mn content of the kernel of AM 2.9.2, and Blue lupin, slightly decreased those of Chittick, Danja, Gungurru (SA), MD92(96), P1993 and P23030, and sightly increased that of Geebung (Table 5.3). Sowing time had significant effects on Zn content in the kernel for all lines. June sowing decreased Zn content in the kernel by 1.6 mg/kg compared with May sowing.

There were no interactions between line and sowing time for mineral content of the seed coat. Sowing time only had a significant effect on the B content of seed coat; June sowing increased B content by 0.7 mg/kg (Table 5.4).

Table 5.3 The mineral content of lupin kernels at two sowing times at Turretfield, SA

	Fe		Mn		B		Cu		Zn		Ca		Mg		Na		K		P		S	
	(mg/kg)										(g/kg)											
ST / Line	1#	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
AM2.9.2	40	33	44	26	18	21	10	8	50	44	0.8	0.9	2.4	2.4	0.2	0.4	11.9	11.5	5.1	4.9	4.1	3.9
Chittick	40	41	25	22	19	22	6	7	38	37	1.1	1.1	2.0	2.1	0.3	0.4	11.0	10.4	3.8	3.8	3.4	3.2
Blue lupin	47	42	81	61	16	16	15	14	54	54	0.4	0.5	1.8	1.8	0.4	0.3	11.8	12.5	6.0	6.1	4.6	4.9
Danja	48	45	21	19	24	23	7	7	39	38	1.1	1.1	2.2	2.0	0.6	0.2	10.9	11.6	4.1	3.6	3.3	3.2
Geebung	50	51	16	18	20	21	6	7	37	34	1.0	0.9	1.9	1.8	0.2	0.5	11.6	10.5	3.8	3.5	3.4	3.2
Gungurru(SA)	43	43	20	17	25	26	6	6	37	35	1.1	1.0	2.1	2.1	0.4	0.5	11.5	10.7	4.3	4.2	3.2	2.8
MD92(96)	42	39	31	28	18	18	10	9	40	38	0.6	0.7	1.9	2.2	0.3	0.4	10.1	10.7	4.6	5.1	4.0	4.3
P1993	67	28	42	34	16	22	9	8	40	40	0.7	0.7	2.0	2.1	0.5	0.6	8.1	7.8	4.0	4.2	3.4	3.3
P23030	32	30	41	34	17	17	9	9	40	40	0.8	0.9	2.1	2.1	0.7	0.5	7.6	8.0	4.2	4.2	3.6	4.0
Mean	45	39	36	29	19	21	9	8	42	40	0.8	0.9	2.0	2.1	0.4	0.4	10.5	10.4	4.4	4.4	3.7	3.6
ST	NS		**		NS		NS		*		NS		NS		NS		NS		NS		NS	
Line×ST	NS		*		NS		NS		NS		NS		NS		NS		NS		NS		*	

1=May sown, 2=June sown.
* $p<0.05$, ** $p<0.01$, NS: no significance.

Table 5.4 The mineral content of the seed coat of lupins at two sowing times at Turretfield, SA .

	Fe		Mn		B		Cu		Zn		Ca		Mg		Na		K		P		S	
	(mg/kg)										(g/kg)											
ST / Line	1#	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
AM2.9.2	22	25	82	55	8.9	9.6	1	1	20	19	5.7	5.3	1.8	1.7	0.4	0.7	3.1	3.2	0.2	0.3	0.3	0.3
Chittick	30	28	23	18	8.5	9.4	1	1	22	22	7.1	7.2	1.1	1.1	0.5	0.7	3.6	3.5	0.5	0.4	0.3	0.3
Blue lupin	31	37	100	77	7.7	8.3	1	2	4	4	3.9	4.9	1.3	1.3	1.2	0.8	3.9	5.0	0.2	0.2	0.3	0.3
Danja	31	36	19	17	9.4	9.5	1	1	25	25	6.9	6.8	1.1	1.0	0.6	0.3	4.3	4.5	0.6	0.6	0.4	0.4
Geebung	30	30	18	22	8.5	9.2	1	1	25	25	6.6	6.4	1.0	1.1	0.3	0.7	4.4	4.3	0.5	0.5	0.3	0.3
Gungurru(SA)	26	27	17	15	9.1	9.5	1	1	22	20	6.8	6.5	1.2	1.4	0.4	0.7	3.7	3.6	0.4	0.4	0.4	0.3
MD92(96)	22	23	69	52	8.4	8.4	1	2	20	17	4.6	4.9	2.4	1.8	0.7	0.8	4.1	3.7	0.3	0.3	0.3	0.3
P1993	22	21	81	59	8.9	11	1	1	16	13	3.9	3.7	1.6	1.7	1.0	1.3	4.5	4.0	0.2	0.3	0.3	0.3
P23030	27	27	90	68	9.2	9.7	1	1	14	13	3.7	4.1	1.4	1.4	1.5	1.1	3.5	3.1	0.3	0.3	0.3	0.4
Mean	27	28	55	43	8.7	9.4	1	1	19	17	5.5	5.5	1.4	1.4	0.7	0.8	3.9	3.9	0.4	0.4	0.3	0.3
ST	NS		NS		*		NS		NS		NS		NS		NS		NS		NS		NS	
Line×ST	NS		NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	

1=May sown, 2=June sown.
* $p<0.05$, NS: no significance.

5.3.4 Genetic variation in mineral content

Species Due to significant interactions occurring between sowing time and line, only the early sowing data were used to evaluate genetic variation. There were significant differences in kernel mineral content except for Fe and Mg (Table 5.5). *L. cosentinii* had the highest Mn, Cu, Zn, P and S content in the kernel, and the lowest Ca content. The opposite results were found for *L. angustifolius*. There was no significant difference between *L. pilosus* and *L. atlanticus* in mineral content except that *L. atlanticus* had higher K, P and S and lower Na content than *L. pilosus* in the kernel.

There were significant differences for minerals in the seed coat among species except for Fe, Cu and K ($p>0.05$). *L. cosentinii* had a high Mn and low Zn content, *L. angustifolius* had relatively higher Zn, Ca, P and S, and lower Mn, Mg and Na in the seed coat than the others.

In addition, the distribution of minerals in the kernel and seed coat was different. The proportion of Fe in the kernel was greater than that in the seed coat for wild species. Mn content in the kernel of *L. cosentinii*, *L. pilosus* and *L. atlanticus* was less than that in the seed coat. In contrast, *L. angustifolius* had less Mn in the seed coat. The content of Cu, P, S and K of the four species was higher in the kernel than in seed coat, while Ca content in the seed coat was much higher than in the kernel.

L. angustifolius had the highest ratio of grain N to S (20:1), but *L. cosentinii*, *L. pilosus* and *L. atlanticus* had a lower N:S ratio, ranging from (15:1 to 17:1). N:S ratio in the seed coat and the kernel also varied. Generally the N:S ratio was higher in the seed coat than in the kernel in the same species except for *L. angustifolius* that had similar ratios in both the seed coat and the kernel (21:1 to 20:1). The ratio of the grain Ca to P of lupin species also varied, *L. angustifolius* had the highest ratio (0.8:1), *L. cosentinii* had the lowest (0.3:1).

Table 5.5 The mineral content of the kernel and the seed coat for 4 species of lupins

Species	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
	mg/kg					g/kg					
						Kernel					
L. cosentinii	47.5	81.1a	16.0b	14.6a	53.9a	0.4c	1.8	0.4ab	11.8a	6.0a	4.6a
L. pilosus	49.6	41.5b	16.7b	8.9b	40.4bc	0.7b	2.1	0.6a	7.9b	4.1bc	3.5c
L. atlanticus	41.7	41.4b	17.9b	9.2b	43.4b	0.7b	2.1	0.3b	10.8a	4.7b	4.1b
L. angustifolius	45.0	22.3c	21.7a	6.6c	38.0c	1.1a	2.1	0.3b	11.5a	4.0c	3.3c
						Seed coat					
L. cosentinii	31.1	99.6a	7.7a	1.5	3.6c	3.9bc	1.3bc	1.2ab	3.9	0.2b	0.27b
L. pilosus	24.8	85.6a	9.0b	1.2	15.2b	3.8c	1.5b	1.3a	4.0	0.2b	0.31ab
L. atlanticus	23.0	82.0a	8.3ab	1.4	21.2a	4.9b	2.0a	0.6bc	3.7	0.3b	0.28b
L. angustifolius	46.2	19.2b	9.0b	1.3	23.2a	6.9a	1.2c	0.4c	3.7	0.5a	0.35a

* Figures in the same column within the kernel or seed coat category with different letters were significantly different ($p<0.01$).
except for B contents in seed kernel and seed coat, S content in seed coat ($p<0.05$).

Cultivar variation Significant differences were found in B, Cu and S content in seed kernels ($p<0.05$). Gungurru (SA) had the highest B content, and Marri had the highest Cu and S content in the kernel. Lowest B, Cu and S contents were for Illyarrie, Gungurru (WA) and Yorrel respectively. There were no significant differences in mineral content of the seed coat between cultivars except for K content ($p<0.01$) (Geebung had 4.4 g/kg K, while Yorrel only had 2.6 g/kg) (Table 5.6).

There was a slight difference in the N:S ratio of *L. angustifolius* cultivars, the values ranging from 17:1 for Marri to 23:1 for Yorrel. The correlation between grain S and grain yield was negative ($R=0.593$, $p<0.01$, $SD=133.3$, $n=22$), but there was no significant correlation between grain N and yield.

There were no significant differences in grain mineral content in cultivars released between 1971 and 1988 except in grain S content. Marri, a cultivar released in 1976 had a significantly higher grain S than cultivars released in 1988 (Gungurru (SA), Gungurru (WA), Warrah and Yorrel) (2.8g/kg vs 2.4 g/kg).

Table 5.6 The mineral contents of the seed kernel and the coat of 11 cultivars of *L. angustifolius*

Cultivar	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
	mg/kg					g/kg					
						Kernel					
Uniharvest	46.3	21.6	20.0	6.9	38.2	1.1	2.2	0.2	11.5	3.9	3.4
Marri	48.1	25.6	20.5	7.2	39.7	1.1	2.1	0.3	12.1	4.0	3.7
Illyarrie	45.6	25.5	19.0	6.8	37.7	1.2	2.0	0.2	12.0	3.6	3.4
Yandee	43.6	21.7	20.6	6.7	39.2	1.2	2.2	0.5	11.8	4.7	3.4
Chittick	39.6	24.6	19.5	6.2	37.6	1.1	2.0	0.3	11.0	3.8	3.4
Danja	48.2	20.8	24.4	6.9	39.1	1.1	2.2	0.6	10.9	4.1	3.3
Geebung	50.4	16.3	20.1	6.4	36.6	1.0	1.9	0.2	11.6	3.8	3.4
Gungurru(SA)	42.6	20.2	25.2	6.4	37.0	1.1	2.1	0.4	11.5	4.3	3.2
Gungurru(WA)	43.0	22.4	24.1	6.1	37.0	1.0	2.0	0.3	11.3	3.9	3.0
Warrah	38.8	23.0	24.7	6.3	36.9	1.1	1.9	0.2	11.8	3.9	3.3
Yorrel	48.6	23.6	20.6	6.7	39.1	1.1	2.1	0.5	10.9	4.1	2.9
SEM	1.11	0.73	0.55	0.08	0.34	0.02	0.03	0.04	0.14	0.73	0.48
						Coat					
Uniharvest	30.2	18.2	9.0	1.3	25.2	7.1	1.1	0.4	3.9	0.6	0.4
Marri	30.4	18.8	9.0	1.2	24.0	6.6	1.3	0.4	3.7	0.5	0.3
Illyarrie	31.1	21.7	8.8	1.2	21.6	7.1	1.2	0.3	3.6	0.6	0.4
Yandee	31.0	16.1	8.5	1.3	22.9	7.1	1.0	0.6	3.0	0.5	0.3
Chittick	30.1	22.7	8.6	1.2	21.6	7.1	1.1	0.5	3.6	0.5	0.3
Danja	30.9	18.9	9.4	1.4	25.0	6.9	1.1	0.6	4.3	0.6	0.4
Geebung	30.0	18.5	8.5	1.1	25.3	6.6	1.0	0.3	4.4	0.5	0.3
Gungurru(SA)	26.3	17.1	9.1	1.4	21.7	6.8	1.2	0.4	3.7	0.4	0.4
Gungurru(WA)	29.8	18.0	9.5	1.4	22.7	6.5	1.5	0.3	3.8	0.4	0.3
Warrah	24.3	21.2	9.6	1.3	23.3	6.6	1.1	0.2	4.3	0.5	0.3
Yorrel	29.6	19.7	8.8	1.2	21.4	7.2	1.0	0.7	2.6	0.5	0.3
SEM	0.63	0.54	0.11	0.04	0.40	0.07	0.03	0.04	0.12	0.02	0.08

5.4 Discussion

The important findings of this experiment were that late sowing decreased the nitrogen content of lupins and affected their mineral content. There was considerable variation in nutrient content between wild lupin (*L. cosentinii*, *L. pilosus* and *L. atlanticus*) and domesticated lupin (*L. angustifolius*). *L. cosentinii* had a higher kernel nitrogen, Mn, Cu, Zn, P and S than the others under the current experimental conditions. There was no marked relationship in seed structure and seed chemical composition between genotype and year of release in this experiment.

Sowing time Sowing time had a significant effect on the N content of the lupins. June sowing resulted in significantly lower nitrogen content. Similar findings were reported for chickpea by Horn *et al.* (1996), who found that seed nitrogen accumulation by chickpea was significantly higher in earlier sowing than for late sowing. In contrast, seed nitrogen content of *L. angustifolius* from late sowing tended to be higher than from early sowing (Withers 1979), and was not influenced by late sowing (Boundy *et al.* 1982). These differences may be a result of the different growing conditions, which has a great influence on crude protein of grain legumes (Kadam *et al.* 1989b and Oluwatosin 1997). In this experiment, a lower seed nitrogen content from June sowing was presumably due to the resulting poor early plant growth and poor grain yield (Chapter 2), and hence poor quality of grain. There is evidence that the nitrogen accumulation of grain legumes is closely linked to biomass accumulation and seed growth (Muchow *et al.* 1993 and Horn *et al.* 1996).

Sowing time only had a significant effect on the Zn content of the kernel and the B content in the seed coat. June sowing decreased the Zn content by 4% and increased the B content by 8%. The reasons for this variation are unknown. There were also significant interactions between line and sowing time in Mn and S content in the kernel. This indicated that lines had different responses to sowing time with respect to these elements. For example, Mn content decreased by 40% for AM2.9.2, 24% for Blue lupin, and increased 8% for Geebung for June sowing. S content was decreased by 0.4 g/kg for Gungurru (SA), by 0.2 g/kg for AM2.9.2, Chittick and Geebung, and increased by 0.4 g/kg for P23030, 0.3 g/kg for Blue lupin and MD92(96) for June sowing.

Species variation Kernel crude protein content of lupin species varied. *L. cosentinii* had a higher crude protein (442 g/kg air dry basis) in the kernel than the others (374, 402 and 412 g/kg air dry basis for *L. pilosus*, *L. atlanticus* and *L. angustifolius* respectively). The value of *L. cosentinii* was higher than that (282 to 401 g/kg in the kernel for *L. cosentinii*) reported by Hill (1977), but kernel nitrogen content of *L. angustifolius* in the current work (412 g/kg) was similar to that (400 g/kg) reported by Hill (1977).

There was a large variation in the fibre content among the four species. Generally, wild lupins had more ADF and NDF in the seed coat and whole seed than domestic types, but this fibre is readily digested by sheep (Chapter 3 and Rowe and Hargreave 1988). However, the high NDF content in the kernel of *L. angustifolius* compared with others in the current study may be a limiting factor for non-ruminant use.

