Selective grazing by sheep to improve the control of weeds of crops

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Declaration

This thesis contains original material that has not been previously presented for any degree at any university or institution. The experimental designs and the preparation of this manuscript were performed by myself, in consultation with my supervisors, Dr John Milton, Professor David Lindsay, Adjunct Professor Mike Ewing and Dr Clinton Revell.

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Summary

With the rapid development of multiple herbicide resistant weeds in crops, it is likely that an important role now exists for new grazing management strategies in farming systems to provide an integrated approach to weed management. In this thesis we examined the general hypothesis that sowing a legume of low preference by sheep relative to the target weeds of crops would improve the control of those weeds in a grazed pasture. To test this general hypothesis, legumes of low preference by Merino sheep were identified and a series of experiments conducted to determine the effect on pasture composition when these less preferred legumes were incorporated into a grazed pasture. We found a learned response that altered forage preference by sheep was important in determining the effectiveness of grazing to reduce seed set by weeds of crops. Investigations on this aspect of the grazing behaviour of sheep were a key part of this thesis.

The short-term relative preference of Merino hoggets among 15 pasture legumes, 4 grain legumes and annual ryegrass was determined by offering adjacent monocultures of each of the forage genotypes to the sheep. The relative preference of the hoggets for each of the 20 forages was determined at three phases of plant growth from estimates of the amount of forage consumed. Sheep showed a low selective preference for Vetch (*Vicia sativa* L.), chickpea (*Cicer arietinum* L.), biserrula (*Biserrula pelecinus* L.), lotus (*Lotus ornithopodioides* L.) and snail medic (*Medicago scutellata* L.) cvs. Kelson and Sava at the vegetative phase of plant growth. An indoor method was also developed to test the relative preference of sheep among forages growing in pots. Using this method chickpea and snail medic, but not biserrula, were found to have a low relative preference by sheep at the vegetative phase.

The short-term relative preference of sheep among annual pasture species did not necessarily reflect their long-term preference by sheep in the field. Sheep grazing an annual pasture sown to Casbah biserrula targeted volunteer ryegrass and its seed set was reduced by more than 90% compared to pastures sown with either snail medic or burr medic. These results demonstrated that short-term relative preference is not a reliable method to identify legumes that will motivate sheep to selectively target weeds in an

annual pasture. Nevertheless, we found that the grazing behaviour of sheep can be modified to achieve a marked increase in the effectiveness of grazing to target weeds of crops.

Sheep that had grazed a pasture containing a high proportion of biserrula avoided grazing biserrula when it was subsequently offered in a preference test, while sheep that grazed a pasture with a lower proportion of biserrula or no biserrula did not. This large shift in selective preference may explain the effectiveness of grazing to reduce annual ryegrass in a pasture sown to biserrula. The preference of sheep among different forages was influenced by their prior grazing experience, so again short-term relative preference may not be a reliable indicator of the grazing behaviour of sheep at pasture.

The selectivity exhibited by sheep among annual pasture plants was related to the nutritive value of the forage, with a preference for plants of high nitrogen, high digestibility and low fibre content at the reproductive and senesced phases of plant growth, but not at the vegetative phase of growth. At the vegetative phase there was a weak negative relationship between compression energy and preference, which suggested that the rate of intake may be partially responsible for selection at this early phase.

The general hypothesis that sowing a legume of low preference by sheep relative to the target weeds of crops would improve the control of these weeds in a grazed pasture was supported. The marked decrease in the seed set of volunteer annual ryegrass in a grazed pasture when Casbah biserrula was introduced, compared to other legumes, demonstrated a clear improvement in the efficacy of weed control. The low preference of sheep for biserrula after they had grazed a biserrula-dominant pasture suggests that sheep developed an aversion and were motivated to seek alternatives, so targeted weeds. The hypotheses addressed in this thesis indicate that legumes of persistent low preference by sheep introduced in a grazed pasture phase could form part of an integrated strategy to manage herbicide resistance in cropping systems.

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Chapter 1

General Introduction

The emergence of herbicide resistant populations of weeds of crops, e.g. annual ryegrass (*Lolium rigidum*) and wild radish (*Raphanus raphanastrum*), in crops in Western Australia has fuelled concern that chemical herbicides are over-prescribed and too heavily relied on as a tool for the management of weeds. Grain producers have found that over time the use of chemical herbicides promotes the survival of strains of weeds that are less affected by the herbicide. As a result the proportion of these “resistant” plants is steadily increasing in cropping regions that rely on herbicides. The lack of viable alternatives to the use of herbicides to control weeds of crops has meant that grain growers are forced to accept the additional cost of carrying herbicide resistant weeds. The need to find viable alternatives to using only herbicides is also being driven by the consumer’s perception that chemicals are overused in agriculture and that this is unhealthy and not environmentally friendly (Lotter 2003).

The purpose of this thesis was to identify legumes of low preference to sheep relative to target weeds of crops and sow these legumes into a pasture that contains these weeds to improve the efficacy of grazing as a tool for weed control. Incorporating alternative methods of weed control into cropping systems, so called “integrated weed management”, is thought to help prolong the effective use of the current herbicides. A grazed pasture phase in the season prior to cropping may be a viable option to avoid losses from the contamination of crops with resistant annual ryegrass. This would be particularly relevant to Western Australian because cropping enterprises rely heavily on chemical herbicides and have a short growing season which is suited to phase farming. Historically, grazing sheep has been a common practice used to control weeds of crops, but grazing has largely been replaced by chemical application, which is usually considered to be more effective. This has meant that management strategies to improve the effectiveness of weed control in grazed pastures have remained underdeveloped.

More recently the concept of ‘phase’ farming has been considered as an alternative to ‘ley’ farming which relies on the persistence of regenerating pastures in a traditional crop-pasture rotation. In phase farming, pasture cultivars (generally aerial seeding annual legumes) are sown to establish pastures intermittently between years of cropping. The legume populations are not required to persist between crop phases because they are re-established at the beginning of each pasture phase (Reeves and Ewing 1993). Soil fertility and weed control should be improved by replacing traditional ley pasture systems with intermittent pasture phases because of the improved productivity from the legumes introduced in the phase. Legumes are considered the most desirable plants to establish in a grazed pasture phase primarily because they increase soil organic nitrogen by symbiotic nitrogen fixation and in many other ways they contribute to improve the physical and chemical fertility of soil (Reeves and Ewing 1993). In addition, legumes are a highly nutritious (protein and energy rich) source of forage suitable for animal production, are not a weed problem in subsequent crops, do not support fungal diseases of cereal crops, provide good ground cover to prevent erosion and improve the sustainability of conventional cropping systems. Compared to annual grasses, the nutritive value of legumes does not decrease as rapidly as the pasture matures (Doyle *et al.* 1989).

Animals can gain weight on mixed legume and grass pastures at up to double the rate of animals grazing on grass pastures with comparable biomass and maturity (Lascano 2000). However, the success of a pasture phase in providing an option for the control of weeds of crops is arguably its most significant benefit as the pasture phase allows a range of points of control for potential weed species, while increasing the seed bank of the annual legumes is not considered a substantial weed risk in subsequent crops (Reeves and Ewing 1993). The introduction of the pasture phase combined with innovative management strategies in grazed pastures offers a significant option to grain producers in combating populations of herbicide resistant weeds.

The success of reducing the grass and broadleaf weed component of a grazed annual pasture is influenced by many factors. The persistence of an annual weed can be simply related to its ability to consistently contribute viable seed to the soil, thus maintaining a seed bank. If this ability is reduced, then the prevalence of the weed should be diminished.

Therefore weed regeneration can be controlled at two important points. One is by removing viable seeds present in the soil and on its surface (Roberts 1981). The other is to stop viable seed from being added to the seed-bank. The primary contribution of grazing to weed control is to reduce viable seed set, although trampling and disturbing the soil by the grazing animal could stimulate germination and therefore also reduce the number of weed seeds in the soil seed-bank.

One aspect of pasture management strategies that promote a desirable change in pasture composition is the specificity by which the grazing animal selects the weeds compared to the legume in a pasture. The relative preference of the herbivore for the weed compared to the legume forage contributes to this. In this thesis, the term relative preference is used to describe the attractiveness of a particular forage to sheep relative to its alternatives and independent of the relative abundance of the forages available. Relative preference has been calculated as a measure of the animal's intake from the forage components available using the Chesson-Manly selection index, and is denoted by α (Chesson 1983).

In a preliminary experiment, sheep clearly exhibited selectivity among a range of annual legume and annual ryegrass forages (Table 1.2, Lindsay, unpublished data). The preference that herbivores exhibit between forage types is determined by many complex and integrated animal and plant factors within the grazing environment.