There was a wide range in the mineral content of the lupins. *L. angustifolius* contained the lowest Mn, Cu and S levels in the kernels, and the highest Ca content both in seed coat and kernel, while, *L. cosentinii* had the highest Mn, Cu, Zn, P and S content in the kernel. Minerals are important for plant growth and animal nutrition. Low seed P (2.1 g/kg) in *L. angustifolius* (cv. Gungurru) limited its establishment (Thomson *et al.* 1991), reduced whole plant weight and increased the root to shoot ratio (Thomson *et al.* 1992). Mn deficiency (8 mg/kg) also caused poor crop establishment and increased seed split (Walton 1978 and Crosbie *et al.* 1994). Higher levels of grain Mn of wild lupins (86.8 mg/kg for *L. cosentinii*, 54.0 mg/kg for *L. pilosus*, and 54.0 mg/kg for *L. atlanticus*) may be good for plant growth and development compared to that of *L. angustifolius* (21.5 mg/kg). These levels are not toxic to animals. The ratios of N:S and Ca:P of *L. angustifolius* were not optimal for sheep growth (Dixon and Hosking 1992 and Murray 1994). *L. cosentinii*, *L. pilosus* and *L. atlanticus* had a more optimum ratio of N:S when compared with *L. angustifolius* (20:1) in the current experiment. The ratio of Ca to P was lower for all species, ranging from 0.3 to 0.8:1.

The distribution of Ca, P and S was similar for all species, with most of Cu, Zn, K, P and S found in the kernel, while most of the Ca was found in the seed coat. This supported the work of Hill (1977), who reported that S and P were mainly concentrated in the kernel and Ca in seed coat for *L. angustifolius*.

Cultivar variation There was large variation in the nutrient content of *L. angustifolius* cultivars. Crude protein content ranged from 396 to 442 g/kg. Such variation in crude protein content was also found in *L. albus* genotypes (Green *et al.* 1977), who found that the crude protein content ranged from 368 to 403 g/kg DM for sweet genotypes, and 381 to 407 g/kg DM for high alkaloid genotypes, and 329 to

420 g/kg DM for their corresponding F3 families. Among *L. angustifolius* cultivars, there was a significant negative correlation between grain S and seed yield, but not between grain nitrogen and yield in the current experiment. This result contrasts with that reported by Green *et al.* (1977), who found that nitrogen content of *L. albus* was negatively correlated with yield. This difference may be a result of the different growing season, soil type and species.

There was no correlation between ADF, NDF and nitrogen content and year of release in this experiment. This was also found by Gladstones (1994), who suggested that the breeding policy for lupins was to maintain or improve protein content, while increasing grain yield. Mineral content was not significantly different between year of release except for grain S, which decreased by 14% for cultivars released in 1988 compared to that cultivars released in 1976. Mineral content of the wild type lupins was equal to or better than *L. angustifolius*. This change in grain S over time indicates a clear need to evaluate such parameters during any breeding program. Low S in particular has long been known as a point of deficiency or imbalance in lupins (Hill 1977 and Dixon and Hosking 1992).

In conclusion, the seed size, seed coat proportion, N, ADF, NDF and minerals varied considerably between species and within species. Sowing time also significantly affected seed coat proportion, N and mineral content. When this variation is related to the large amount of grain lupins being used for animals, considerable variation in animal production could occur. The following Chapter will further explore the variation in nutrient content of lupins when they are grown in different growing seasons and sites.

CHAPTER 6
VARIATION IN CHEMICAL COMPOSITION OF LUPINS GROWN IN DIFFERENT YEARS AND SITES

6.1 Introduction

Seasonal conditions and growing sites have strong effects on the grain yield of legume crops. For example, Erskine and Khan (1977) found that environmental effects (eg rainfall and soil conditions) on grain yield of cowpeas accounted for 82% of total variance. Similar results were reported by Bliss *et al.* (1973) for cowpeas and Tapscott *et al.* (1994) for *L. angustifolius*. However, the interactions between seasonal conditions and variety or growing sites and variety on grain yield were significant (Tapscott *et al.* 1994 and Redden *et al.* 1997) due to differences in diseases, temperature, moisture and soil conditions. For example, Merrit and Gungurru had the highest seed yield per plant at Mt Barker and South Carrabin, but Danja had the highest seed yield per plant at Mullewa (Tapscott *et al.* 1994).

Growing conditions also have a strong influence on grain quality of legume crops. Seed of *L. angustifolius* from Western Australia often contains low Mn due to the Mn deficiency in the soil (Crosbie *et al.* 1994). Low Mn content in seeds (8 mg/kg) could cause poor crop establishment and increased seed split, hence lower seed quality (Walton 1978 and Crosbie *et al.* 1994). On the other hand, protein content varied according to locations (Krober *et al.* 1970). Similar findings for *L. albus* were reported by Jimenez *et al.* (1991). In addition, anti-nutritional factors, eg alkaloids, in *L. angustifolius* grown in southern Western Australia exhibited large variation (0.002 - 0.131%) between growing locations (Harris 1994).

Most of the early research concentrated on the effects of environmental factors on grain yield, protein and oil quality and quantity and anti-nutritional factors. Nutrient content (eg fibre and mineral contents) has been less researched and there is also less information on the impact of growing environment (year, location) on the nutrient

contents of *L. pilosus*, *L. atlanticus* and *L. cosentinii*. Thus the following experiment was conducted to determine variations in the chemical composition and mineral content of *L. pilosus*, *L. atlanticus* and *L. cosentinii* grown in 1992 (except MAR5300, which grew in 1993) at Yeelanna and 1993 and 1994 at MRC. Both these locations are on the Eyre Peninsula, a major cropping region of South Australia.

6.2 Materials and methods

6.2.1 Materials

L. atlanticus (P22924 and MAR6034), *L. pilosus* (P20954, P23341 and P23342) and *L. cosentinii* (cv. Erregulla) were grown in 1992 (except MAR5300 grown in 1993) at Yeelanna, and in 1993 and 1994 at Minnipa Research Centre. At Yeelanna, the soil was a light sandy clay loam over a medium clay beginning at 8-9 cm depth (field pH 8.5 to 9.5). Minnipa had the same soil type as Yeelanna, but over a sandy clay loam commencing at 20 cm (field pH 9). Yeelanna in 1992 was wet compared to Minnipa in 1993 (Egan and Hawthorne 1994). The seed was provided by Jim Egan, SARDI, Port Lincoln, SA. Hundreds of seeds in each line were hand separated into coat and kernel for measurement of the seed coat proportion of whole seed weight and the chemical composition of the seed coat and seed kernel.

6.2.2 Methods

NDF and ADF content of the seed coat and seed kernel were determined in duplicate using the methods described by Harris (1984). N content was determined using a Leco 2000 combustion system (Anon. 1994) and mineral content was determined using an inductively coupled plasma (ICP) analyser digested by nitric/perchloric acid.

6.2.3 Statistics

The experiment was a factorial design. The variance in chemical composition of lupins due to locations, the year of growth and cultivar was analysed by ANOVA using Systat (Wilkinson 1996).

6.3 Results

6.3.1 The variation in seed structure and chemical composition at the two locations

Seeds grown at MRC were heavier (48.5 g/100seed) and had a lower seed coat proportion (257 g/kg) than seed grown at Yeelanna (38.8 g/100 seed and 296 g/kg respectively). There was little difference in the N content of the seed coat and the kernel between the two locations (4.7 vs 4.7 g/kg and 59.4 vs 60.3 g/kg for the seed coat and the kernel respectively). Seeds grown at Yeelanna had slightly higher fibre content of both seed coat and the kernel than those grown at MRC (Figure 6.1).

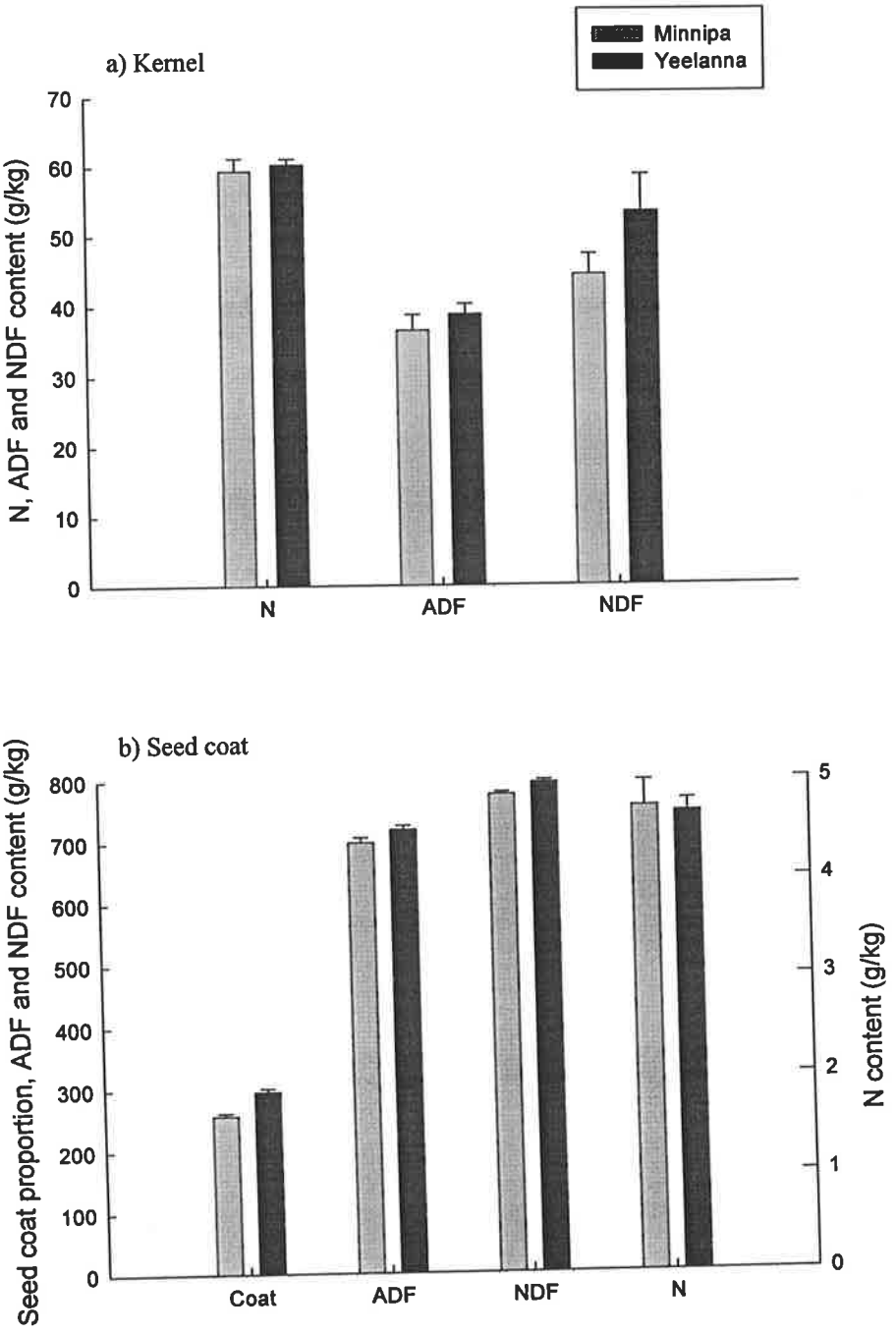


Figure 6.1 The nutrient contents of the seed kernel (a) and the seed coat (b) of lupins grown at two locations on the Eyre Peninsula, SA.

6.3.2 The mineral content of lupins grown at two locations

There were differences in mineral content at the two locations. Generally, seeds grown at MRC had higher Fe, Mn, B, Ca and K content and lower Cu, Zn, Mg and Na in the seed coat than seeds grown at Yeelanna, but the S and P content of the seed coat were similar at the two locations (Figure 6.2b). Seeds grown at MRC had higher levels of Fe, Mn, Ca and P and lower levels of B, Cu, Mg, K and S in the kernel than those grown at Yeelanna (Figure 6.2a) (Na and B are not shown in the Figures).

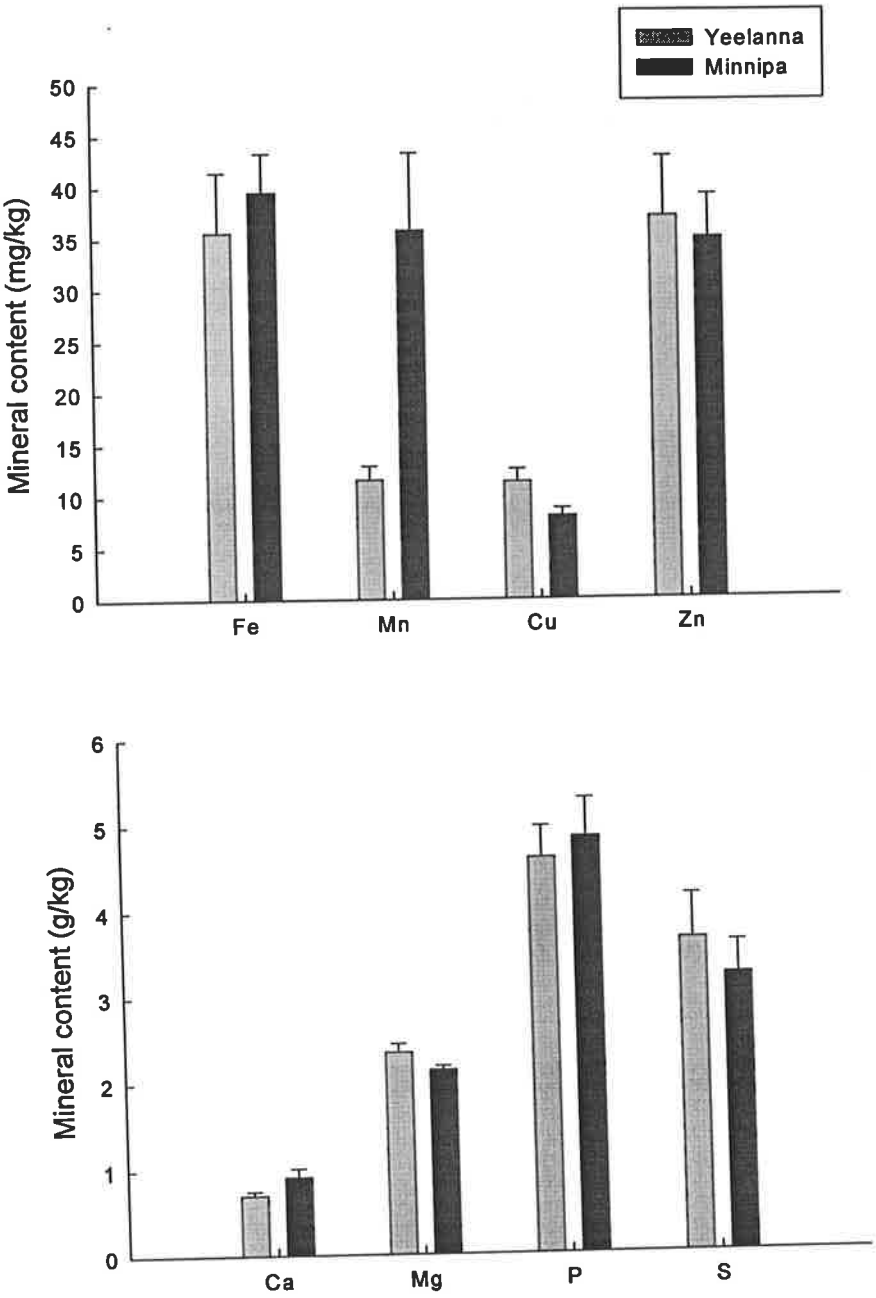


Figure 6.2a The mineral content of seed kernels of lupins grown at two locations on the Eyre Peninsula, SA (bar = s.e.).

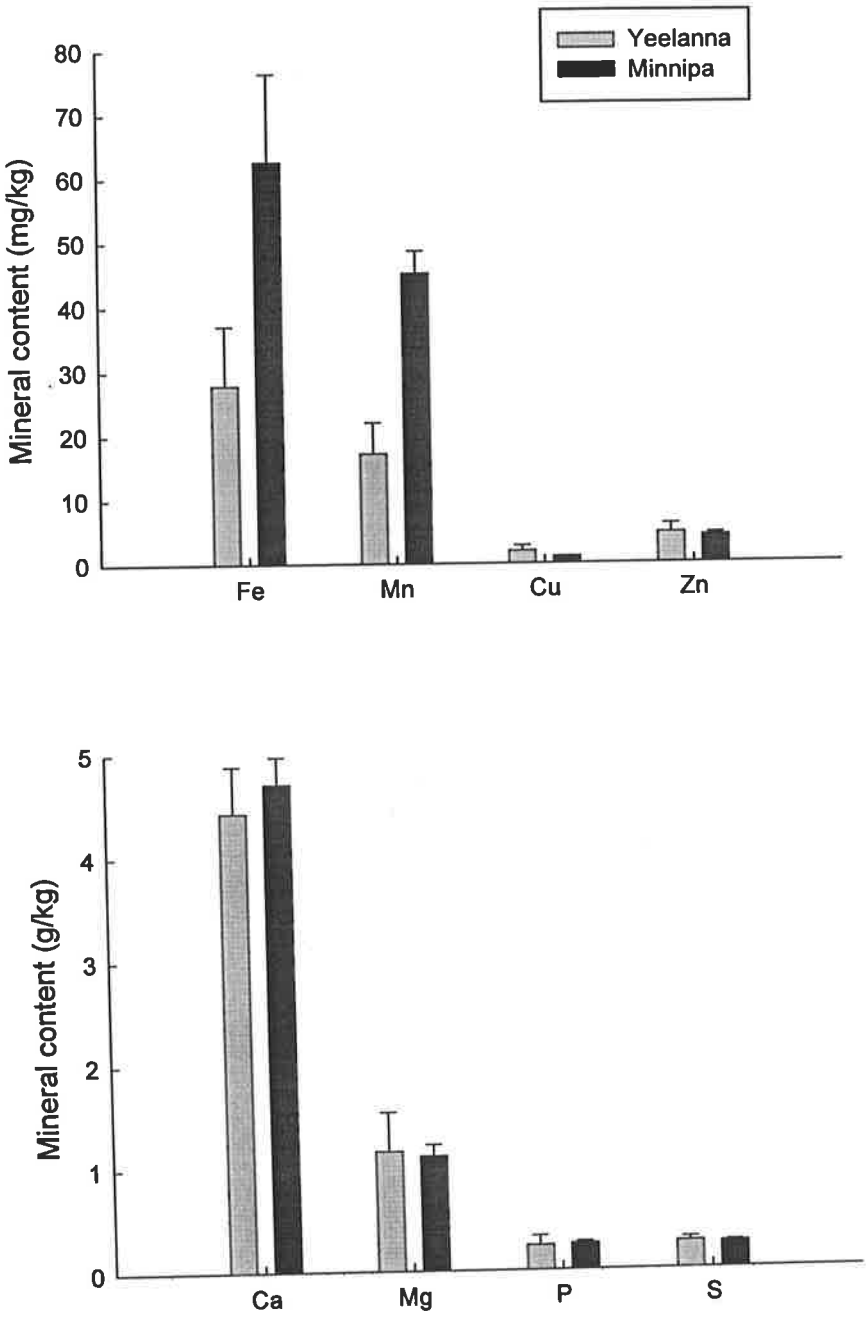


Figure 6.2b The mineral content of seed coats of lupins grown at two locations on the Eyre Peninsula, SA (bar = s.e.).

The mineral content of whole seeds differed between the two locations. Generally, the Fe, Mn and Ca contents of whole seeds were higher in seeds grown at MRC, and lower in Cu, Zn, Mg, P and S content compared with seeds grown at Yeelanna (Table 6.1).

Table 6.1 The seed mineral content of lupins grown at MRC and Yeelanna

	Fe	Mn	Cu	Zn	Ca	Mg	P	S
		mg/kg				g/kg		
MRC	35.9	25.9	5.3	24.5	2.0	1.8	3.2	2.2
YEE	33.8	13.1	8.1	26.5	1.8	2.0	3.4	2.7

6.3.3 Variation in the seed structure and chemical composition in different growing years at same location (MRC)

There was a significant interaction between year of growth year and line for 100 seed weight, coat proportion, N and NDF content in both seed coat and seed kernel (Table 6.2). Seed weight in 1994 was significantly lower than that of 1993, an average decrease of 42.7%. While the seed coat proportion of the whole seed was greater for all lines except for P22924 where the seed coat proportion was decreased by 10% for seeds grown in 1994. Kernel nitrogen content was 30% more on average for seeds grown in 1994, but line responses to the growing year varied. For example, MAR6034 had 37% more kernel nitrogen for seeds grown in 1994 than in 1993, while P22924 had 24% more kernel nitrogen in 1994 than in 1993. NDF content in the seed coat decreased for all lines grown in 1994, but the magnitude of the decrease varied among lines. For example, NDF content decreased by 32 g/kg for MAR5300 and only 1 g/kg for Erregulla. Kernel NDF content increased for all lines grown in 1994 except for P23342 where it decreased by 15%. There was no interaction between line and year of growth in kernel and coat ADF content, but year of growth had a significant effect on kernel and coat ADF content. Seeds from 1994 had 23 and 3.7 g/kg lower ADF content in the seed coat and seed kernel respectively than those from 1993.

Table 6.2 The seed structure and chemical composition of seeds grown in 1993 and 1994 at MRC (g/kg except for 100 seed weight)

	Weight of 100seeds (g)		Coat proportion		Coat						Kernel					
					N		ADF		NDF		N		ADF		NDF	
Year / Line	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
MAR5300	48.0	30.2	251	262	4.7	6.1	706	678	774	742	58.1	77.6	45.8	38.9	38.2	47.3
MAR6034	46.2	30.6	243	263	5.2	6.0	683	642	757	735	56.4	77.3	45.8	36.3	35.5	42.5
P22924	28.9	22.7	293	266	4.1	4.6	700	679	781	778	64.0	79.7	32.7	27.9	38.3	45.7
P20954	59.9	26.0	258	293	4.7	5.9	701	669	782	779	54.1	69.0	30.9	32.2	50.0	72.1
P23342	68.9	32.1	233	269	3.7	6.1	696	688	804	787	55.6	73.6	32.5	32.1	66.4,	56.2
Erregulla	27.5	18.3	260	289	3.9	5.0	680	670	773	772	73.0	92.1	26.0	23.9	36.1	43.8
Mean	46.6	26.7	256	274	4.4	5.6	694	671	778	765	60.2	78.2	35.6	31.9	44.1	51.3
Year		**		**		**		*		**		**		*		**
Year×Line		**		**		**		NS		*		**		NS		**

* p<0.05,** p<0.01, NS: no significance.

6.3.4 The mineral content of seeds grown in different years at MRC

The mineral content of the seed coat and seed kernel varied in different growing years (Figure 6.3). Generally, seeds had higher levels of Fe, Mn, Cu, Zn, K, P and S in the seed kernel, and higher levels of Fe, Mn, Zn and K in the seed coat when grown in 1993 compared to 1994.

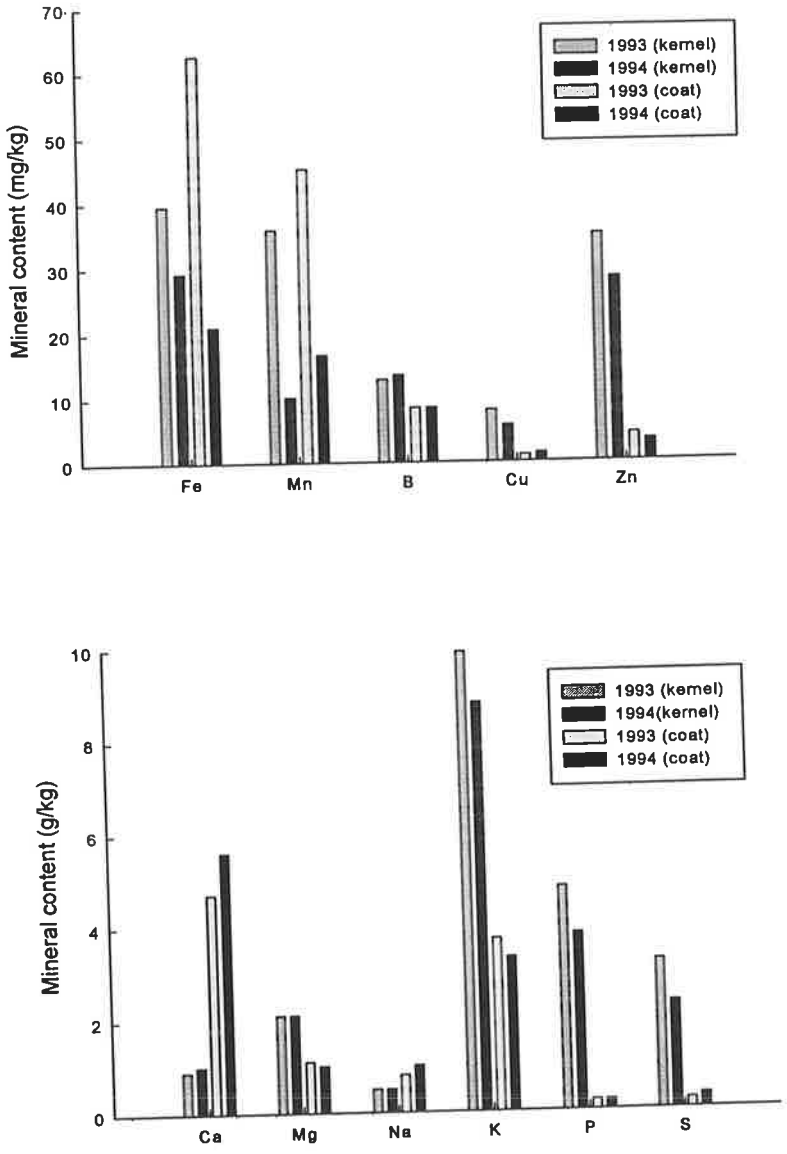


Figure 6.3 The mineral content of the seed coat and kernel of lupins grown in 1993 and 1994.

Seeds grown in 1993 had higher levels of Fe, Mn, Cu, Zn, K, P and S in the whole seed than did those in 1994 (Table 6.3).

Table 6.3 The mineral content of lupin seed grown in 1993 and 1994

	Fe	Mn	Cu	Zn	B	K	P	S	Ca	Na	Mg
	mg/kg					g/kg					
1993	43.7	37.9	6.2	27.1	11.6	3.5	3.7	2.4	1.9	0.58	1.88
1994	26.8	11.9	4.3	21.5	11.8	3.3	2.8	1.8	2.2	0.64	1.83

6.3.5 Genetic variation in the seed structure and chemical composition of seeds grown in 1992 at Yeelanna

There was large variation in seed structure and seed chemical composition among lines that were grown in the same year and same location. Hundred seed weight of P23342 and P23341 was more than twice of that of Erregulla. Seed coat percentage of P22924 was 55 g/kg more than that of MAR6034. ADF and NDF content of the seed coat of P23342 were the highest, while MAR6034 had the lowest ADF and NDF content in seed coat. Nitrogen in the seed coat of all lines ranged from 4.0 g/kg to 5.4 g/kg. Erregulla had the highest nitrogen and lowest NDF in the seed kernel (Table 6.4).

There was little difference in ADF, NDF and nitrogen content of the grain, which ranged from 205-255, 227-295 and 41.0-46.6 (g/kg) respectively.

Table 6.4 The seed structure and chemical composition of lupin lines grown in 1992 at Yeelanna (g/kg)

Line	Weight of 100seed (g)	Coat proportion	Coat			Kernel		
			N	ADF	NDF	N	ADF	NDF
MAR6034	41.4	258	4.0	692	781	57	36	34
P22924	25.1	313	5.4	710	782	63	36	50
Erregulla	19.7	305	4.7	700	783	65	43	32
P20954	45.8	306	4.6	731	798	57	37	73
P23341	50.0	304	4.8	740	806	59	43	64
P23342	50.5	289	4.4	744	815	60	36	61
SEM	5.37	8.2	0.19	9.0	5.9	1.2	1.4	6.8

6.3.6 The mineral content of lines

The mineral content of lines varied both in the seed coat and the seed kernel (Figure 6.2a and b). Fe, Mn and Ca content of the seed coat and Fe, Mn, B, Cu, Zn, K, P and S content of the seed kernel of the lines were as follow: P23341 had the highest Fe content, MAR6034 had the highest Mn, and P22924 had the highest Ca content in the seed coat, while Erregulla had the highest Fe, Mn, B, Cu, Zn, K, P and S content in the kernel.

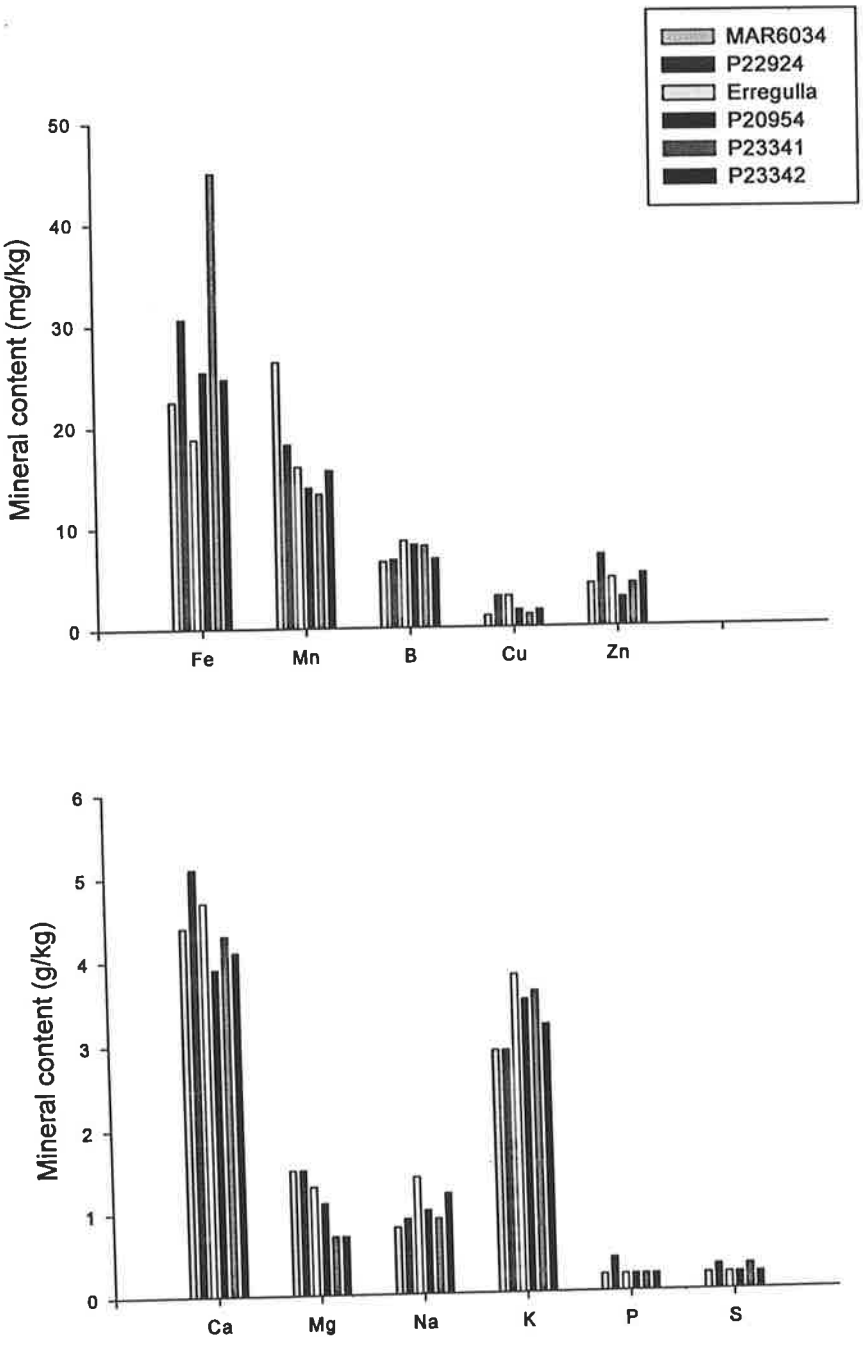


Figure 6.4a The mineral content of the seed coat for lines of lupins grown in 1992 at Yeelanna.