The preference shown by sheep between different legumes is important for essentially two reasons. First, the sheep's choice of forage may affect the productivity of the animal either positively or negatively. Second, the sheep's choice of forage may affect the productivity of the pasture either positively or negatively because species that are not eaten will be favoured, while the preferred species will be disadvantaged (Rousset and Lepart 2002). It is commonly assumed that high preference by sheep is an important attribute of legumes for grazing. In this thesis we propose that for the control of weeds of crops, and possibly to improve the productivity of the legume in a grazed pasture, a legume that is preferentially grazed by sheep is at a distinct disadvantage. We hypothesised that sowing a legume of low preference to sheep relative to the target weeds will improve the efficacy of weed control in a grazed pasture phase. We expected that incorporating a legume that sheep avoid into a pasture phase would stimulate selective grazing by the sheep toward the weeds, thereby reducing the competitiveness of the weed plants in the pasture and their subsequent ability to contribute to the weed seed bank. This dual attack on weeds from inter-plant competition and selective grazing of weeds by sheep presents a promising strategy to reduce the seed set of weeds without relying on the effectiveness of herbicides.

Table 1.2. The relative preference (α) for each of 12 different forages offered to Merino ewe hoggets at the vegetative, reproductive and senesced phases of plant growth at Allandale farm, Wundowie, Western Australia in 1998. Parallel monoculture stands (1 meter in width) were established for each forage and then grazed by sheep until about 50% of the forage was removed. Forage intake was estimated from paired pasture cuts taken before and after grazing (Lindsay, unpublished data)

Type of Forage	Phase of Plant Growth		
	Vegetative	Reproductive	Senesced
Trifolium incarnatum cv. Caprera	0.24	0.16	0.09
Trifolium subterraneum cv. Dalkeith	0.14	0.05	0.06
Lolium rigidum cv. Conquest	0.11	0.08	0.14
Trifolium glanduliferum acc. CPI 87182	0.09	0.07	0.01
Trifolium subterraneum cv. Dinninup	0.09	0.14	0.11
Ornithopus compressus cv. Santorini	0.07	0.07	0.05
Biserrula pelecinus cv. Casbah	0.06	0.08	0.03
Medicago polymorpha cv. Santiago	0.06		0.03
Medicago scutellata cv. Robinson	0.04	0.01	
Medicago scutellata cv. Sava	0.03	0.01	0.11
Lathyrus	0.02	0.16	0.19
Vicia sativa subsp. *Amphicarpa*	0.02	0.14	0.02
Neutral relative preference (α)	0.08	0.08	0.08
P-value	0.000	0.036	0.050

This strategy should also reduce the grazing pressure required to achieve successful weed control. Heavy grazing can be an effective tool to control weeds that persist in pastures under typical grazing conditions, but this is not always practical or desirable. If there is a limited number of sheep on the farm a high grazing pressure can only be applied to a relatively small area. In addition, paddocks that are grazed heavily are more susceptible to erosion and the productivity of the sheep is also likely to be compromised by highly variable nutrition. The lower grazing intensity required for the strategy proposed addresses these issues.

To test the general hypothesis that sowing a legume of low preference to sheep relative to the target weeds of crops will improve the efficacy of weed control in a grazed pasture phase this thesis consists of three sections. First, a process is described to identify a number of legumes that sheep show a low preference for relative to 2 weeds of crops. Of the legumes that were least preferred by sheep, those that were considered best in terms of meeting the criteria of a potential ‘companion’ legume were tested in the second part of the thesis. Here the legumes were incorporated into grazed pastures and their capacity to assist in reducing the seed set of the weed was assessed. Third, some of the limitations of the methods used to identify the potential legume candidates warranted further experimentation in order to develop a better understanding of the changes in preference exhibited by sheep. Changes in preference are due to the different situations/environments in which a sheep exhibits its selection, specifically its previous grazing experience.

Chapter 2

Literature Review

2.1 Introduction

Grazing behaviour and its application to the ecological management of plant populations has been widely investigated. Livestock managers and scientists alike have developed an appreciation and fascination with the function and complexity of the plant-animal interface in grazing systems. As a result of a greater understanding of this interface, tools to manage and manipulate plant-animal interactions to improve pasture and animal productivity have emerged. With the rapid development of multiple herbicide resistance in weeds of crops, it is likely that an important role now exists for new grazing management strategies and their incorporation into systems of integrated weed management. The scope of this review is to identify some potential pasture management tools, particularly to manage plants that are a component of Mediterranean-type pastures but become weeds of crops grown in rotation.

2.2 Grazed pasture systems of southern Australia

A large portion of southern Australia has been devoted to broad-acre agricultural systems. Land-use systems range from continuous cropping to permanently grazed pastures and include strategies involving combinations of crops and pastures in rotations (Puckridge and French 1983; Reeves and Ewing 1993). These systems are continually evolving to meet the challenge associated with combining the physical human and financial resources of a farm to produce a profitable outcome within a context of technological and economic change.

2.2.1 *Climate*

Southern Australia, particularly southwestern Australia, is considered to have a Mediterranean-type climate, which is characterized by mild wet winters and hot dry summers, with a total annual rainfall between 200 mm and 1000 mm (Rossiter 1966). As a result, annual pasture species are dominant in Mediterranean-type climates and the feeding value of the forage for livestock varies markedly at different times during the season (Baker and Dynes 1999).

2.2.2 *Animal Production*

The ideal pasture plant for animal production was described by Doyle *et al.* (1989) as abundant, able to sustain a high intake rate, highly digestible and comprise the appropriate nutrients to meet the animal's requirements. The seasonality in the Mediterranean-type climates of southern Australia means that annual pasture quantity and quality is highly variable and, particularly during autumn, sustaining animal production from pastures is difficult. Sheep can be expected to lose weight and have reduced wool growth rate and fibre diameter during autumn unless appropriate supplementary feeds are made available (Doyle *et al.* 1989) or pastures from the previous season of suitably high quality are conserved.

2.2.3 *Pastures*

Pasture communities in the developed Mediterranean-type regions of southern Australia are typically annual legumes, grasses and broadleaf species. A number of these plant species have been introduced, developed and established in the pasture regions of southern Australia to meet the demand for forage production from pastures. Many other species have been introduced inadvertently and persisted in favourable areas, originating from the Mediterranean basin or other Mediterranean climates (e.g. California, Chile and South Africa; Rossiter 1966).

Legumes are considered the most desirable plants to establish in a grazed pasture phase primarily because they increase soil organic nitrogen by symbiotic nitrogen fixation and in many other ways they contribute to improve the physical and chemical fertility of soil. In addition, legumes are a highly nutritious (protein and energy rich) source of forage suitable for animal production, are not a weed problem in subsequent crops, they do not support fungal diseases of cereal crops, provide good ground cover to prevent erosion and improve the sustainability of conventional cropping systems. Compared to annual grasses, the nutritive value of legumes does not decrease as rapidly as the pasture matures (Doyle *et al*. 1989).

2.2.3.1 Permanent pasture systems

Permanent pasture systems primarily support animal production and are typically found in higher rainfall areas with longer growing seasons that are less suited to cropping. Permanent pasture species must be able to tolerate grazing and set seed consistently over many years to persist in the pasture. *Trifolium subterraneum* L. (subterranean clover) has been introduced successfully to many permanent pastures in Western Australia along with some annual and perennial grass species, but broadleaf weeds and grass weeds that have been accidentally introduced are also common.

2.2.3.2 Ley farming systems

'Ley farming' systems differ from permanent pastures in that the pasture must regenerate between crops in various combinations of crop-pasture rotations. Reeves and Ewing (1993) describe ley farming as "a 'traditional'… system based on self-regenerating annual legume pastures and cereal cropping". The pasture rotation in ley farming systems complements cropping by providing a window to control weeds and diseases, while soil fertility can also be improved. A pasture rotation is generally used to produce livestock and is a potentially profitable strategy for diversifying the production from a farm.

2.2.3.3 Phase farming systems

In phase farming, pasture cultivars (generally annual legumes) that are sown to create the pasture phase are not required to persist between crop phases because they are re-established at the beginning of each pasture phase (Reeves and Ewing 1993). In such systems the length of the cropping period (often 5 years or more) is such that seed reserves of the pasture species would be depleted before they have an opportunity to regenerate a new pasture (Ewing, pers. comm.). Pasture phases are used as a break in crop rotations to improve soil fertility (Reeves and Ewing 1993) and assist in weed (Friend and Kemp 2000) and disease management (MacNish and Nicholas 1987) while facilitating livestock production. The multi-functional nature of a grazed pasture phase in cropping rotations and the development and commercialization of a number of new annual legume species such as *Ornithopus compressus* L. (yellow serradella), *O. sativus* Brot. (French serradella), *Biserrula pelecinus* L. (biserrula) and *T. glanduliferum* L. (gland clover) that suit this system has improved the economic viability of adopting this practice (Pannell 1992). In a recent model, occasional 3-year pasture phases were considered one of the most promising tools for the management of herbicide resistant weeds in cropping rotations (Monjardino *et al.* 2004).

2.3 Weed management in grazed pastures

A producer can employ two strategies when using grazing as a tool for weed management in crop rotations. Either the grazing animal is introduced to remove weeds selectively from the crop itself or from a pasture phase preceding the crop. Periods of pastures are well suited to the use of grazing to control weeds although sometimes grazing is possible within cropping or forestry systems (Popay and Field 1996). However, selectivity between crop and weed species is generally not sufficiently contrasting and most crops are readily defoliated when livestock are introduced so this option is not commonly used (personal observation). Therefore in this review only the use of grazing to control weeds in pasture systems will be reported.

In grazed annual pasture systems weed control is achieved by a reduction in viable seeds returned to the soil. Grazing affects both root and top growth in plants and depletes the plants' carbohydrate reserves, which generally reduces the plant's capacity to produce seeds (Friend and Kemp 2000). Any removal of live plant foliage by herbivores will result in reduced plant growth and indirectly reduce seed production, while grazing senesced pastures prior to the seeds being shed will result in a direct decrease in seed production if the plant material is edible.

Animal species that have been used for weed control include goats, sheep and cattle. Numerically, sheep are the most commonly used for biological control of weeds in Victoria (Amor 1987) and most likely in all other southern Australia States, and are probably the most suitable tool for weed control in grazed pasture because of their availability and effectiveness. However, different animal species vary in their effectiveness in controlling different weed types and these differences may be important in developing a range of grazing strategies for weed management. For example, goats can be used to target thorny weeds (Pierce 1990).

A pasture phase provides an ideal opportunity to manage weeds because there are many options for control. These include the use of herbicides, cutting hay or silage and grazing the pasture. In phase farming, weed control strategies that reduce seed set are not a negative cost in the last year of a pasture phase because the cultivars (generally annual legumes) that are sown to create the pasture phase are not required to persist between crop phases (Reeves and Ewing 1993).

Pastures are usually comprised of several desirable species and many less productive and desirable species, sometimes called weeds. This raises the contentious issue of defining a weed. Some species may be desirable pasture species, but weeds in crops. Annual ryegrass (*Lolium rigidum*) is a good example of this class of plant. On the other hand, some species may be unproductive in the pasture phase and considered a weed, but not pose a significant threat to the productivity of a crop grown in rotation. So, Carter's description of a weed as a plant growing where it is not desired (Carter 1990) seems more appropriate that to arbitrarily classify a plant as a "weed" without regard to where it is growing.

Grazing in pastures provides an excellent opportunity for the control of weeds in both pasture and crops and is commonly recommended as a tool of integrated weed management strategies. However, sheep graze pastures selectively and weeds are rarely eaten in preference to the desirable species in the pasture (Friend and Kemp 2000). Under these conditions weeds often persist with the dual drawback of decreasing the feeding value of the pasture, especially at the end of the season, allowing the weed population to carry over into the following crop or pasture. The persistence of weeds under grazing is generally explained by their high tolerance of grazing and/or their unattractiveness to the grazing animal. There are many examples where selective grazing has resulted in one or several plant species that are less preferred by an introduced herbivore becoming dominant because they are avoided during grazing (Harper 1969; Broom and Arnold 1986; Olson *et al.* 1996; Augustine and McNaughton 1998; Lorimer 1999). To use grazing animals to control weeds effectively, a combination of appropriate management and an understanding of the complex mechanisms of the plant-animal interface is required. Pasture plants differ in their sensitivity to grazing (Broom and Arnold 1986; Bouton 2001). The stress that is put on a plant by grazing depends on growth cycles, soil fertility and seasonal conditions (Kemp *et al.* 1996). The greatest impact on the weed plant is achieved by grazing when the weed plant is germinating, regenerating from buds or during flowering. Sheep have been shown to reduce the seed set by annual ryegrass by up to 70% by the removal of mature seed heads (Anon 1971; Gramshaw and Stern 1977).

A ‘phase’ pasture in a cropping rotation may provide an effective option to improve weed management compared to ‘ley’ pasture. Ley farming systems rely on pasture regeneration in the pasture phase and there is often a trade off between weed management and pasture seed set. The need to provide continuity of seed of the desirable species may limit the extent of seed set control of weeds in the ‘ley’ pasture. Consequently, a limited array of legumes can be incorporated into ley farming systems, as only the species that persist under heavy grazing are usually successful. The purpose of this section of the review is to outline research dealing with grazing pastures to manage weeds and to identify specific areas where additional research could improve grazing strategies for weed control in a pasture phase.

Many components that contribute to changes in pasture composition under grazing are closely linked and these linkages and interactions are outlined in Figure 2.1. To achieve favourable changes in pasture composition the pasture manager should be familiar with the natural drivers of change in pasture composition and impose management interventions to these drivers that precipitate change in the desired direction.

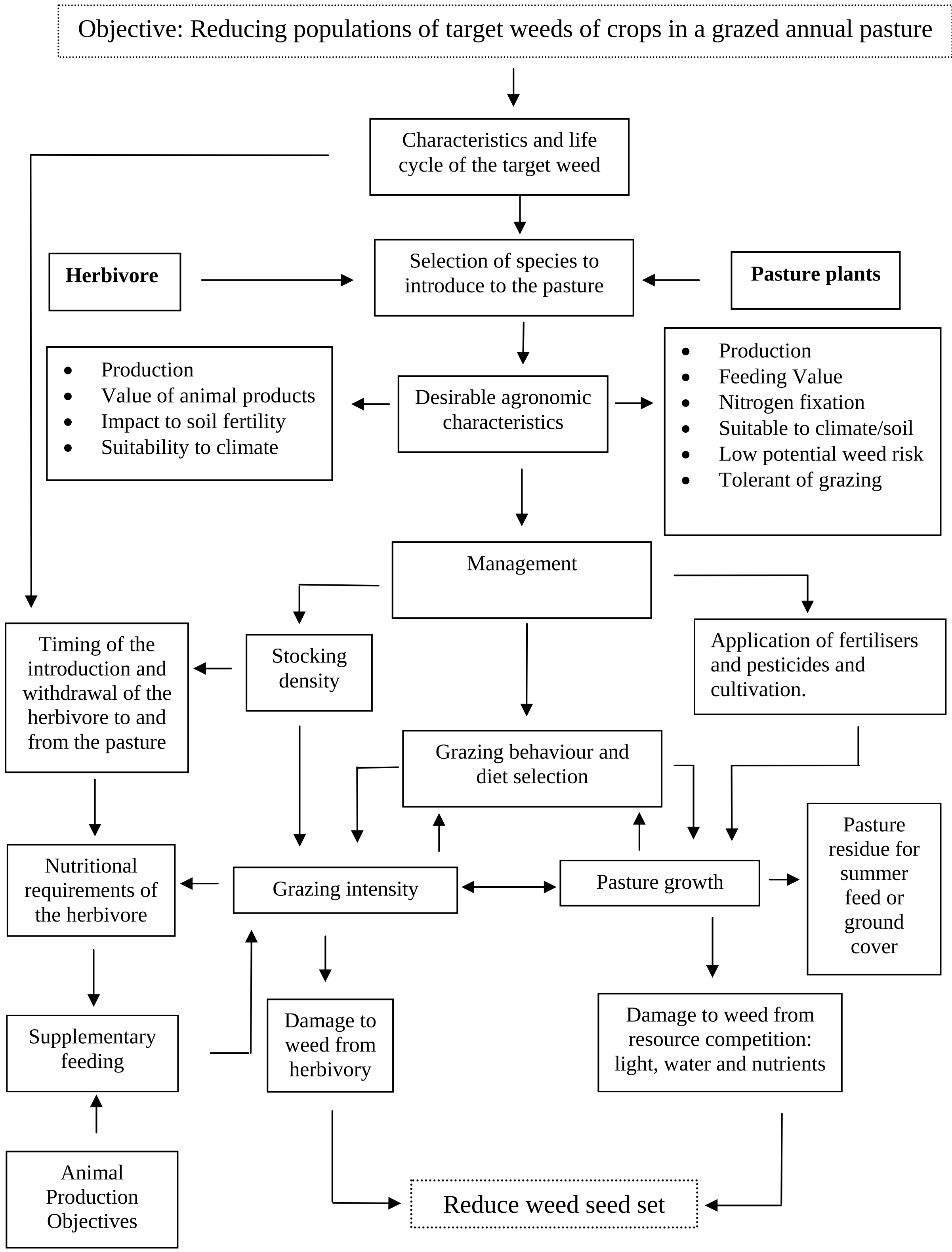

Figure 2.1. Summary of the concepts and linkages of grazing and plant competition to reduce weed seed set in a pasture, taken for the literature that is cited in the text.