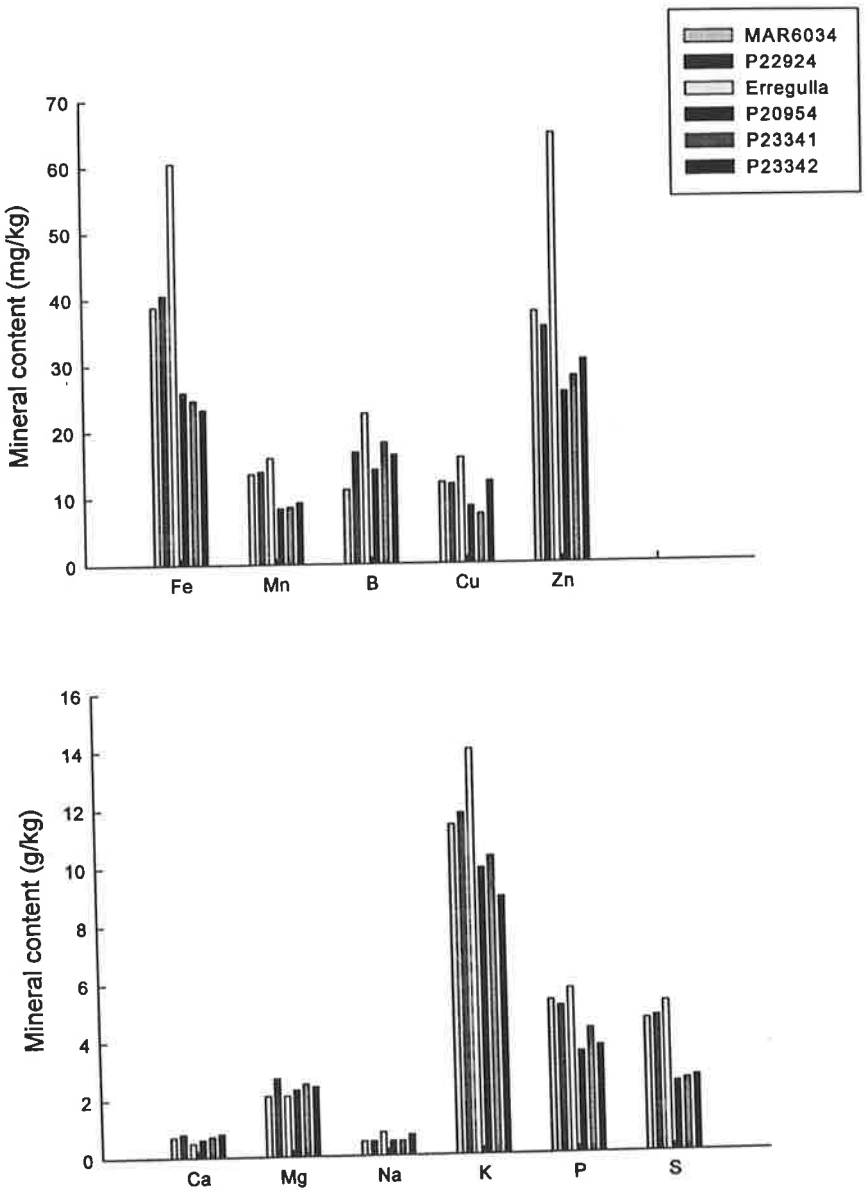


Figure 6.4b The mineral content of seed kernels for lines of lupins grown in 1992 at Yeelanna.

Figure 6.4a and b also show that most of the minerals were concentrated in the seed kernel rather than seed coat except for Ca.

On a whole seed basis, Erregulla had the highest content of almost all minerals except for Mn, Ca and Mg. P22924 had the highest Ca and Mg content of the lines.

The content of Fe, Mn, B, Cu, Zn, Ca, Mg, Na, K, P and S in the whole seed is shown in Table 6.5.

Table 6.5 The mineral content of lupin seeds

	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
Line	mg/kg					g/kg					
MAR6034	34.6	16.9	9.9	9.3	29.2	1.6	1.9	0.6	3.7	4.0	3.4
P22924	37.4	15.3	13.6	9.1	26.6	2.2	2.3	0.6	4.6	3.6	3.3
Erregulla	47.8	15.9	18.3	11.9	46.5	1.8	1.9	1.0	5.4	4.0	3.7
P20954	25.7	10.1	12.3	6.4	18.6	1.6	1.9	0.7	4.1	2.5	1.7
P23341	30.8	10.0	15.1	5.5	20.8	1.8	2.0	0.7	4.2	3.0	1.8
P23342	23.7	11.1	13.6	9.2	23.2	1.7	1.9	0.9	3.5	2.7	1.9

6.4 Discussion

The important findings in this experiment were that environmental factors (growing sites and growing seasons) had a significant effect on seed quality. Seeds grown at MRC were heavier, had less fibre and less seed coat than those grown at Yeelanna. N content was similar at both locations. Dry conditions in 1994 resulted in smaller seeds, hence lower macro- and micro-nutrient content per seed. There were variations in seed size and seed chemical composition of lupin lines. Erregulla had the smallest seed, but the highest kernel nitrogen, and a higher kernel mineral content, except for Ca and Mg.

Growing sites Growth site had a significant influence on seed quality. Seeds grown at MRC had more kernel nitrogen than those grown at Yeelanna in the current study. Little difference in the crude protein content resulting from location was reported by Bacon *et al.* (1995) for peas, White *et al.* (1981) for *L. angustifolius*, and Oluwatosin (1997) for cowpeas. Large variation in crude protein content for different locations was reported by Jimenez *et al.* (1991) for *L. albus*, Gottschalk *et al.* (1976) for peas,

Tandom *et al.* (1957) for Phaseolus, and Krober *et al.* (1970) for pulses (including pigeon-pea, black-gram, green-gram, lentil, cowpea and pea). Jimenez *et al.* (1991) grew 17 lines of *L. albus* at two locations and found that the protein content was 37.43% and 32.41% respectively. This difference may be because of different seasonal conditions, soil types and different crops tested in different experiments.

Seeds grown at MRC had less seed coat and fibre than those grown in Yeelanna. This may be because the seeds grown at MRC were heavier than those grown at Yeelanna, resulting in a lower proportion of seed coat, hence a lower fibre content due to the fibre being mainly concentrated in the seed coat.

The mineral content of the seeds varied at the different growing sites. Seeds grown at MRC had higher levels of grain Mn than those grown at YEE in the current study. This result is supported by Schultz and French (1978) and White *et al.* (1981). The latter found that Mn varied from 6 mg/kg to 118 mg/kg DM of *L. angustifolius*. The variations in other elements was supported by White *et al.* (1981), who collected seeds from 23 sites and found Cu, Zn and S contents varied from 1.7 to 5.3 mg/kg DM, 19 to 38 mg/kg DM and 0.18% to 0.31% respectively.

The variable results found in chemical composition and mineral content due to locations suggested that the effects of soil conditions on chemical composition of the different genetic sources were complex. For example, the protein content of soybean tended to increase with increasing phosphorus levels in the soil (Kapoor and Gupta 1977). *L. albus* has been known to accumulate Mn from the soil (Hill 1986). In contrast, Gottschalk *et al.* (1975) grew peas in variable soil conditions and found that the protein content showed no variation. This may be because soil conditions were also affected by weather (McNair 1945).

Growing years Year of growth only had strong effects on ADF content both in the seed coat and in the kernel. Effects on other parameters could not be identified due to the significant interaction between growth year and line. Generally, seeds grown in 1994 were small, thin-coated, contained higher levels of grain nitrogen, had a lower level of fibre except for NDF in the seed kernel, and were lower in the level of

most mineral elements regardless of the interaction. These results suggest that the seed size and nutrient content were strongly affected by moisture because 1994 was extremely dry in south eastern Australia. This was supported by Withers and Forde (1979), who found that seed weight was reduced by 43% to 72% due to water stress. Below average rainfall resulted in a significantly lower seed nitrogen than above average rainfall, 38 vs 177 kg N/ha from Beech and Leach (1988) and 96 vs 232 kg N/ha from Horn *et al.* (1996) for chickpea. In contrast, Oluwatosin (1997) found that there was not much difference in protein content of cowpea that was grown in different years at the same location. These differences may be due to different seasonal conditions and the different species tested.

Genetic variation There was considerable variation in seed size, ranging from 19.7 g per 100 seeds for Erregulla to 50.5 g per 100 seeds for P23342. This was supported by Erskine and Khan (1977), who reported that 100 seed weight of genotypes of cowpea varied from 8.1 to 14.1g. The protein content of lines in this study were similar, but considerable variation in protein content of genotypes of cowpeas was found by Oluwatosin (1997), ranging from 20.8 to 35.9%. Mineral content also varied among lines in this study. Erregulla was highest for most of the elements in the seed kernel. These variations create greater opportunity for breeders to select more desirable characters, in terms of seed quality, for animal production.

In conclusion, growing year and growing site had strong effects on seed size, seed coat proportion and nutrient content (N, ADF, NDF and minerals). Therefore, when crops are grown over large areas, variation in grain quality is likely due to environmental variation. Selection of species or cultivar might reduce this variation. The consequence of this large variation in seed quantity and seed quality of lupins under different growing condition could be reflected in animal production. The effect of nutrient variation in utilisation efficiency by animals will be determined in Chapter 7.

CHAPTER 7
NUTRITIVE VALUE OF LUPINS FOR SHEEP

7.1 Introduction

Lupins are an excellent feed for sheep due to their high crude protein content (28% to 35%) and metabolisable energy (12.5 MJ/kg) (Murray 1994). Lupins are readily accepted and digested by sheep. Although they contain relatively high fibre, it is readily fermented in the rumen and produces valuable metabolisable energy, and the risk of developing lactic acidosis is low when lupin grain is included in sheep diets (Dixon and Hosking 1992 and Murray 1994). Feeding experiments (Brown *et al.* 1986, and Dixon and Hosking 1992) have shown that lupins fed as a supplement can increase intake, live weight gain, wool growth and efficiency of feed conversion of sheep feeding or grazing low quality roughage.

With these outstanding features, lupins are used widely in sheep production systems in Australia, especially Western Australia. In the 1990's around 800,000 tonnes of *L. angustifolius* grain were produced each year, 500,000 tonnes were exported and more than 200,000 tonnes were used for sheep feed. Large amounts of lupin plant residues including seed lost at harvest are grazed by sheep (Nelson and Delane 1991 and Murray 1994). As much as 200-350 thousand tonnes of seed of self generating sand plain lupins (*L. cosentinii*) or 'bitter lupin' are also eaten by sheep annually in Western Australia (Murray 1994). Associated with this seed are also much larger quantities of plant residues.

L. cosentinii is an example of a wild lupin adapted to local growing conditions being used as sheep feed. Other wild lupins such as *L. atlanticus* and *L. pilosus* could be cultivated for animal feed in areas with fine textured and alkaline soils (Buirchell and Cowling 1992 and Egan and Hawthorne 1994). However, the nutritive value of these wild lupins has not been well studied, and knowledge of their effects on animal performance is almost unknown. This study was therefore conducted to determine

the chemical composition and the characters of degradation of wild lupins in the rumen of sheep using a few lines due to the small volumes of material available. This study also determined the nitrogen balance of sheep when larger quantities of these lupins became available. To evaluate their potential use in animal production, the nutritive values of *L. pilosus* and *L. atlanticus* were also compared with that of *L. angustifolius*, which is commonly used in animal production of Australia.

7.2 Materials and methods

This study consisted of three parts:

1. determination of the chemical composition of *L. pilosus*, *L. cosentinii*, *L. atlanticus* and *L. angustifolius*,
2. measurements of degradation of DM and N of lupins in the rumen of sheep and,
3. measurements of digestibility and N-balance when these lupins were used in diets for sheep.

7.2.1 Part 1: The seed structure and chemical composition of lupins

The lupin lines The seeds of 19 lines of lupins including 12 lines of *L. angustifolius*, 2 lines of *L. pilosus*, 3 lines of *L. atlanticus and* 1 line of *L. cosentinii* were collected from WA and one line of *L. angustifolius* from SA. Six hundred seeds in each line were separated into coat and kernel by hand for measurements of the seed coat percentage of whole seed weight and the chemical components of the seed coat and seed kernel.

Chemical component analysis NDF and ADF content of the seed coat and seed kernel were determined in duplicates using the methods described by Harris (1984). N content was determined using the Kjeltec Auto 1030 (Kjeldahl method) and crude protein was calculated as N x 6.25, and mineral content was determined using an inductively coupled plasma (ICP) analyser digested by nitric/perchloric acid.

7.2.2 Part 2: The degradability of DM and N of lupins in the rumen of sheep

The lupin lines A total of 11 lines of lupins, including 2 lines (P1993 and P23030) of *L. pilosus*, 3 lines (AM2.9.2, P22927 and MD92(96)) of *L. atlanticus*, and 6 lines (cvv. Danja, Gungurru, Illyarrie, Uniharvest, Warrah and Yandee) of *L. angustifolius* from WA were used in this experiment.

Animal management Three mature Merino wethers fitted with rumen cannula were kept in individual pens and fed twice daily at 8:30 and 16:00 with a ration consisting of 65% oaten chaff, 15% lucerne chaff, 15% lupin seed (cv. Gungurru) and 5% mineral mix. A period of 7 days prior to the incubation was allowed for the animals to adapt to the diet and the animal house. Water was available at all times. The cannula of each animal was routinely cleaned to ensure it remained secure and stable.

Sample incubation procedure in sacco The degradability of DM and N was determined using the method described by Mehrez and Ørskov (1977), and Huntington and Givens (1995). For each of the incubation runs, approximate 5 g samples in duplicates from each lupin line were weighed into the previously dried and weighed nylon bags (9×17 cm, pore size of 44 μm, Swiss Screens). The bags with sample and one glass marble were fastened with fishing line and wetted before inserting into the rumen. Nine bags were inserted into the rumen via the cannula at 8:00 am on the first day of any given incubation period. Samples were incubated in the rumen for periods of 2, 4, 8, 12, 24, 36, 48 and 72 hours.

After removal from the rumen, the bags were immediately washed under running water until the rinse water was clear. On average, this took about 3 minutes per bag. The bags were dried in an oven at 65 °C for 48 hours, then weighed. All residues were collected for nitrogen analysis. The difference between the sample weight and the residues was considered as DM loss over the incubation period, and used for describing the DM and N degradation patterns of lupins using Equation 1 (Ørskov and McDonald 1979):

Equation 1 ..... $p=a+b(1-e^{-ct})$

p: DM or N degradability

a: soluble and completely degradable material that is rapidly washed from the bag

b: the insoluble but potentially degradable material which is degraded by the microorganisms according to first-order kinetics

c: rate constant of b function

t: incubation time (hour)

Ground lucerne chaff (5 g) was used as a uniform standard and incubated for 48 hours in all animals and runs. This provided a control both within time periods and between animals, and also for longer term variation over the experimental period.

Chemical analysis N content was determined using a Leco 2000 combustion system (Anon. 1994).

7.2.3 Part 3: Digestibility and N-balance of lupins fed to sheep

Animals and feed Twenty four Merino wethers (approx. 12 months of age, mean weight 43 ± 0.87 kg) were housed in individual pens and fed a diet containing 30% *L. angustifolius* (cv. Gungurru), 67% barley straw, 3% mineral mix (White *et al.* 1992). After 4 days adaptation to the animal house, the intake of each sheep was measured for 7 days. Based on their intake and live weight, 16 sheep were finally selected for the *in vivo* study.

Three species of lupins, *L. pilosus* (P23030), *L. atlanticus* (P22927) and *L. angustifolius* (cv. Gungurru), and barley straw were used in this experiment. Lupins were crushed and barley straw was cut to around 5 cm long before being fed to the sheep. A mineral mix was supplied and fresh water was available at all times.

N-balance study procedure This study included two total excreta collection periods. In the first period, 16 sheep were selected from the intake study and separated into 4 groups (average weight 44 kg for each group). The composition of diets are given in Table 7.1 and expressed as per 1000 g for clarification. Diets are

referred to as Diet 1 (barley straw only), Diet 2 (barley straw + *L. angustifolius*), Diet 3 (barley straw + *L. atlanticus*) and Diet 4 (barley straw + *L. pilosus*).

For the second collection period, 12 sheep from the first period were used with the same basic dietary materials as for Period I. Diets were fed to provide the level of lupin supplementation from 150g/head/day to 300g/head/day. The diets in the second collection period were referred to as Diet 5 (barley straw + *L. angustifolius*), Diet 6 (barley straw + *L. atlanticus*) and Diet 7 (barley straw + *L. pilosus*).