2.3.1 *Natural drivers of change in pasture composition*

In a pasture, the populations of different species change within and between seasons under the influence of many interacting factors. Vickery (1981) has postulated four mechanisms that drive vegetational change in grazed pastures independent of human interventions. First, physical and chemical attributes of the plant affecting its resistance to damage by herbivores. Second, the species of herbivores that have access to the pasture and their grazing behaviour. Third, spatial distribution of plant nutrients in the sward. Fourth, the climate and the physical environment can significantly affect the botanical composition of a pasture.

Botanical composition is impacted by the grazing habit of herbivores because defoliation affects different plant species with different severity and is spatially heterogeneous (Bouton 2001). In addition, herbivores graze selectively and this favors some plant populations while disadvantaging other competing populations. The selection by sheep of their preferred forage components can markedly affect the composition of pasture species (Harper 1969; MacNish and Nicholas 1987; Olson *et al.* 1996). In general, plants that sheep prefer will be selected in the pasture and their growth and seed production will be reduced compared to less preferred plants. If this continues over a number of seasons the preferred plant species is likely to be reduced in or eliminated from the pasture.

Rossiter (1966) reported that in California ungrazed pastures quickly become grass dominant. Conversely, heavy grazing can result in a marked reduction in the grass component of subterranean clover pastures (MacNish and Nicholas 1987). Subterranean clover is uniquely adapted to grazing because of its prostrate growth habit and the position of its inflorescence relative to the leaf canopy and also because it buries its seed (Rossiter 1966). The seed production of aerial seeding legumes can be severely reduced by grazing. Some species that do not bury their seeds have inherent unpalatability in the developing inflorescence as another mechanism for resisting the damage due to grazing (Rossiter 1966).

At the animal level, selectivity and food preference varies between species, herds and individuals. Pastures with populations of the annual herb *Echium plantagineum* (Patterson's curse) can rapidly become dominant when grazed by cattle or horses only. However, Patterson's curse rarely becomes dominant in pastures that are frequently grazed by sheep (personal observation).

There is a clear effect of soil type on species persistence (Rossiter 1966). A common example among annual legumes is that *Medicago* spp. are dominant on medium to heavy textured neutral to alkaline soils, while *Trifolium subterraneum* cultivars are better suited to light to medium textured acidic soils (Cocks *et al.* 1980). Due to their symbiotic nitrogen fixing ability, legumes are more competitive than grasses and broadleaf species in nitrogen deficient soils (Cocks *et al.* 1980). Rossiter (1966) reports on the spectacular effect that trace elements can have on pasture composition. Subterranean clover can be distinctly disadvantaged in soils deficient in molybdenum, zinc and particularly potassium and phosphorous (Rossiter 1966).

Different plants have different ranges of rainfall and temperature for which they are most competitive. A common characteristic of successful pasture plants is their wide climatic tolerance (Rossiter 1966). There are differences between species in their capacity to cope with climatic stresses such as drought and extreme temperatures. Mechanisms such as hardseededness allow some species to deal better with climate variability. Variability in rainfall can affect pasture composition between seasons. Plants that mature earlier may be more competitive in short seasons. Grazing can directly delay flowering in ryegrass by one month (Davidson 1990) and the implications of time to maturity may be even more important.

Some plants are allelopathic, producing organic materials that inhibit the growth of other plants. These plants can have a competitive advantage in pastures (Leigh *et al.* 1995) and are cited as a possible mechanism for the displacement of desirable pasture species by weeds (Sanford *et al.* 2003). Root exudates from *Lespedeza cuneata* have been found to inhibit the establishment and growth of some grasses (Kalbertji and Mosjidis 1993).

Annual ryegrass has been found to have allelopathic activity and influenced the emergence and growth of Italian ryegrass, cocksfoot and lucerne (San Emeterio *et al.* 2004).

2.3.2 ***Management interventions that change pasture composition***

One of the most significant applications of research on the grazing behaviour of domestic animals has been to improve the strategies that are employed to manage plant composition in grazed pastures. The proposed benefits of implementing grazing management strategies are to increase the productivity (feeding value) of the pasture and improve soil fertility (Kemp *et al.* 1996). Management strategies that speed up the rate of change toward the desired pasture composition are the key tool that assists in making effective use of grazing management. Using grazing management for weed control involves identifying the desired species mix, particularly identifying undesirable species, and designing a grazing management strategy to change composition in that direction. Varying the timing and intensity of grazing, understanding the differing preferences for pasture components, understanding the distribution and diversity of pasture plants, understanding the reproductive cycle of the pasture plants and timing are important in achieving the desired changes in pasture composition (Kemp *et al.* 1996) (Figure 2.1).

2.3.2.1 Grazing management

To achieve effective weed management in a grazed pasture, a useful and effective herbivore is required. Given that livestock populations at a farm scale are usually fixed, the decision about which animal species should be used is perhaps less important than how best to use the animals available. Popay and Field (1996) state that "for grazing animals to be useful for weed control, such animals must be available for use, and they must be able to be fenced onto or off an area in order to adjust grazing pressure".

Managing the grazing animal involves controlling the timing, frequency and intensity of grazing (Carter 1990; Dowling *et al.* 1996; Kemp *et al.* 1996; Popay and Field 1996). Kemp (1994) states that "in practice, the tools available to producers for grazing management are the stocking rate, time and duration of grazing and time and duration of rest periods". An important additional consideration is the grazing behaviour of the animal. Grazing behaviour of individual animals or groups of animals is by no means static and could be considered as a component of pasture management decisions. With this in mind, a better understanding of the grazing behaviour of herbivores will be an important tool for the astute producer seeking to manage the pasture composition for animal production and other objectives such as weed control.

Another potential tool is to introduce additional plant species into a pasture that encourage the grazing animal to remove the target weed by shifting preferential grazing patterns. To exploit this possibility it is necessary to have a detailed understanding of the preferential grazing behaviour of sheep in relation to key pasture species and aspects of this are covered later in the review.

Forage preference depends on the animal's grazing experience as well as the animal's natural behaviour (Provenza 1995). As a result there is scope to manipulate patterns of food learning to make animals more effective weed control agents. Given the potential for field scale application of learned forage preference in weed control, under-pinning research has not received the attention that it deserves. Walker *et al.* (1992) demonstrated that familiarizing lambs with a soluble extract of the *Euphorbia esula* L. (leafy spurge) weed increased the intake of leafy spurge when they were introduced into an infested pasture. Lambs that had not been exposed to the soluble extract of leafy spurge generally avoided eating the weed. The exploitation of independent and social learning cues in sheep could be an important tool for weed management in southern Australia.

2.3.2.2 Managing the pasture plants

In addition to managing grazing, many other interventions can be imposed to facilitate desirable changes in pasture composition. The introduction of new pasture species that are more productive and competitive than the existing species is an effective tool to improve the overall productivity of the pasture.

2.3.2.3 Other tools to manage pasture composition

Selective herbicides are one of the most effective tools of manipulating composition. Many different modes of action in these herbicides mean that many weeds can be exclusively targeted by the herbicide in the pasture with a much lower impact on non-target weeds. Selective herbicides often become less effective over the longer-term as many populations of weeds readily develop resistance.

Fertilisers can have a marked affect on pasture composition and these interactions have been covered extensively by Rossiter (1966). Fertiliser application typically speeds up the rate of change in the pasture, favouring higher producing species (Kemp *et al.* 1996). In general, the application of nitrogen favours non-legume species, while the application of phosphorus favours the performance of legumes relative to grass (Rossiter 1966). However, the nitrogen fixed by the improved production of legumes when phosphorus is applied may improve the performance of grasses and other non-legumes in the longer term (Cocks 1980). Papers published in 1933 by Martin Jones describe the effects of various fertilizer applications and grazing management strategies on the composition of grazed pastures (Harper 1969). Harper (1969) reports that Jones' studies highlighted the practical implications of combinations of fertilizer and the timing and duration of livestock introductions and states that "so impressive was this achievement in agronomic practice that for years afterwards many farmers seemed to be managing their livestock to create the image of a perfectly composed pasture [reduction in grasses] rather than using their pastures to grow the most perfect livestock". To enhance the effectiveness of a pasture phase to reduce the seed set by non-legume weeds application of phosphorus and potassium, but not nitrogen should be considered.

2.4 Diet selection in sheep (and ruminants)

Voluntary food intake in sheep grazing annual pastures is the result of complex behavioural decisions by which a diet is selected that should, on average, meet their nutritional requirements. Intake is the result of bite size, biting rate and feeding time (Hodgson 1985). The intake of forage by sheep is generally not representative of the total plant material that is available in the pasture. Sheep are able to select the more nutritionally beneficial components of the available forage, and by doing this can increase the efficiency of their grazing (Provenza 1995). To achieve this, sheep exhibit forage preferences between the plant patches, plants and plant components that are available within the area to which they are confined.

The selectivity of sheep ranges from that of a mower to that of a selective herbicide and selection between pasture plants ranges from total avoidance to total intake of a single kind of forage plant. The animal's physiological status and its grazing environment influence selectivity. Food selection can be misinterpreted when considered independently of this complexity. Arnold (1981) states that "there is no doubt that failure to understand how ruminants select their food has led to enormous wasted effort in seeking 'palatability factors' in pasture plant species". An outline of the wide range of interacting factors that contribute to diet selection is given in Figure 2.2.

Although the diet selected tends to match a diet that is most suitable in meeting a sheep's nutritional needs, the cues that a sheep uses to identify preference obviously do not directly measure nutrition. Rather, the sheep has a number of sensory mechanisms that are used in differentiating between grazing options. Sheep are able to discriminate between potential forages by detecting various sensory stimuli using taste, smell, sight and touch (Arnold 1966a, 1966b). These senses interact with the sheep's 'foraging memory', which is continually reviewed each time the individual grazes, so that the sheep consumes what it perceives to be an optimal diet, within the constraints of its mental and physical ability. Dietary selection involves a complex interaction of animal behaviours where sheep readily discriminate between plant species and plant parts in a variable and constantly changing manner (Heady 1964). In this review the capacity of sheep to graze selectively, and the implications of this for grazing management, specifically, the manipulation of populations of pasture species under grazing will be discussed.

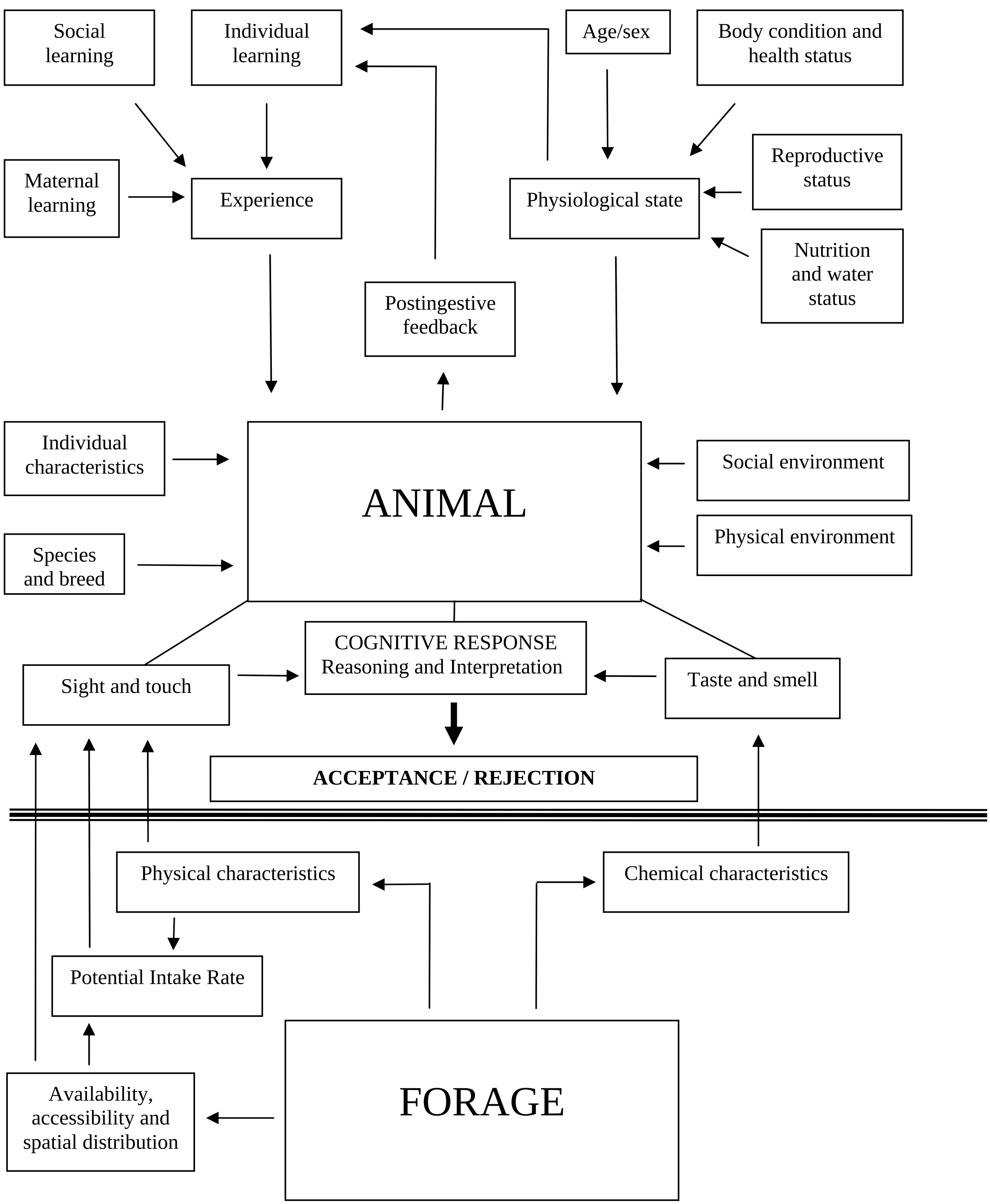

Figure 2.2. Summary of the concepts and linkages that influence plant-animal interactions in diet selection, taken from the literature that is cited in the text.

Stoddart *et al.* (1975) define preference as "the selection of plants by animals". However, the diet selected by the grazing sheep does not necessarily represent its true preference for the available forage components. Diet selection is the result of a compromise between the animal choosing the most desirable material of that available and how exclusively the most desirable components can be targeted given the amount and quality of the forage that is available and the animal's physiological status. Many other factors contribute to the forage that a grazing sheep selects from the pasture. The definition offered by Parsons *et al.* (1994) that preference is "what the animals would select given the minimum of physical constraints" is perhaps the most relevant. This definition gives two mechanisms that drive diet selection, motivation or preference of the animal among the available forage and constraints to the animal exercising its preference. However, this structure may not adequately apply to all aspects of diet selection since many components of diet preference are closely linked to the physical constraints (e.g. spatial availability), and therefore require some arbitrary means of separation. The inter-relatedness of factors contributing to diet selection can confound considerations about preference. For example, it is often reported that leaves are eaten in preference to stems but it is difficult to distinguish whether this is because leaves are preferred to stems or because they are more accessible and it is difficult for the animal to eat stems without eating leaves. Identifying the contribution of each inter-related factor is often difficult to quantify and this may confound some results.

2.4.1 ***Constraints to diet selection***

The physical environment of grazing animals is made up of many interrelated and interacting components that assist or limit their capacity to select their preferred diet. The time sheep spend grazing and their diurnal grazing pattern is affected by the climate and the amount of feed available (Arnold 1981). During summer in southern Australia sheep tend to graze in the early morning and mid to late afternoon, while in winter sheep graze almost continuously during daylight (Arnold 1981).

Sheep are most selective when the amount of forage available is neither too high nor too low. When the quantity of available forage is low and sheep fail to meet their nutritional requirements they become less selective. Hamilton *et al.* (1973) have shown that below 550 kg/ha of green dry matter, sheep fail to select a diet with a digestibility higher than that of the herbage available. When food availability is high, and typically as forage changes between vegetative and reproductive phases of growth, patch grazing by sheep often occurs (Dumont *et al.* 1995). Total herbage availability, species availability and the availability of plant components were found to be significant constraints to diet selection by sheep grazing tussock grassland (Stevens 1979). As forage quality declines sheep selection intensity increases sharply, but at very low pasture quality their selectivity diminishes (Jung and Sahlu 1986).

The gregarious nature that is common in sheep potentially reduces the efficiency by which it selects its diet from the food that is available. Gregariousness refers to “the desire to be with one’s own kind and to engage in similar behaviors” (Provenza and Balph 1988). Although to what degree gregariousness affects diet selection is unknown, it is thought that the behaviour restricts variation in dietary choice compared to animals foraging in different locations (Provenza and Balph 1988). The implications of gregariousness are likely to be most evident in environments where sheep are able to express their natural behaviours more freely (such as when grazing in a paddock) rather than in intensive systems (like a feedlot).