Table 7.1 Ingredient and nutrient composition of diets fed to sheep in the N-balance study

	Period I				Period II		
Item	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6	Diet7
Barley straw (g)	973	773	773	773	644	644	644
L. angustifolius (g)	-	200	-	-	333	-	-
L. atlanticus (g)	-	-	200	-	-	333	-
L. pilosus (g)	-	-	-	200	-	-	333
Mineral mix* (g)	27	27	27	27	22	22	22
Total (g)	1000	1000	1000	1000	1000	1000	1000
DM (g/kg)	910	910	911	913	910	910	916
N (g/kg of DM)	3.6	15.2	14.5	14.2	23.0	21.8	21.3
GE (MJ/kg of DM)	17.3	17.7	17.6	17.8	18.5	18.2	18.5

* Siromin : Na 158.0-176.0, Ca 43.0-48.0, S 35.0-39.0, P 13.5-15.0, Mg 3.5-4.0, K 104.0-116.0 (g/kg) and Fe 1746-1940, Mn 520-580, Zn 1040-1160, Cu 104-116, Mo 36-40, I 3-4, Co 72-80, Se 5.4-6, Ni 3-4, Cr 3-4, V 3-4 and B 3-4 (mg/kg).

For both collection periods, the sheep were weighed at the beginning and end of the measurement period, which lasted 7 days. For the first period, the sheep were fed for 8 days before being transferred to metabolism cages, then fed the same diet for a further 7 days for adaptation to the cages. For the second collection period, The sheep were allowed a period of 10 days to adapt to the diets.

Each sheep was fitted with a harness and faecal collection bags for 3 days before starting collections. Total faeces were collected and weighed daily. A 10% sample of total faeces was stored in a freezer at -10 °C. After each 7 day collection period, the sample from each day was bulked and mixed, then sampled and dried in an oven at 60 °C for chemical analysis.

All urine was collected and weighed daily in the morning using 4.5 L plastic containers with meshed covered funnels on the top to prevent contamination. At the uranic collecting time, 50 ml of 6 N HCI was added to each container to keep the pH below 2.5. 10% of total urine was sampled daily and stored at 4 °C for nitrogen analysis.

Feed residues were collected at two daily intervals, and mixed prior to sampling for chemical analysis.

The chemical analysis Feeds, residues and dried faeces were ground through a 1-mm screen for DM, ash, N, and GE analyses. DM was determined after 12 hours in an oven at 105 °C. Ash was determined in a muffle furnace at 550 °C for 6 hours. N content of dried faeces, feeds and residues was determined using a Leco 2000 combustion system (Anon. 1994). The N content of urine was determined using the Kjeltec Auto 1026 (Kjeldahl method). GE was determined by using the Parr 1261 Adiabatic Bomb Calorimeter.

Calculations The daily N-balance for each diet was calculated by subtracting the total faecal N and total urinary N excretion from the total N intake.

The DMD for each species of lupins (*L. angustifolius*, *L. atlanticus* and *L. pilosus*) was calculated using the formula (Equation 2) of Charmley and Greenhalgh (1987):

Equation 2 ..... **a=(v-b*p)/(1-p)**

a: the coefficient for lupins

b: the coefficient for barley straw

v: digestibility of the diet

p: the proportion of barley straw in diet

This formula assumes that the digestibility of the roughage in the diet is not changed when partially substituted with lupins. Therefore, any changes in the digestibility of the diet result from the replacement of lupins.

7.2.4 Statistical analysis

The experiments were a factorial design and all data were analysed using a general linear model from Systat (Wilkinson 1996).

7.3 Results

7.3.1 The seed structure and seed chemical components of lupins (seeds from WA)

There were significant differences between species in 100 seed weight and seed coat proportion in the whole seed (Table 7.2). Seeds of *L. pilosus* and *L. atlanticus* were much heavier than those of *L. angustifolius* ($p<0.01$), and had a higher proportion of seed coat, but there was no marked relationship between seed coat percentage and 100 seed weight. For example, *L. pilosus* had the highest 100 seed weight, but relatively lower seed coat proportion in whole seed than *L. cosentinii* that had a smaller seed and high proportion of seed coat in the whole seed weight. *L. angustifolius* had the smallest seeds and the lowest seed coat proportion of all of species.

The seed coat fibre content of the four species varied ($p<0.01$). Generally, wild species had higher levels of ADF and NDF in the seed coat than *L. angustifolius*. For

example, *L. pilosus* had 10% more of both ADF (727 g/kg) and NDF (811 g/kg) in the seed coat than *L. angustifolius* (603 and 721 g/kg respectively). Nitrogen content of the seed coat ranged from 3.9 g/kg (*L. atlanticus*) to 7.5 g/kg (*L. cosentinii*).

Kernel NDF content, rather than kernel N and ADF content showed variation between species. *L. cosentinii* and *L. angustifolius* had the highest NDF content of the kernel. There was little difference in ADF content of kernels between species with a range of 27 to 35 g/kg. *L. cosentinii* had the highest kernel N content and *L. pilosus* had the lowest.

The distribution of the chemical components in the seeds was different. Most fibre was concentrated in the seed coat, rather than in the kernel, while most of the N was in the kernels.

Table 7.2 The seed structure and seed chemical composition of 4 lupin species (g/kg) (means ± s.e.)**

				Seed coat			Seed kernel		
Species	n*	Weight of 100seed (g)	Coat proportion	N	ADF	NDF	N	ADF	NDF
L. cosentinii	1	17.8c**	323a	7.5b	699ab	765b	70	27	55cb
		±2.06	±13.5	±0.6	±15.2	±14.6	±2.8	±6.4	±4.0
L. pilosus	2	41.9a	283b	5.2ac	727a	811a	62	31	41a
		±1.46	±9.6	±0.4	±10.8	±10.3	±2.0	±4.5	±2.8
L. atlanticus	3	23.8b	309a	3.9c	677b	788ab	68	35	47ac
		±1.19	±7.8	±0.3	±8.8	±8.4	±1.6	±3.7	±2.3
L. angustifolius	13	15.5c	239c	5.4a	603c	721c	68	28	55b
		±0.57	±3.7	±0.2	±4.2	±4.0	±0.8	±1.8	±1.1

* n = number of lines.
** Figures in the same column with different letters were significantly different ($p<0.01$).

7.3.2 The mineral contents of lupin species

The seed coat mineral content was quite variable between the different lupin species (Table 7.3). *L. cosentinii* had higher levels of Fe, Mn, Cu, Na, K, P and S of seed coat than the others. While *L. angustifolius* had higher Zn and Ca content of the seed coat, *L. pilosus* had the highest B and the lowest P, and *L. atlanticus* had the highest Mg.

The mineral content of the kernels was also quite variable with *L. cosentinii* having the highest Fe, Mn, Cu, Zn, Na, P and S content, and the lowest Ca and B content. *L. angustifolius* had the highest level of Ca in the kernel , and the lowest levels of Mn, Cu and S of in the kernels.

The distribution of minerals in seed varied. Fe, B, Cu, Zn, P, S and K were mainly in the kernel, and Ca was mainly concentrated in the seed coat for all species.

Table 7.3 The concentrations of mineral elements in the seed coat and the seed kernel of lupins

Species	n*	Fe	Mn	B	Cu	Zn	Ca	Mg	Na	K	P	S
		mg/kg					g/kg					
							Coat					
L .cosentinii	1	-	54.3	7.2	5.0	13.8	3.1	1.8	1.4	5.7	0.6	0.6
L. pilosus	2	15.8	36.2	9.7	1.5	9.1	3.8	1.4	1.4	3.6	0.2	0.3
L. atlanticus	3	22.1	47.1	7.9	1.7	9.1	4.4	2.0	0.5	3.2	0.3	0.3
L. angustifolius	11	29.0	17.4	8.3	0.9	23.0	6.4	1.3	0.2	3.1	0.4	0.3
							Kernel					
L. cosentinii	1	51.0	30.9	15.6	12.4	64.1	0.4	2.0	1.0	10.7	4.8	4.3
L. pilosus	2	28.7	24.9	22.1	7.6	34.6	0.7	2.4	0.7	9.2	4.0	3.8
L. atlanticus	3	44.9	20.9	20.5	7.6	34.1	0.8	2.5	0.3	10.8	4.3	4.3
L. angustifolius	11	46.8	18.1	24.2	5.7	40.0	1.0	2.0	0.2	10.9	4.0	3.5

* number of lines.

7.3.3 The degradability of DM of lupin species

The disappearance of DM of lupins incubated in nylon bags in the rumen of sheep was very high for all species, with around 50% disappearing after 2 hours incubation and over 90% disappearing after 48 hours incubation (Table 7.4).

Table 7.4 The disappearance of DM (%) from lupins incubated in the rumen of sheep (means ± s.e.)

	Time of incubation (hours)							
Species	2	4	8	12*	24	36**	48**	72
L. pilosus	49.6	54.3	68.5	74.2ab	80.9	89.0b	94.1b	97.9
	C2.60	±0.11	±1.61	±2.69	±0.45	±0.72	±0.27	±0.27
L. atlanticus	51.9	59.1	67.3	72.2b	81.9	86.9b	94.0b	97.5
	±1.41	±2.31	±0.63	±1.69	±3.03	±1.75	±0.88	±0.33
L. angustifolius	49.6	58.0	70.0	78.0a	88.2	96.0a	97.8a	98.3
	±1.11	±1.66	±1.13	±0.67	±1.13	±0.28	±0.12	±0.09

Figures in the same column with different letters were significantly different (* $p<0.05$, ** $p<0.01$).

The degradability of species showed maximum differences after 12, 36 and 48 hours incubation. *L. angustifolius* had a significantly ($p<0.01$) higher degradation rate after 36 and 48 hours incubation than *L. atlanticus* and *L. pilosus*, and a higher ($p<0.05$) degradability after 12 hours incubation than *L. atlanticus*. There was little difference between *L. atlanticus* and *L. pilosus* in overall degradability of DM. However, the DM degradation patterns were not similar for the 3 species (Figure 7.1).

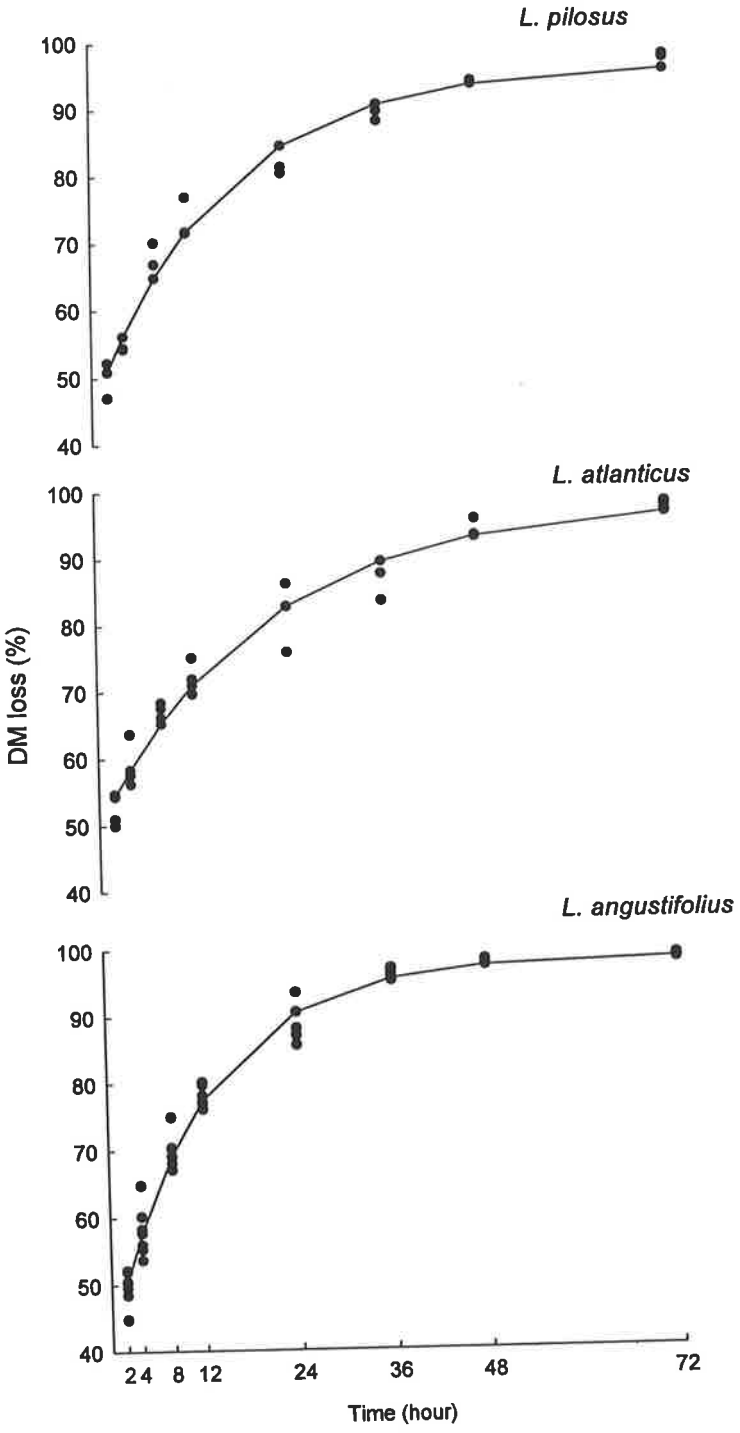


Figure 7.1 DM loss from lupins fermented *in sacco* in the rumen of sheep.

7.3.4 Parameters to describe the degradation pattern of the DM of lupin seed incubated in the rumen of sheep

The parameters defined in the model $p=a+b(1-e^{-ct})$ (Equation 1, Ørskov and McDonald 1979) for lupin degradation are shown in Table 7.5.

Table 7.5 The parameters describing the patterns of degradation rate of lupin seed incubated in the rumen of sheep (means ± s.e.)*

Species	a (%)	b (%)	c	100-(a+b)**
L. pilosus	44.9	51.7ab	0.061ab	3.4
	±1.54	±2.40	±0.004	±0.85
L. atlanticus	48.0	49.4b	0.060b	2.6
	±2.03	±2.10	±0.006	±0.15
L. angustifolius	41.6	56.8a	0.083a	1.6
	±1.61	±1.51	±0.005	±0.29

* Figures in the same column with different letters were significantly different ($p<0.05$).

**100-(a+b)= the undegradable portion of a sample.

There were no significant differences in the highly soluble fraction (a) of lupin species, averaging 44.8%. *L. angustifolius* had a higher ($p<0.05$) insoluble but potentially degradable component (b) than *L. atlanticus*. No significant difference was found between *L. angustifolius* and *L. pilosus* or between *L. pilosus* and *L. atlanticus* in a, b and c. The proportion that was undegradable was low for all species and ranged from 1.6% to 3.4%.

7.3.5 The nitrogen degradability of lupin species

Nitrogen degradation rate of lupins showed a similar trend to dry matter disappearance, where over 70% of nitrogen disappeared after 2 hours incubation, and nearly completely disappeared from the nylon bag after 24 hours incubation (Table 7.6). The degradation patterns of lupin nitrogen were quite similar (Figure 7.2).