The balance that is made between maintaining social contact and the herbivore selecting its preferred forage is influenced by many factors. There is a trade-off between the perceived benefit of staying with the group and benefits associated with exploring forage away from the group. There are also a number of factors that influence the cohesiveness of a particular group, for example the animal’s maturity, feeding motivation, genotype, time spent in the group and the age of the cohort when it was formed. Whether an animal initiates feeding is influenced by the degree of anxiety or comfort experienced by the animal. The social environment has been found to influence the grazing behaviour and feeding preferences of sheep (Winfield *et al.* 1981; Scott *et al.* 1996). The incentive for the animal to graze away from the group is influenced by its relative preference for the forage near the flock compared to that further away. By staying within a group each individual limits its ability to explore and subsequently choose forage from its total range.

The extent of this instinct-driven trade-off has been explored by (Dumont and Boissy 2000). Increasing the distance of a preferred grazing site from the socially attractive site results in an individual sheep spending less time grazing at the preferred site and this effect is reduced when companion sheep are introduced to the site (Dumont and Boissy 2000). Group size can also influence grazing behaviour in a group. Penning *et al.* (1993) demonstrated that the time spent grazing by sheep in a group is reduced if the group size is less than four. In view of this we have ensured that in our experiments the size of groups of sheep was greater than four and where sheep were individually penned they were kept proximate to other sheep.

2.4.2 ***Forage preference***

Grazing herbivores usually have access to a range of different edible herbage options and like many grazing animals they distinguish between the food, thus displaying preference, indifference or avoidance for the potential food. For example, sheep generally prefer green leaf material in preference to stem and dead material (Guy 1981).

There are differing views on how preference motivation develops. Arnold (1981) considers diet selection to be a principally the animals' innate ability to choose on the basis of desirable and undesirable physical and chemical plant characteristics using the senses of sight, smell, touch and taste. Provenza (1995) has proposed that food preferences are the result of "neurally mediated interactions between the senses (i.e., taste and smell) and the viscera [which] enable ruminants to sense the consequences of food ingestion, and these interactions operate in subtle but profound ways to affect food selection and intake, as well as the hedonic value of food". This is referred to as postingestive feedback (Provenza 1995) and is discussed further in a later section of this review. In support of this view Gietzen (1993) observed that rats use taste as a marker to identify acceptable or unacceptable food, after their feeding preference has been previously distinguished through metabolic feedback. There is evidence to support both of these views, animals display preference between novel foods after briefly sampling them. However, it is clear that relative preference of animals between foods is also modified by the experience they gain after the ingestion of the food combined with learned eating habits, particularly while the animal is young (Arnold and Maller 1977). Provenza (1996a) incorporates inherent grazing preferences by grouping the mechanisms for aversion response in animals into three groups; sensory, nutrient and toxin specific aversions. Thus defined, sensory specific aversions would tend to be inherent to the animal, but they are likely to change with experience as the animal familiarizes itself with the metabolic consequences of eating the food. On the other hand, nutrient and toxin specific preferences rely on the animal being able to associate a specific component of its diet as the causative feedback agent and then alter its grazing behaviour appropriately.

The response of an animal to the sensory stimulus given by a food can be described simply as the degree of acceptance for a positive response or rejection for a negative response. Tribe and Gordon (1950) use the term 'palatability' to describe "the sum of the factors which operate to determine whether and to what degree a food is attractive to an animal". Food that is desirable to a grazing animal has a high palatability and food that is undesirable has a low palatability. Terms such as edibility, acceptability and attractiveness are also used to describe the differences in sensory stimulus that different forage types provide to the herbivore.

That herbivores prefer some forages and avoid others is clear. However, the appropriate terminology for describing the selectivity that herbivores exhibit when eating is as difficult as it is contentious. McClymont (1967) stated that "[The] differences in relative amounts of foods eaten by a particular animal when they are equally available, or in the absolute amounts eaten when they are the only food available, are usefully described, respectively, as the relative and absolute edibilities of the foods for those animals". McClymont (1967) believed that "describing these qualities as differences in 'palatability' or 'acceptability' as is commonly done is less preferable". McClymont (1967) reasons that the terms 'palatability' and 'acceptability' do not differentiate between whether the food preference is being measured in relative or absolute terms.

Palatability has been defined as “the overall sensory impression an animal receives from its food” (Forbes 1986), which limits the concept of palatability to being determined primarily by the inherent physical and chemical characteristics of the food. However, to be useful palatability should be understood as a combined characteristic of the plant and the animal and could be described universally as the preference for forages by herbivores. Unlike the other plant characteristics the palatability of the plant depends primarily on the perception of the herbivore. Rook *et al.* (1997) argue that because it is hard to build a case that palatability confers any survival value for the grazing animal over the longer term that it can be concluded “Palatability is a concept of limited usefulness which is best avoided in intake and diet selection studies”.

In this thesis the term preference is generally used to describe selectivity of sheep among forages as it includes the influence of animal behaviour on diet selection, rather than the inherent characteristics of the plant for which acceptance by the herbivore can be variable. Many plant and animal factors that have been found to be associated with preference have been identified and are discussed in this review.

2.4.2.1 Plant factors affecting preference for forages

Many plant characteristics contribute to a plant being either selected or being avoided by sheep. These characteristics can influence both the location at which the animal makes its selection and the selection it makes from the forage at the location. Milne *et al.* (1979) characterize this distinction by introducing the concepts of “site selection” and “bite selection”. The plants physical and chemical properties influence the animal’s site and bite selection depending on the sensory stimulus they elicit to the grazing herbivore (Baker and Dynes 1999). For example, plant chemical characteristics such as low palatability or mild toxicity can reduce their removal by grazing (Norman 1957; Burritt and Provenza 2000). Plant physical characteristics such as low accessibility (Kydd 1966) or thorniness (Cooper and Owen-Smith 1986) can reduce their removal by grazing. Hodgson (1982) states that it is usually possible to explain the avoidance of plants or plant parts by grazing animals in terms of particular chemical constituents or the surface characteristics of the plant. The digestibility, and therefore feeding value, of annual pasture plants declines rapidly as the plants mature and senesce due to changes in the chemical composition of the forage (Purser 1981). The decreasing acceptability of annual plants as they mature has been associated with this declining digestibility (Carter 1990).

2.4.2.1.1 Physical plant characteristics

Plant physical characteristics refer to the spatial, surface and structural characteristics of the plant and pasture sward and influence the physical mechanisms of food intake by the animal (collection, prehension, communition and mastication). Physical characteristics thereby determine the potential intake rate of the forage, and potential intake rate has been shown to have an independent effect on preference (Kenney and Black 1984). Spatial characteristics such as poor accessibility or low density, which are known to reduce selection of some plants by sheep, could also be classified as constraints to diet selection by the animal.

2.4.2.1.1.1 Forage availability, accessibility and spatial distribution

The accessibility of pasture plants can have either a positive or negative influence on whether they are preferred by sheep. Plants with an erect growth habit are more susceptible to grazing while plants with a prostrate growth habit are less easily grazed. The persistence of subterranean clover, capeweed and erodium under heavy grazing has been attributed to their capacity to assume a prostrate growth habit (Rossiter 1966). The degree to which the plant's growth habit affects whether or not it is grazed is affected by the physical specificity of prehension that the herbivore can apply to the available forage. Sheep are able to graze forage at a closer distance to the soil surface than cattle and therefore have a greater capacity to prehend prostrate plants.

The spatial arrangement of forage can affect selection by herbivores (Hodgson 1982). The preference and selectivity of sheep depends upon the relative distribution of preferred and less preferred plant species. Where sheep select preferred forage from among less preferred forage their intake rate is likely to be reduced and this may result in either less selective grazing or the animal selecting an alternative site. Typically, sheep will consume most of the plant material within a certain plane in mixed swards, particularly if the acceptability of the forage is not greatly different (Hodgson 1982).

Plants can be protected from herbivory because of the presence of a neighbouring plant that is avoided by the grazing animal (Rousset and Lepart 2002). A higher density of less preferred plants in a patch can result in less intense grazing of proximate plants of higher preference (Owens and Norton 1992). Protection provided by unattractive neighboring plants has also been shown for insect herbivores (Hjalten and Price 1997). However, the selectivity of insects for grazing sites is likely to be very different from that of ungulates because of their relative body size. Ungulates are not likely to have the same selective capacity therefore neighboring plants may have a greater effect on grazing selectivity.

The spatial interaction of the herbivore with its potential food has clear implications for food selection within a site and selection of a site. In a study in the African Serengeti McNaughton (1978) showed that grazing ungulates preferentially graze the more palatable grasses but the grazing behaviour the animals exhibited in order to achieve this selectivity differed markedly between species. The zebra and Thompson's gazelle were able to select the preferred grass from amongst the unpalatable grass more efficiently than the wildebeest and buffalo, that is, they had greater bite selectivity. Whereas the wildebeest herds continually moved between locations with a low representation of unpalatable plants while the movements of zebra and Thompson's gazelle was "completely chaotic" and not related to the proportion of unpalatable plants (McNaughton 1978), so the wildebeest exhibited greater site selectivity. The ungulates selected a higher quality diet of grass than the average of what was available. However, this was achieved in two distinctly different ways. While it is widely agreed that sheep exhibit both bite selection and site selection, the factors determining this behaviour are largely unknown. Ridout and Robson (1991) found that when sheep are offered alternative monoculture rows of grass and clover, more clover is selected than when sheep graze a mixed grass and clover pasture. The relative spatial distribution of the preferred and non-preferred species in combination with the physical capacity of the herbivore to select its preferred diet from what is available are likely to influence the relative importance of site selection and bite selection on total diet selection. The size and shape of the ruminant's mouth parts, particularly its incisor arcade structure, affect its capacity to graze selectively (Gordon and Illius 1988) and this capacity may influence whether the herbivore has greater site or bite selectivity (Milne 1991).

Selection by sheep for patches of the same species varies depending on height. Hutchings *et al.* (2000b) found that sward height strongly influenced the grazing decisions made by naive Texel and Scottish crossbred Greyface lambs. Hodgson and Jamieson (1981) found that sward height, and not bulk density, was an important factor in grazing preference but he conceded that to find no effect of bulk density was difficult to understand and may have reflected the overriding influence of sward height.

Plants respond to grazing by adopting a more prostrate growth habit compared to ungrazed pasture. Kydd (1966) found that perennial ryegrass (*Lolium perenne*) developed horizontal tillers after the plant had been grazed to ground level repeatedly. The prostrate growth habit of the grasses (*L. perenne* and two *Poa* species) was linked to their increase in a grazed pasture over several seasons. Annual ryegrass (*Lolium rigidum*) also adapts and grows closer to the ground surface in response to grazing (personal observation).

The relative preference of sheep between leaf and stem may vary between plant genotypes due to differences in the physical and chemical characteristics between the forage (Ru and Fortune 1999). This adds complexity to determining relative preference between whole plants, as the animal may make its choice on the basis of differences between particular components of the forage. The lower stem is generally more lignified than the upper stem in both grasses and legumes (Wilson 1993) and the lower nutritional value may reduce its preference by sheep. The proportion of the preferred plant components in the plant's total biomass is likely to vary between different plant genotypes (e.g. leaf:stem ratio) and there may be a continuous rather than discrete variation between the components. Sheep defoliate the preferred components of a plant first, and the attractiveness and therefore potential rate of intake of the remaining plant decreases. Since relative preference is affected by potential intake rate, the preference of sheep for the plant relative to an alternative could be considered continuous. Similarly, younger plant leaves have physical and chemical characteristics that are different from older leaves, and this affects preference (Bland and Dent 1964, Fontenot and Blaser 1965 cited by McClymont 1967; Wright and Illius 1995). Therefore the age of the leaves could be considered to provide a continuously changing scale of relative preference between the leaf forage. On the other hand, gross distinctions are often made between plant components in order to compare plant parts, particularly leaf, petiole and stem (Ru and Fortune 1996). In this case these plant components may be considered discrete. Because leaves are usually eaten in preference to stems the plant:stem ratio of a plant would have a considerable bearing on determining the relative preference between different plant genotypes.

2.4.2.1.1.2 Plant surface characteristics

Many plants produce sharp thorns and spines and which deter herbivory and afford the species a competitive advantage in grazed plant communities. It is likely that thorns act in two ways to deter grazing animals. First, the thorns inflict pain and have the potential to injure the animal's mouth and digestive tract (Cooper and Owen-Smith 1986). Second, in order to avoid the thorns the animal is forced to reduce its rate of intake, an important determinant of food preference (Kenney and Black 1984). The thorns of spinescent species vary morphologically including variations from straight spines (e.g. *Rhus pyroides*) to mixed hooked and straight paired thorns (e.g. *Acacia tortilis*) (Cooper and Owen-Smith 1986). The saffron thistle (*Carthamus lanatus*) is a thorny annual weed and as it matures it becomes increasingly resistant to herbivory by sheep, on the other hand goats are able to graze the thistle effectively and are suggested as a tool for weed management (Pierce 1990). It is suggested that the decrease in the acceptability of annual plants with maturity might be partly due to troublesome seed-heads, e.g. *Hordeum leporinum* (barley grass) and *Erodium moschatum* (Carter 1990).

2.4.2.1.2 Chemical plant characteristics

The chemical attributes of forage are often linked to their preference by herbivores. This is not surprising since the chemical composition of forage ultimately determines its nutritive value. Chemicals contained in forage and their capacity to be taken up by the herbivore for use as energy or building body tissue determine the feeding value of the forage to the animal (Baker and Dynes 1999). Irrespective of how the sheep detects feeding value, sheep generally do not prefer poorly digestible foods that are low in energy (Provenza 1995). In general terms, sheep avoid forage with a low feeding value, particularly when their average diet does not meet their nutritional requirement. Feeding value is defined as the production response by animals when feed available is non-limiting (Purser 1981).

The chemicals contained in forage also enable the animal to recognise and distinguish between forage types creating an olfactory stimulus that the herbivore can taste and/or smell. Specific chemical compounds may singularly contribute to the acceptance or rejection of forages (Gherardi and Black 1991). Examples of compounds known to increase preference include soluble carbohydrates, protein, salts and those known to decrease preference include condensed tannins, cyanogenic glucosides and alkaloids. At very high concentrations, compounds that are commonly measured to be attractants can become aversive (Arnold 1981). This supports the view of Provenza (1995) that energy and nutrient balance is important and when consumed in excess or deficit the animal receives metabolic signals to modify its diet (Figure 2.3).

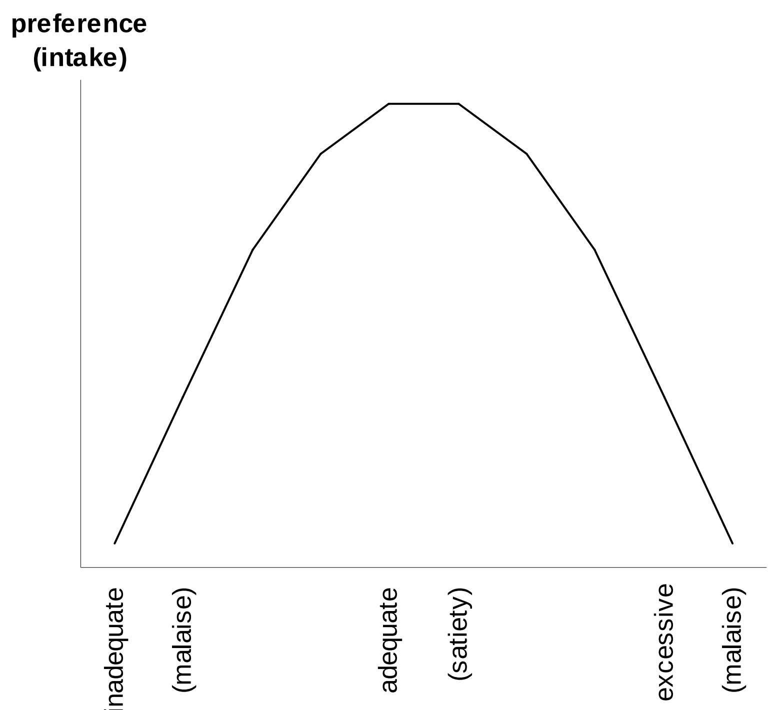

Figure 2.3. Intake of forage is depressed if the forage contains either inadequate or excessive levels of a compound. Forage that has an adequate nutritional balance is preferred. Adapted from (Provenza 1995).