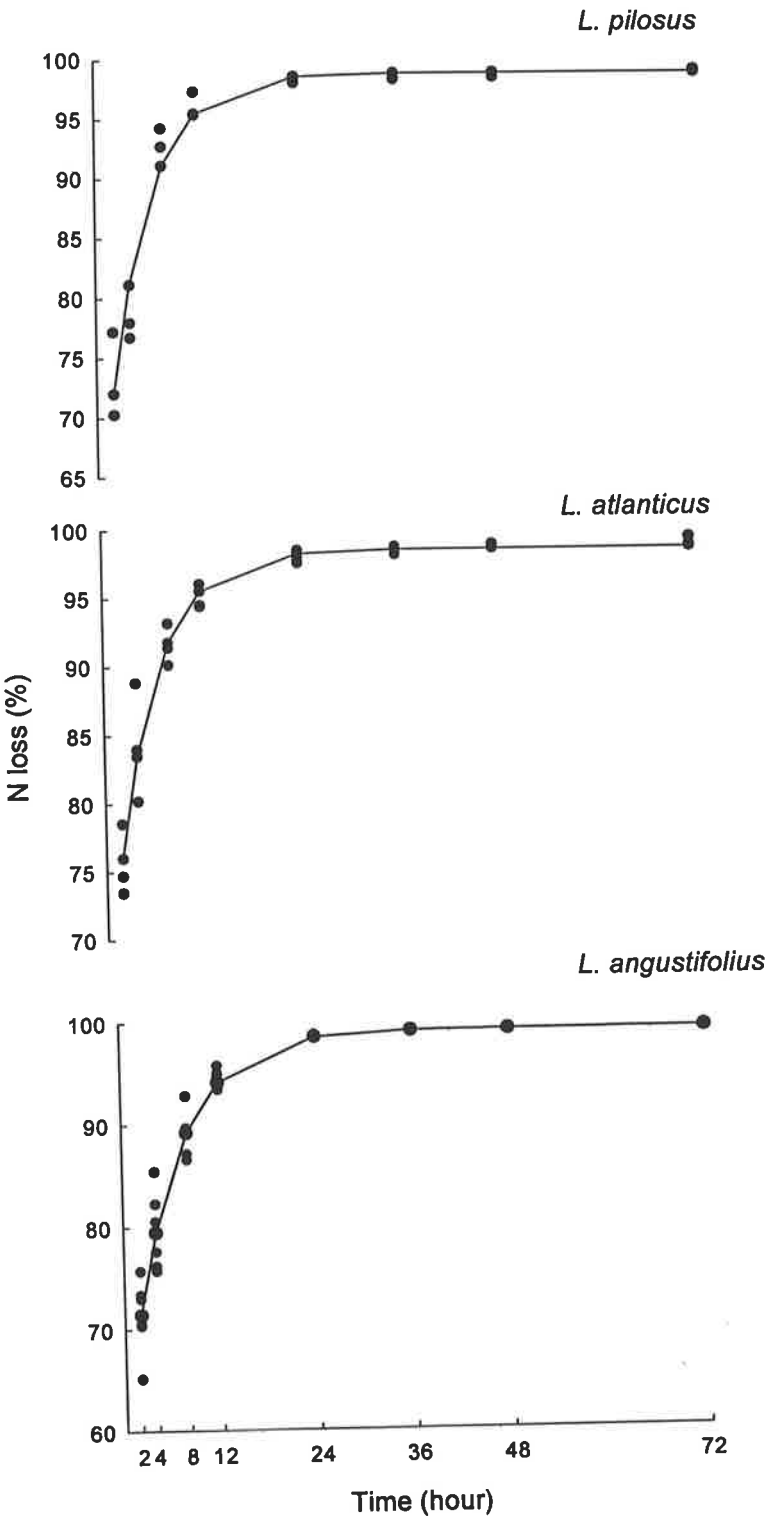


Figure 7.2 N loss from lupins fermented *in sacco* in the rumen of sheep.

Table 7.6 The disappearance of N from lupins incubated in the rumen of sheep (means ± s.e.)

	Time of incubation (hours)							
Line	2	4	8*	12	24*	36**	48**	72
L. pilosus	73.7	77.3	93.4a	96.3	98.1ab	98.3b	98.5b	98.9
	±3.47	±0.61	±0.74	±0.93	±0.11	±0.07	±0.10	±0.11
L. atlanticus	75.5	84.3	91.5ab	95.0	97.9b	98.3b	98.7b	99.0
	±1.53	±2.49	±0.88	±0.53	±0.27	±0.16	±0.09	±0.18
L. angustifolius	71.3	79.6	88.8b	94.4	98.5a	98.9a	99.1a	99.1
	±1.47	±1.56	±0.96	±0.36	±0.05	±0.05	±0.04	±0.04

Figures in the same column with different letters were significantly different (* $p<0.05$, ** $p<0.01$).

7.3.6 Parameters for describing N degradation patterns of lupins

Nearly 60% of N was readily soluble in the rumen for *L. pilosus* and *L. angustifolius* and over 65% was soluble for *L. atlanticus* (Table 7.7). The potential degradable nitrogen of lupins was over 98% (a+b) for all species, with only 0.9% to 1.3% N being undegradable.

Table 7.7 The parameters of N disappearance from lupins incubated in the rumen of sheep (means ± s.e.)

Species	a (%)	b (%)	c	100-(a+b)*
L. pilosus	58.0	40.9	0.208	1.1
	±4.91	±4.74	±0.014	±0.17
L. atlanticus	65.5	33.2	0.191	1.3
	±2.53	±2.60	±0.009	±0.10
L. angustifolius	59.2	39.9	0.179	0.9
	±2.56	±2.61	±0.018	±0.12

* 100-(a+b)= the undegradable portion of a sample.

7.3.7 The nutrient intake and digestibility for different lupin varieties fed as supplements at two levels

There was no significant difference in DMI, OMI, GEI, DMD, AED and N-balance ($p>0.05$), except for NI and AND ($p<0.05$) between different lupin varieties at the low level of supplementation. The NI of *L. pilosus* as a supplement was lower than that of *L. angustifolius* and AND of *L. atlanticus* as a supplement was lower than that of *L. angustifolius* at the low level of supplementation (Table 7.8).

There were significant differences in DMI ($p<0.05$), NI ($p<0.05$), GEI ($p<0.01$), AND ($p<0.05$), AED ($p<0.05$) and N-balance ($p<0.05$) between *L. angustifolius* and *L. pilosus* as supplements at the high level supplementation. Generally, the diet containing *L. angustifolius* seeds had a higher DMI, OMI, NI, GEI, AND, AED and N-balance than the diet containing *L. pilosus*. However, there were no significant differences between *L. angustifolius* and *L. atlanticus* as supplements. There was no significant difference between *L. atlanticus* and *L. pilosus* in the parameters measured except for GEI ($p<0.05$), where the diet containing *L. atlanticus* had a higher GEI than that for *L. pilosus* at the high level of supplementation.

Table 7.8 The nutrient intake and digestibility for Merino wethers fed different lupin species at two levels of supplementation to a basal diet of chopped barley straw (means ± s.e.)*

	Low (150 g/head/day)			High (300 g/head/day)		
	L. angustifolius	*L. atlanticus*	*L. pilosus*	*L. angustifolius*	*L. atlanticus*	*L. pilosus*
DMI (g/day)	605 ±23.0	656 ±9.7	602 ±28.4	767a ±25.1	738ab ±44.1	643b ±16.4
OMI (g/day)	566 ±22.0	614 ±6.6	564 ±29.8	721a ±23.8	697ab ±39.0	609b ±12.7
NI (g/day)	10.0a 0.16±	9.6ab ±0.23	9.1b ±0.17	18.4a ±0.12	15.7ab ±1.10	10.6b ±1.40
GEI (g/day)	10.8 ±0.42	11.8 ±0.13	10.8 ±0.56	14.0a ±0.45	13.3a ±0.75	11.6b ±0.28
DMD%	57.9 ±1.02	56.2 ±1.75	57.0 ±0.93	60.5 ±0.65	57.4 ±1.98	54.0 ±1.81
AND%	68.3a ±0.66	64.3b ±0.65	64.5ab ±1.57	76.6a ±0.70	74.5ab ±1.14	64.2b ±4.53
AED%	56.5 ±1.40	55.1 ±2.51	55.4 ±0.78	59.3a ±0.71	55.8ab ±1.97	52.5b ±1.69
N-balance(g)	1.28 ±0.26	0.80 ±0.25	0.79 ±0.16	6.01a ±1.46	3.42ab ±0.41	1.85b ±0.68

* Figures in the same row within each supplement level with different letters were significantly different ($p<0.05$).

7.3.8 Differences in DMD between lupin species fed at two levels

There were no significant differences in DMD between the lupin species fed at the two levels. The mean DMD was 86.6% for *L. angustifolius*, 74.5% for *L. atlanticus* and 75.4% for *L. pilosus*. There was no interaction between the lupin species and supplement levels. However, compared to the low level of supplementation, the digestibility of each species significantly decreased at the higher level of supplementation by 8.9% for *L. angustifolius*, 11.0% for *L. atlanticus* and 25.7% for *L. pilosus* (Table 7.9).

Table 7.9 DMD (%) of lupins at two supplement levels (calculated using Equation 2) (means ± s.e.)

	Supplement level	
Species	Low (150 g/head/day)	High (300 g/head/day)
L. angustifolius	90.7 ± 0.04	82.6 ± 0.02
L. atlanticus	83.0 ± 0.08	73.9 ± 0.05
L. pilosus	86.6 ± 0.04	64.3 ± 0.05

7.3.9 The effect of supplement level on nutrient intake and digestibility of diets

Intake Supplementation with lupin grain significantly increased the total DMI, OMI, NI and GEI ($p<0.01$) (Table 7.10). The total DMI increased by 45.7% when 150g/head/day of lupin seed was fed and by 67.7% when 300g/head/day was fed. If it is assumed that all the lupin seed was consumed (indicated by the low residual N of 0.39 g/day at the low level, 3.12 g/day at the high level and 0.53 g/day when no supplement was fed), the intake of barley straw was slightly increased at the low level of supplementation (about 50 g/day/head), but there was no increase in barley straw intake at the high level of supplementation.

Table 7.10 Nutrient intake of sheep supplemented at two different levels with lupins (means ± s.e.)*

Item	Barley straw only	Low (150 g/head/day)	High (300 g/head/day)
DMI (g/day)	427c	622b	716a
	±22.3	±14.0	±22.7
OMI (g/day)	395c	582b	676a
	±20.3	±13.3	±20.4
NI (g/day)	1.7c	9.6b	14.9a
	±0.1	±0.14	±0.12
GEI (MJ/day)	7.4c	11.1b	13.0a
	±0.38	±0.25	±0.41
ASH (g/day)	32.2	40.6	40.6
	±2.40	±2.36	±2.53

* Figures in the same row with different letters were significantly different ($p<0.01$).

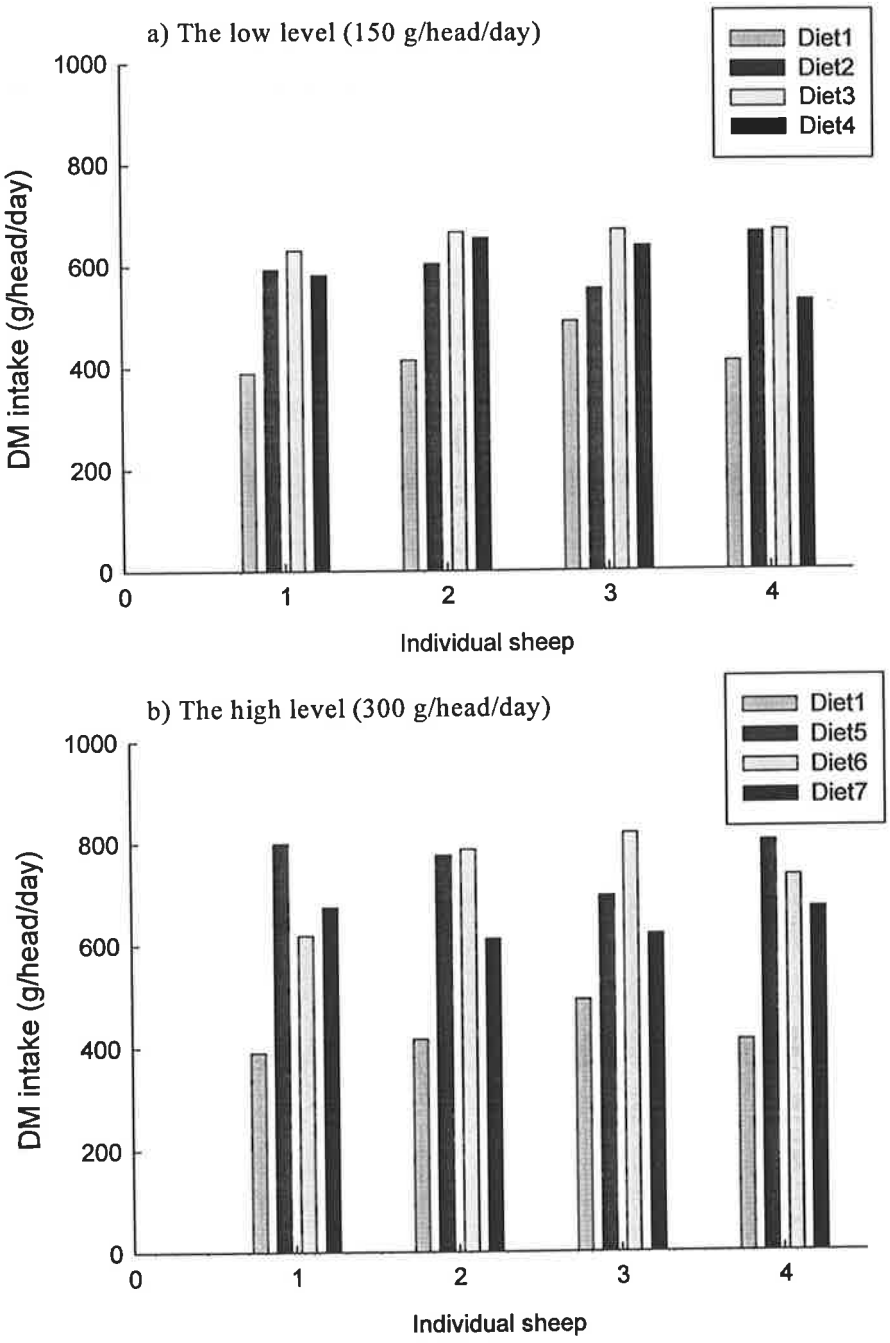


Figure 7.3 Mean daily DM intake for individual sheep fed each diet at two levels of lupin supplementation.

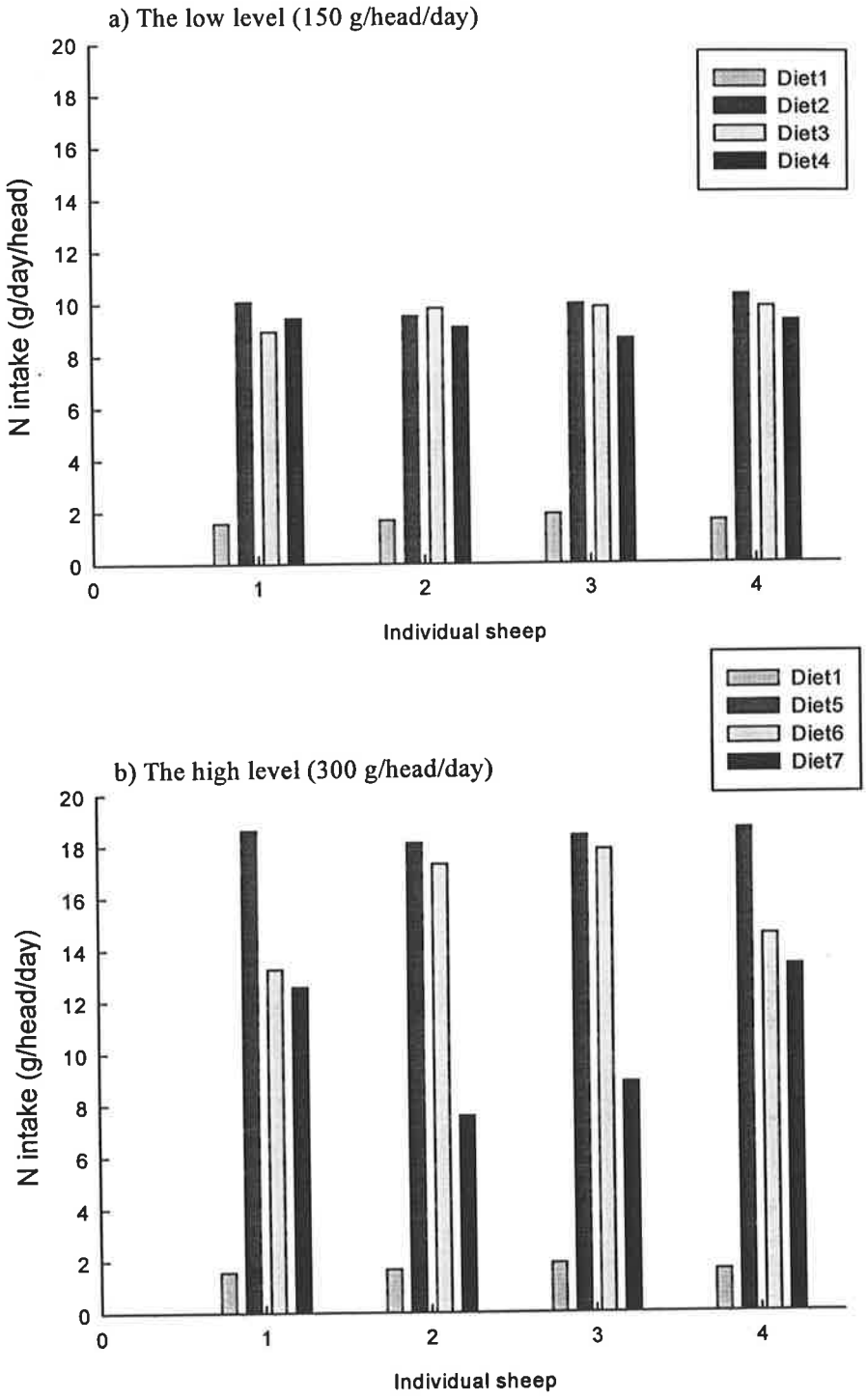


Figure 7.4 Mean daily N intake for individual sheep fed each diet at two levels.

At the low level of supplementation, there was little variation in DMI between individual sheep, but there was large variation between sheep at the high level of supplementation (Figure 7.3). For example, the DMI of Diet 6 (barley straw+*L. atlanticus*) varied from 617 for sheep 1 to 817 (g/head/day) for sheep 3. The NI of each sheep also varied quite widely (Figure 7.4). At the low level of supplementation, there was little difference in NI between sheep, but there was considerable variation in NI at the high level supplementation. For diet 7 (barleystraw+*L. pilosus*), The NI intake of two sheep (Number 2 and 3) was significantly lower than that of the others.

Digestibilities and N-balance Feeding 150 g/head/day of lupin seed significantly improved DMD by 18.0%, OMD by 16.7% and AED by 25.7% compared with no supplementation. N-balance was also significantly improved by 150 g/head/day of lupin grain supplement (Table 7.11). However, no further improvement in DMD, OMD, AED was achieved at the high level of supplementation. However, the high level of supplementation significantly increased N retention compared with the low level of supplementation (3.76 vs 0.96 g N/day), even though the N excreted in the urine (UN) increased with increasing NI (Figure 7.5). The correlation between NI and UN was significant and positive (UN=0.557 + 0.484 NI, R^2=0.947).

Table 7.11 The digestibility and N-balance for sheep supplemented at two levels with lupins (means ± s.e.)*

Item	Barley straw only	Low (150 g/head/day)	High (300 g/head/day)
DMD%	48.3b	57.0a	57.3a
	±0.97	±0.70	±1.16
OMD%	50.9b	59.4a	59.3a
	±0.90	±0.79	±1.11
AED%	44.3b	55.7a	55.9a
	±1.26	±0.92	±1.16
N-balance (g)	-2.31c	0.96b	3.76a
	±0.15	±0.14	±0.72

* Figures in the same row with different letters were significantly different (p<0.01).

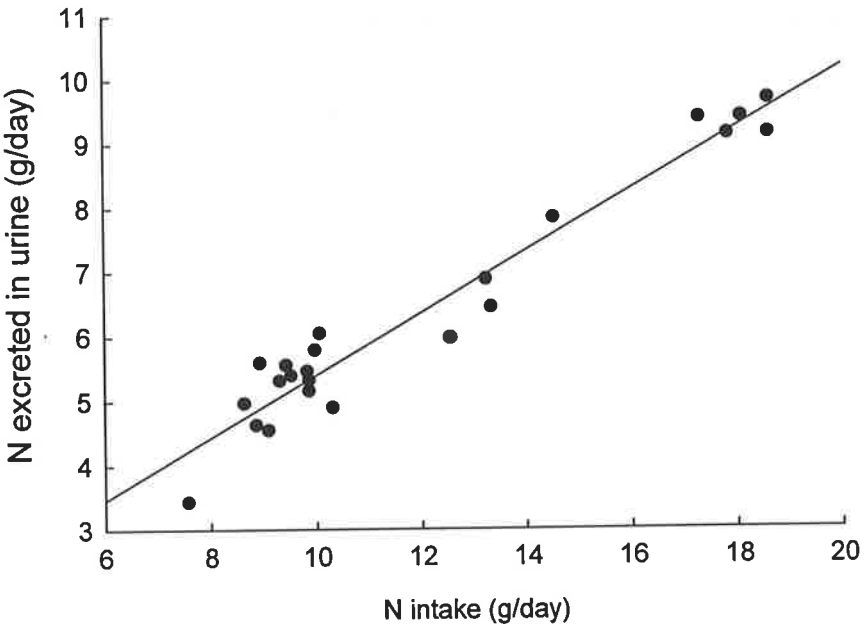


Figure 7.5 The relationship between N intake and N excreted in urine for sheep fed barley straw with different lupin species.

7.4 Discussion

The important findings in this experiment were that wild lupins had a greater proportion of seed coat and a higher seed coat fibre content than domesticated lupins and the N content of the kernels of the different lupin species were similar. The high seed coat fibre content did not appear to be a limiting factor for ruminant nutrition as evidenced by the high degradation rate of DM and N for all lupins. Supplementation with lupin seed significantly improved DMI, DMD, AED and N-balance. However, high level supplementation was not an efficient method to increase animal production as there was only a small increase in the DMD and AED of the overall diet, with a reduction in the DMD of the lupin component. That there were no significant differences between *L. pilosus*, *L. atlanticus* and *L. angustifolius* in DMI, DMD, AED and N-balance suggests that *L. pilosus* and *L. atlanticus* could be used in place of *L. angustifolius* as a feed supplement at low levels. *L. atlanticus* could

substitute for *L. angustifolius* at high levels of supplementation as there were no differences in DMI, DMD, AED, AND and N-balance when theses species were fed as a supplement to barley straw.

7.4.1 The seed structure and chemical composition of lupins

Seed structure There was variation in the size of seed and the proportion of seed coat between the species studied. Wild lupins had larger seeds with a thicker seed coat than the domesticated species. The reduction in thickness of the seed coat resulted in the seed coat being a lower proportion of the whole seed for domesticated lupins. However, a thick seed coat was not a limiting factor for ruminant nutrition as it is highly degradable in the rumen (as discussed in Chapters 3 and 5).

Nutrient components There was little difference in CP content of the lupin seeds studied (range from 28.7% to 33.1% on air dry dasis) from Western Australia. This range was narrower than that reported by Hill (1977), Pate *et al.* (1985) and Buirchell and Cowling (1992) (26% to 40.1% of DM). These differences may be associated with differences in cultivars, growing seasons and analytical methods. The seed ADF content (air dry basis) varied from 16.5% for *L. angustifolius* to 24.4% for *L. cosentinii*, the corresponding values for NDF content were 21.4% and 28.4% in the current experiment. Wild lupins had a higher fibre content than the domesticated species as a result of the thicker seed coat of the wild lupins (as described in Chapter 5).

There were differences in nutrient distribution within the whole seed. Most of fibre was concentrated in the seed coat and most of the CP was concentrated in the kernel for all species. Similar findings were reported by Hill (1977). The distribution of ADF and NDF in the seed coat of all lupins ranged from 87.1% to 92.5% and 80.4% to 88.6% respectively in the current experiment. This indicates that a high CP and low fibre feed can be achieved by dehulling lupins because of the low fibre and high CP in the kernel.

Mineral contents of lupins *L. cosentinii* had a higher Mn, P and S content and lower Ca content than *L. angustifolius*. *L. pilosus* and *L. atlanticus* had similar levels of these elements to those of *L. angustifolius* except for the lower seed coat P content for *L. pilosus* and *L. atlanticus*. The distribution of minerals in the whole seeds was similar for all species. Most of the Ca was in the seed coat and most of the B, Cu, Zn, Mg, K, P and S were in the seed kernel (as described in Chapter 5). This is in agreement with Hill (1977), who reported that most Ca in seeds appeared to be in the seed coat and most P appeared to be in the seed kernel. Mn in the wild species was concentrated in the seed coat rather than in the seed kernel, but there was not much difference in Mn content of the seed coat and the seed kernel for *L. angustifolius* in the current study.

The differences in the N and the Mineral content of the seeds studied in Chapter 5 and those studied in this Chapter are attributed to the seeds being grown at different locations. The seeds in Chapter 5 were from Turretfield, SA, while the seeds studied in this Chapter were from the Department of Agriculture, WA. This provides further evidence that location can lead to differences in the nutrient content of lupins.

7.4.2 DM and N degradability of lupins

DM degradability Lupin grain was highly degraded in the rumen with over 97% of the seed disappearing after 72 hours incubation in the rumen. This agrees with the results of Dixon and Hosking (1992), who found that more than 80% of the DM of lupins (*L. angustifolius*) was fermented in the rumen with a rumen fractional outflow rate of 0.02 kgh^{-1}. There was little difference in DM degradability between the wild and domesticated species. The potentially degradable proportion (a+b) was similar, 96.6% for *L. pilosus*, 97.4% for *L. atlanticus* and 98.4% for *L. angustifolius*. This value of 97.4% for *L. angustifolius* was similar to that reported by Domingo (1990) (99%), but higher than that reported by Hosking (1987) (88%). These differences may reflect differences in the basal diet fed in these studies. Lucerne hay was fed as the basal diet in Hosking's experiment, whereas a low quality roughage was fed in Domingo's study and a low quality roughage plus *L. angustifolius* was fed in the current study.

The high degradability determined *in sacco* for lupins ground through 1-2 mm in this study may differ from *in vivo* results, in practice with coarsely ground lupin seed or whole grain fed to sheep. There is evidence that the rapid solubilisation of DM of *L. angustifolius* was reduced from 46% to 3% as the particle size of the sample increased from "fine" (ground through at 0.8mm screen) to "coarse" (samples passed through a mill without a screen) (Freer and Dove 1984). Therefore, it seems likely that increasing the particle size of lupins as occurs under *in vivo* conditions may reduce the degradation rate of lupins and possibly increase the efficiency of utilisation lupins by the rumen microbial population.

Lupin N was rapidly degraded in the rumen. The potentially degraded part (a+b) for N averaged 98.8% for all species. This result was supported by Kibelolaud *et al.* (1993), who found that the degradability of lupin CP in the rumen was 93.4% with ruminal outflow rate of 0.06 kgh^{-1}. On average, 73.5% of the lupin N disappeared after 2 hours incubation in the rumen in the current experiment. Guillaume *et al.* (1987) and Huguet *et al.* (1984) reported that more than 75% of lupin CP was degraded in less than 2 hours incubation in the rumen for a ground through 1 or 2 mm screen. Robinson and McNiven (1993) reported values of 80% for *L. albus in sacco.*

N degradation for *L. pilosus* after 8 hours incubation in the rumen was greater than that for *L. angustifolius*, but there was little difference between the species in the extent of degradation after 12 hours incubation (mean=95.2%). Although lupin N is highly degradable in the rumen, it has a higher value as a N supplement than urea. This is not only because lupins have high buffer-soluble CP content (true protein) (66%) (Robinson and McNiven 1993), but the protein, peptides and amino acids are better sources of rumen degradable N than urea in terms of the efficiency of rumen microbial protein synthesis (Hume 1974, Broderick and Craig 1989, Chen *et al.* 1992 and Singh *et al.* 1995). Furthermore, Hume (1974) compared fishmeal and soybean meal with lupins and found that lupins produced the highest level of ammonia N and the lowest total non-ammonia nitrogen, but the flow of microbial N was not significantly different between the treatments.

7.4.3 The effect of lupin supplement on intake and the efficiency of nutrient utilisation

Intake Lupin supplementation significantly increased the total DMI of the diets with variable effects on the intake of the roughage component. A low level of lupin grain supplementation slightly increased the roughage intake, but had no effect at a high level of supplementation. Similar results were reported by Roberts *et al.* (1979), Butler (1981), Foot *et al.* (1983), Smith and Warren (1986), Smith and Kenney (1987), Curtis and Mavrantonis (1990), and Margan (1994). Margan (1994) found that 100g/day of lupin seed supplement increased the total DMI of sheep by 11%, but increased wheaten hay intake by only 4%. Wheaten hay intake was decreased by 14% when the level of lupin supplement was increased up to 400g/day. The results in the current study contrast with those reported of Rowe and Aitchison (1987) and May *et al.* (1993). May *et al.* (1993) reported that DMI was not affected by the addition of lupins when fed to cows.

Stimulation of roughage intake with low levels of supplementation may reduce the retention time of the diets and balance the diet nutrients for sheep. Foot *et al.* (1983) found that the retention time of ^{103}Ru-P in the rumen of Merino weaners was 20.0 hours for herbage alone, 16.6 hours for 120 g lupin grain supplementation, and 12.7 hours for 190 g lupin grain supplementation, but the retention time did not decrease with a further increase in lupin grain supplementation to 250 g. In addition, high levels of supplementation may not improve the roughage intake since the deficiency of protein in the diet may have disappeared at a low level of supplementation. Minson and Milford (1967) supplied a legume to low protein Pangola grass and found that 100 g/day of the legume supplement increased both total voluntary intake, and Pangola grass intake. Further increasing legume supplementation from 200 to 400 g/day depressed the voluntary intake of Pangola grass, thus they (Minson and Milford) concluded that the increase of grass intake was due to the increase of crude protein intake. Once this supplementation overcame the deficiency of CP in the diet, a further increase in the legume supplement may have resulted in a depression in the voluntary intake of roughage due to direct substitution.

Nutrient utilisation efficiency Supplementation significantly improved nutrient utilisation efficiency across supplementation levels. This was indicated by an increase in DMD of 18.0% and 18.6%, AED by 25.7% and 26.2% and apparent N-balance by 3.3 and 6.1 g/day for the low and the high levels of supplementation respectively. This result was similar to that reported by Margan (1994), who found that lupin supplements (at 100 g/day and 400 g/day) had a positive effects on digestibility of the diets, and increased AND and AED by 38% and 16% at low level and by 36% and 19% at a high level of supplementation respectively. The results of Margan (1994) suggest that high levels of supplementation did not further improve DMD, AED and AND, but N-balance did improve by 2.8 d/day in the current experiment. This result is in contrast with that reported by Minson and Milford (1967), who found that "DMD was linearly correlated to the legume content when the proportion of legume in the diet was more than 10%, but the increase in digestibility caused by the addition of the first 10% was greater than that obtained when more legume was added". However, their results also showed that a high level of supplementation was not efficient.

7.4.4 The differences between lupin species as supplements

DMD of lupins DMD was not significantly different between species, but was 90.7% for *L. angustifolius*, 83.0% for *L. atlanticus* and 86.6% for *L. pilosus* at the low level of supplementation. DMD of *L. angustifolius* was similar to that reported by Murray (1992) (90%), and higher than that reported by Foot *et al.* (1983) (87.7 %). This difference may be a result of the different basal diets fed in the different experiments.

DMD was affected by the level of lupins in the diets for all lupin species. The DMD of the lupins decreased when the proportion of lupins in the diet increased. This may be because the different nutrient composition of the base diets resulted in different DMD of the supplementary feed. This was supported by Valentine and Bartsch (1986), who found that the DMD of lupins increased as the proportion of lupin

increased in oaten hay based diets (DMD =63.8+13.7X, X=proportion of lupin grain), but decreased on oaten pasture (DMD=81.2-1.0X) for Friesian cattle.

Digestibility and N-balance of diets The effects on the DMI, DMD, AED and N-balance of the whole diets were similar for all 3 lupin species at the low level of supplementation. However, *L. angustifolius* was superior in AND, AED and apparent N-balance than *L. pilosus* at higher supplementation levels. This may have been associated with the intake of *L. pilosus* seed being lower than that of *L. angustifolius* at the high level of supplementation (indicated by there being 0.32 g of N residue for *L. angustifolius* and 6.94 g of N residue for *L. pilosus* each day). Alternatively the relatively higher levels of alkaloids of *L. atlanticus* and *L. pilosus* compared to *L. angustifolius* may have altered N digestion and metabolism. This result contrasts with the grazing study reported by Arnold *et al.* (1976), who suggested the nutritive value of the *L. angustifolius*, *L. luteus* and *L. cosentinii* were similar in terms of digestible organic matter intake. Nevertheless sheep weight gains were better on *L. angustifolius* than on *L. cosentinii* over three years. However this lower weight gain appeared to be related to differences in the initial amounts of grain present in the field as much as palatability which is suggested in the current study.

N retention was 12.8%, 8.3% and 8.7% of the NI for *L. angustifolius*, *L. atlanticus* and *L. pilosus* at the low level of supplementation. Corresponding values were 32.7%, 21.8% and 17.4% at the higher level of supplementation. The value for N retention for *L. angustifolius* at the low level of supplementation in this study was lower than the 22.4% reported by Kung *et al.* (1991), when lambs were supplemented with lupins. This was higher than that reported by Margan (1994), who found only 8% for lupin seed alone and 12% for lupin (*L. angustifolius* cv. Uniwhite) when supplemented with roughage. These differences may reflect variation in grain quality and the demands of the animals being fed, particularly the lambs in the study of Kung *et al.* (1991).

7.4.5 The efficiency of lupins as protein supplements

A number of researchers (Thompson and Curtis 1990, Morcombe and Ferguson 1990, Dixon and Hosking 1992 and Margan 1994) have shown that supplementing with low levels of lupin grain may be more efficient. The current study also supports this view. This is supported by the result that UN increased with increasing levels of NI (Figure 7.5). A low efficiency of utilisation of lupins at the high levels of supplementation may be related to the longer retention time of diets with high levels of lupins and the high degradability of lupins in the rumen. Ganev *et al.* (1979) reported that as the proportion of dietary concentrate increased, the fractional rumen outflow rate of protein supplements decreased. If this is the case, a high level of supplementation with lupins may have a lower fractional rumen outflow rate resulting in more lupins being degraded in the rumen. In addition, Faichney (1996) reported that for diets containing 8% and 20% CP, with CP intakes (g/day) of 34.4 and 86.3, the amount of non-ammonia CP leaving the stomach of sheep remained very similar (50.0 g/day vs 55.0 g/day), the CP being fermented in the rumen.

In conclusion, the feeding value of *L. angustifolius*, *L. pilosus* and *L. atlanticus* were similar when fed as a supplement to barley straw at 150 (g/day/head). This suggests that direct grazing of standing crops of *L. atlanticus* or *L. pilosus* should be both possible and valuable to sheep. Harvested grain could also be readily utilised as a supplement on poor summer pastures. These alternatives reflect management practices that are wide spread in southern Australia where *L. cosentinii* is grown for grazing as a standing crop or *L. angustifolius* is grown as a crop to harvest.

CHAPTER 8
GENERAL DISCUSSION

8.1 Effects of domestication on seed structure, yield and chemical composition

Seed colour and seed size Domesticated lupins had lighter coloured seed coats than wild lupins (Chapter 3). The change in colour is a result of reduction in phenolics in the seed coat (Plitmann and Kislev 1989 and Smartt 1990). Smartt (1990) suggested that a reduction in the level of phenolics in the seed coat improved flavour of seeds in many species of grain legumes. However, this selection may also reduce the ability of cultivars to adapt to the environment because phenolics tend to have anti-fungal properties and are protective colorants for grain legumes (Johns 1994). Presumably, breeding and selection of grain legumes that changes the colour of the seed coat may result in reduced adaptability to variable environmental conditions.

Domestication has had some major effects on the seed size of lupins. The recently released cultivars of *L. angustifolius* (1987 and 1988) had smaller seeds than cultivars released in 1971 (Chapter 3). This finding is also supported by the work of Landers (1991), Gladstones (1989) and Gladstones (1994). A reduction in the seed size with the breeding and selection of *L. angustifolius* may be a consequence of the significant reduction in the alkaloid content of *L. angustifolius* (Gladstones 1989) as small seeds have a lower alkaloid content (Watad 1980).

Seed coat structure, thickness and seed coat proportion Domestication reduced the seed coat thickness of lupins due to a thinner palisade layer and a thinner hourglass layer for soft-seeded seed and a thinner palisade layer for smooth-seeded seeds (Chapter 3). Although there is little information available for lupins, Lush and Evans (1980) and Plitmann and Kislev (1989) found that domesticated varieties of legumes had thinner seed coats than wild ones due to shorter and thinner-walled palisade cells.

Seed coat thickness was significantly and positively related to seed size for wild lupins (Chapter 3). This suggested that selection for smooth-seeded seeds of wild lupins may result in small seeds as smooth-coated seeds had a thinner seed coat than rough-coated seeds (Lush and Evans 1980 and Chapter 3). With the reduction in seed coat thickness through domestication, the seed coat as a proportion of the seed has been reduced. This is supported by the data where wild lupins had a greater proportion of seed (55 g/kg) coat than domesticated lupins across all species (Chapter 5 and 7).

N, fibre and anti-nutritional factors Domestication significantly reduced the fibre content of the seed coat, slightly increased kernel NDF content, and had no significant impact on kernel N and ADF content (Chapter 5 and 7). The reduction in fibre content was a result of the reduction in seed coat thickness through breeding. However, no significant changes in kernel N content may appear to have been made through breeding. Gladstones (1994) indicated that the breeding policy for lupins was to maintain or improve protein content, while increasing yield. However, there is evidence that the variability in seed protein of *Phaseolus vulgaris* was reduced as evidenced by isoenzyme studies in comparison to wild populations (Gepts 1990). For example, a lectin-like seed protein (arcelin) is present in wild, but not cultivated accessions of *P. vulgaris*. This protein provides resistance to some specialised seed predators. Such a change in the protein component would not be evident from the sample measurement of N to determine CP, so changes of this nature may need further consideration for lupins.

Selection of lupin seed for low alkaloid content has been one of the major targets for breeding and selection of lupins. This was because of the bitter taste and potential toxicity of the alkaloids. Wild lupins had a higher alkaloid content that domesticated lupins (eg *L. angustifolius*) (Gladstones 1989). Johns (1994) suggested that selection for reduced allelochemicals has been an important objective in the breeding and selection of grain legumes.

Minerals Domestication of *L. angustifolius* has resulted in low seed S content (Chapter 5). This was indicated by a 0.4 g/kg reduction for cultivars released in 1988 compared to the cultivar released in 1976. Wild lupins (*L. cosentinii*, *L. pilosus* and *L. atlanticus*) had higher levels of Mn, Cu and S, both in the seed coats and the kernel, than domesticated lupins (*L. angustifolius*) (Chapter 5 and 7). High Mn content assists in crop establishment and reduces split seeds (Crosbie *et al.* 1994 and Walton 1978). Wild lupins had a better ratio of N:S (16:1) than domesticated lupins (20:1). The N:S ratio is important for optimum microbial growth in ruminants (Murray 1994).

Yield Recently released cultivars of *L. angustifolius* which have been improved in some agronomic characteristics did not have a significantly higher yield, but had lower numbers of seeds per pod than early released cultivars under Turretfield environmental conditions in 1995 (Chapter 4). The cultivar released in 1976 had significantly higher numbers of seeds per pod (4.2 seeds/pod) than cultivars released in 1980, 1982 and 1988 (3.2, 3.4 and 3.6 seeds per pod respectively). The cultivar released in 1979 (357 g/m^2 for Illyarrie) had a similar yield to that released in 1987 (average 324 g/m^2 for Danja and Geebung) and 1988 (average 348 g/m^2 for Gungurru, Warrah and Yorrel). This result contrasts with that reported by Cowling and Speijers (1994), who suggested that yield of cultivars of *L. angustifolius* increased annually by 2.4% from 1973 to 1991. These differences may be a result of different environmental conditions. Another example is that, for the same cultivars, seeds grown for the current study were lighter than those from Western Australia (13.1 vs 15.5 g/100seed). The variation in seed weight and yield for *L. angustifolius* under the different growing conditions probably indicates that the cultivars are sensitive to changes in environment. This may also, in part, be a result of a significant reduction in the level of alkaloids. Alkaloids, like other antinutritional factors, play an important role in protecting plant growth and development (Gladstones 1970, Pate 1983 and Johns 1994). For example, alkaloids reduce susceptibility to disease and insect pests (Gladstones 1970) and the non-protein amino acid canavanine is catabilised to respiratory carbon and ammonia nitrogen in developing plants (Johns 1994). As Pate (1983) suggested "amongst cultivated plants the sad state of many grain legumes in terms of yield losses through disease

and predation by animals may be directly attributed to the removal by selective breeding of a range of toxic and anti-nutritional factors".

8.2 Effects of environmental factors on seed structure, yield and chemical composition

8.2.1 Seed size, seed coat proportion and structure

Sowing time Sowing time did not significantly influence seed size, seed coat structure and thickness in the current study. However, the proportion of seed coat was significantly affected (Chapter 3 and 4). This finding is contrary to previous work which showed that high temperatures and low moisture stress at late growing season, due to a late sowing, resulted in poor seed filling (Delane and Gladstones 1988) and terminated the growth of lupins prematurely (Perry and Poole 1975). The difference may be because a late sowing in the current experiment did not result in a large enough variation in temperature and moisture at the stage of seed maturation, or because of the different growing conditions between Western Australia and South Australia. For example, most of *L. angustifolius* is grown on acid, sandy soils in Western Australia, whereas the soil in this experiment was a clay loam with a pH of 7.1. The late sowing however did reduce the proportion of seed coat in the lupins by 8 g/kg (Chapter 5). The low rainfall in November in the current study may have contributed to this reduction in the proportion of seed coat. Plitmann and Kislev (1989) suggested moisture stress might be a limiting factor for the development of the seed coat.

Year of production Growing year had significant influences on seed size, seed coat structure and seed coat proportion (Chapter 6). The dry conditions of the 1994 season significantly decreased seed size by 43% compared to 1993, decreased seed coat thickness by 17% and increased seed coat proportion by 7% across species (Chapter 3 and 6). This provides further evidence that moisture may affect the longevity of each phase during seed development (Plitmann and Kislev 1989). That seed coat proportion increased under dry conditions in 1994 may be a result of the

significant reduction in seed size for seeds grown in the year acting to increase the ratio of the seed coat to the kernel.

Location Seeds grown at MRC were heavier than those grown at Yeelanna (48.5 vs 38.8 g/100seed) and had less seed coat (257 vs 296 g/kg). Similar findings for soy beans were reported by McNair (1945), who found that seed size varied largely due to growing locations. The differences in seed size and the proportion of the seed coat for the different locations may be a result of differences in soil type and climatic conditions affecting plant growth and development. For example, fine-textured, alkaline soils reduced nitrogen fixation by *L. angustifolius*, hence its growth (White 1990).

8.2.2 Grain yield

Sowing time Early sowing significantly increased the seed yield of lupins (Chapter 4). This was probably because a late sowing resulted in poor early growth, poor vegetative and reproductive development on the branches and poor pod formation, hence poor yield (Perry 1975, Perry and Poole 1975 and Noffsinger and van Santen 1995). Low moisture levels in November may be a major factor contributing to this poor yield as pod and seed filling would have been much faster and the duration of filling was shortened in lupins when moisture stress began after the beginning of pod and seed filling (Dracup and Kirby 1996).

8.2.3 Chemical composition

Sowing time Environmental conditions not only affected seed yield, but also influenced the chemical composition of the lupins (Chapter 5). Late sowing significantly decreased the kernel N content but had no significant effects on grain ADF and NDF content. This was presumably associated with the late sowing causing poor yield (Chapter 4). There is considerable evidence that the N accumulation in grain legumes is related to seedling growth and biomass accumulation (Muchow *et al.* 1993 and Horn *et al.* 1996).

Sowing time also affected the mineral content of the lupin seeds (Chapter 5). Late sowing decreased the kernel Zn content by 4% and increased the coat B content by 8%. Sowing time interacted with line of lupins for the kernel Mn and S content. This is supported by the result that kernel Mn content decreased by 40% for AM2.9.2, 24% for Blue lupin, and increased 8% for Geebung in the June sowing. S content decreased by 0.2 g/kg in AM2.9.2, Chittick and Geebung and increased by 0.4 g/kg in P23030, 0.3 g/kg in Blue lupin and MD92(96) (Chapter 5). The reasons for this are unknown.

Year of production The dry conditions of 1994 significantly reduced nitrogen production per 100 seeds (1.6 g vs 2.1 g N/100seed) and decreased Fe, Mn, Cu, Zn, K, P and S content compared to 1993 (Chapter 6). This is consistent with reports that soil moisture greatly affects plant growth and development (Withers and Forde 1979, Plitmann and Kislev 1989 and Gregory *et al.* 1997). Possible reasons for this poor growth and development were that moisture stress limited the chemical transport and the biological processes that affect nitrogen availability and, hence reduced nitrogen absorption and accumulation in the plants (Gregory *et al.* 1997).

Location Except for grain N content, the growing site had a strong effect on the chemical composition of the lupins. This was supported by the finding that more grain ADF and NDF and less grain Mn was found in seeds grown at Yeelanna than in seeds grown at Minnipa Research Centre (Chapter 6). Small differences in protein content for different locations was reported by Bacon *et al.* (1995) for pea, White *et al.* (1981) for *L. angustifolius*, Oluwatosin (1997) for cowpeas, while large variations were reported by Jimenez *et al.* (1991) for *L. albus*, Gottschalk *et al.* (1976) for peas and Tandom *et al.* (1957) for *Phaseolus*. Different soil types and climatic conditions have also been shown to contribute large variation in oil content of soy beans (McNair 1945). This suggests that the effects of growing site on the chemical composition of different genetic material is complex because soil conditions are also affected by weather (McNair 1945).

8.3 Potential of wild lupins as animal feed

Adaptation to soil conditions *L. angustifolius* can be grown on coarse-textured, well-drained soils of acid-neutral reaction (Gladstones 1970 and 1982b). However, this species does not perform well on heavy textured alkaline soils (Reeves 1974, White 1990 and Landers 1991) whereas *L. atlanticus* and *L. pilosus* are adapted to this type of soil. With around 9 million hectares of this type of soil in temperate Australia, *L. atlanticus* and *L. pilosus* are potentially valuable 'rough' seeded species for these soils (Buirchell and Cowling 1992 and Egan and Hawthorne 1994).

Chemical composition Compared to cultivars of *L. angustifolius*, wild lupins (*L. atlanticus* and *L. pilosus*) had a similar nutrient content (protein and minerals) but a higher fibre content in the seed coat (Chapter 5 and 7) and higher grain alkaloids (0.36% for *L. atlanticus* and 0.55% for *L. pilosus*) (Buirchell *et al.* 1994). However, the higher concentration of alkaloids and fibre in these species are unlikely to be major limiting factors for ruminants as 200-350 thousand tonnes of *L. cosentinii* seed (bitter lupin) are consumed by sheep annually in Western Australia (Murray 1994). The seed coat of lupins studied was highly degraded in the rumen (Chapter 3 and Rowe and Hargreave 1988) and it is known that alkaloids can be removed by washing with water (Johns and Kubo 1988 and Kadam *et al.* 1989a).

Nutritive value The DM digestibility (as calculated from the low level of supplementation treatment) for the wild lupins did not differ significantly from that of domesticated lupins (83.0% for *L. atlanticus*, 86.6% for *L. pilosus* and 90.7% for *L. angustifolius*) (Chapter 7). Wild lupins as a supplement fed at low levels had similar values as *L. angustifolius* in terms of DMI (629 vs 605 g/day), DMD (56.6 vs 57.9%), AED (55.2 vs 56.5%) and N-balance (0.8 vs 1.28 g/day). *L. angustifolius* had a higher AND (68.3%) than wild lupins (64.4%) (Chapter 7). This provides some evidence that wild lupins can substitute of *L. angustifolius* when fed at low levels as a supplement for sheep. *L. angustifolius* was superior to *L. pilosus* and similar to *L. atlanticus* at high levels of supplementation in terms of DMI, AND and AED. However, high level supplementation was not efficient because the N in the

lupins is highly degraded in the rumen and the digestibility of the basal diet is not further improved (Chapter 7).

In conclusion, this work provides evidence that domestication of *L. angustifolius* induced numerous changes in seed structure and chemical composition. With these changes, cultivars may vary in their tolerance to growing conditions and disease. Thus if the "model" of lupin production used for *L. angustifolius* is adopted for commercial testing of *L. atlanticus* and *L. pilosus*, ruminant animals can utilise grain from relatively "wild type" germplasm. This would enable large scale testing of new lines as the development of these species as crop plants progresses. This could see two possible end points. The first would be a crop like *L. cosentinii* which would remain essentially a green manure or forage crop, with little harvested grain. The second is like *L. angustifolius* where there is a more mature industry with grain trading and a focus towards the quality demands, and price differentials for monogastric systems.

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