Concentrations of nitrogen, water soluble carbohydrates, fibre and digestibility have been linked to diet selection in sheep. Lambs have been found to select for a specific protein intake of around 14 to 17 % (Kyriazakis and Oldham 1993). The ability of sheep to select for their preferred protein concentration was linked to improved growth. Sheep offered a choice of high and low protein foods grew more quickly than those offered a single feed (Kyriazakis and Oldham 1993). However, for sheep grazing pastures “the higher relative edibility of high-protein, high-energy pasturage cannot be interpreted as evidence of innate ability to select for nutrients, since such material is also probably more palatable" (McClymont 1967). Although this view may be confounded because higher palatability may be a result of the animal’s grazing experience and therefore be linked to the animal’s innate ability to modify its selection to improve the nutritive value of its diet. Forage preference of sheep for grasses can be increased by the application of a nitrogen fertiliser (calcium ammonium nitrate) and thereby increasing plant nitrogen (Edwards *et al.* 1993). Plants grown in leached soils with poor nitrogen fertility are less preferred by sheep than plants grown in soils with higher nitrogen levels (Hutchings *et al.* 2000b). However, if nitrogen intake is excessive, sheep may then select foods lower in nitrogen to balance their nitrogen intake, if these foods are available (Kyriazakis and Oldham 1993). Sheep have been found to strongly reverse their preference from clover to grass when they were infused with ammonia (Cosgrove *et al.* 1999), although the severity of this treatment meant that this was measured in only two sheep in the treatment group, which were considered to be in a suitable state to continue the experiment. Despite the experimental complications, this suggests that sheep may have the capacity to regulate protein intake to avoid excessive levels of rumen ammonia. Therefore, we would expect sheep to be most selective for high nitrogen feeds when the average nitrogen of the plant material available is lower than its requirement or if the sheep has been eating a diet deficient in nitrogen.

The concentration of non-structural carbohydrates in plant material has been linked to sheep preferences. Sheep that are offered forage that differs only in its non-structural carbohydrate composition select the material with the highest concentration of non-structural carbohydrate (Michell 1973; Ciavarella *et al.* 1998; Fisher *et al.* 1999). Spraying pastures with glyphosate increased the concentration of water soluble carbohydrate in ryegrass stems and treated pastures are grazed preferentially by sheep (Leury *et al.* 1999). Similarly, the total non-structural carbohydrates of an annual ryegrass-silver grass pasture that was spray-topped with glyphosate was higher than non spray-topped pasture and sheep exhibited a preference for the spray-topped forage (Siever-Kelly *et al.* 1999). However, subsequent to spraying, the pasture increased in nitrogen and digestibility so it is unclear whether one or a combination of these factors increased its preference by sheep (Siever-Kelly *et al.* 1999). Sheep preferred *Phalaris aquatica* L. forage with higher water soluble carbohydrate levels (126 *v* 62 mg/g DM) in shaded *v* unshaded pastures that only differed significantly in water soluble carbohydrate concentration (Ciavarella *et al.* 1998).

The level of total non-structural carbohydrates in grasses varies during the day and is highest at some time in the afternoon (Fisher *et al.* 1999). Ciavarella *et al.* (2000b) found that the sucrose and starch carbohydrate components increased most markedly during the day. This has been attributed to the accumulation of sucrose in the leaves during photosynthesis. The eating preferences of sheep have been observed to coincide with the subtle differences in total non-structural carbohydrates in plant material that have been recorded diurnally (Fisher *et al.* 1999). Harvey *et al.* (2000) found that the intake of clover by sheep remains relatively constant throughout the entire day, whereas the intake of grass varies widely throughout the day, peaking in the mid to late afternoon. This may be related to greater diurnal variability in total non-structural carbohydrates in grass compared to clover.

Ruminants are also known to select among forages for specific macronutrients. The acceptability of pastures fertilized with sodium has been found to increase the acceptability of forage to dairy cows (Chiy *et al.* 1998) and sheep prefer pastures fertilized with phosphorus (Ozanne and Howes 1971). However, this may be confounded by the higher digestibility of phosphorus fertilized pasture (Missen 1991).

Many legumes and some grasses contain secondary compounds that reduce their intake by grazing herbivores (Baker and Dynes 1999). The current literature on the instances of interactions between plant secondary compounds and herbivores has been reviewed recently (Hegarty 1982; Bryant *et al.* 1992; Smith 1992; Craig 1995; Reed 1995). The specific compounds involved are difficult to identify because of the large number of these compounds that are synthesized by plants. Francis (1973) suggested that the lack of β-glucosidase was associated with low palatability in a mutant strain of subterranean clover. The absence of β-glucosidase was suggested to result in the sheep ingesting higher levels of unhydrolysed glycosides and that this mechanism may explain the reduced preference for subterranean clover cultivars that have high levels of isoflavones (such as Dinninup and Yarloop). Secondary compounds can act to reduce the herbivores preference for the forage by reducing its palatability due to an objectionable taste or smell, or by stimulating a negative postingestive experience when the animal eats the forage and thereby indirectly influencing preference by initiating an aversion response. Adverse affects in the herbivore that ingests plant secondary compounds vary in their intensity and concentration required to cause toxicity. The effect can occur shortly after feeding or result from a cumulative build up of a toxin over a much longer period of time (Baker and Dynes 1999).

2.4.2.2 Animal factors affecting forage preference

Although animals have some capacity to select forage on the basis of the inherent chemical and physical characteristics of the plant material, there is an increasing body of work supporting the view that learning combined with physiological and behaviour interactions contribute to preference in sheep. Some useful tools of grazing management have been developed from a better understanding of these processes. The animal factors affecting preference are an additional driver of diet selection acting to influence selection in conjunction with, rather than independently of, the plant factors.

2.4.2.2.1 Morphophysiology/species/breed

The adaptation of species of herbivores to diets that vary widely, representing the differing ecological niches that they occupy, is reflected in their differing feeding preferences. Morphopysiological variations and adaptations are related to diet selection among ruminants (Hofmann 1989). Hofmann (1989) classifies these variations according to three feeding types; concentrate selectors, intermediate mixed feeder and grass and roughage eaters. Ruminants that exhibit greater selectivity (concentrate selectors) tend to have a larger but narrow mouth, mobile lips and large salivary glands. While grass and roughage eaters have a wider mouth, thicker tongue, heavy jaw muscles and a larger rumen (Hofmann 1989). Therefore sheep could be considered grass and roughage eaters, despite clearly exhibiting grazing selectivity. Between genus (Mills 1977), species (Aldezabal and Garin 2000) and probably breed, ruminants exhibit different feeding preferences.

2.4.2.2.2 Reproductive status

Both the increased nutritional requirements and decreased rumen capacity of pregnant ewes are known to influence their forage preference. The uterus of a pregnant ewe can account for up to 70% of the total glucose turnover of the animal (Silver 1976). During the final two months of pregnancy ewes select feeds with higher levels of protein, provided the food has a high metabolisable energy (Cooper *et al.* 1994).

2.4.2.2.3 Age/sex

There is some evidence to suggest that the period of time where livestock learn diet selection most efficiently is during weaning. After weaning the animal's willingness to sample unfamiliar foods decreases (Provenza and Balph 1988). The experience of the foetus through the mother's diet is also subject to speculation, and is currently being investigated in sheep. Selective grazing behaviour in adults is thought to be more stable compared to that of young herbivores (Provenza and Balph 1988).

2.4.2.2.4 Feeding motivation (appetite)

Appetite or the degree of hunger or satiety influences the food selection of grazing ruminants. Fasting affects the intake rate and grazing preferences of sheep (Newman *et al.* 1994; Villalba and Provenza 1999). Newman *et al.* (1994) found that fasted sheep spend less time grazing clover compared to non-fasted sheep, but differences in grazing preference were typically small and often not significant (Newman *et al.* 1994). It is a widely held view that animals with a greater feeding motivation tend to be less selective and conversely that animals become more selective as they reach satiety [Ivlev 1961, cited by Jung and Koong 1985]. However, evidence on the effect of hunger on selectivity is not consistent. Newman *et al.* (1994) found that fasted sheep increase their intake of grass relative to clover, while Hutchings *et al.* (2000a) found that fasted sheep selected a higher proportion of clover relative to grass. Hutchings *et al.* (2000a) suggested that this apparent contradiction could be explained by the length of time over which the experiments were conducted. The study by Newman *et al.* (1994) was conducted over several days compared to the longer term study by Hutchings *et al.* (2000a). In another study, Jung and Koong (1985) found that the composition of samples collected from esophageal fistulated sheep was the same as unfasted sheep, but the fasted sheep that were subsequently fed a high quality feed (corn 45% and alfalfa hay 45%) selected pasture of about 5% higher crude protein at the 2 highest levels of supplementation. Owing to the variable effect of fasting on selectivity, we have avoided fasting sheep for extended periods prior to determining preference in experiments reported in this thesis.

2.4.2.2.5 Diet diversity

Where ruminants have a choice of diet between a number of forages, it is uncommon that only one forage will be grazed exclusively. For example, sheep generally prefer white clover to perennial ryegrass, but they prefer to include ryegrass in their diet rather than to exclusively eat clover (Penning *et al.* 1997). Sheep were also found to prefer ryegrass when grazed exclusively on clover on the previous day (Newman *et al.* 1992). An extensive review of the herbivores preference for a varied diet is presented by Provenza (1995), who suggested that animals may develop mild short-term aversions to nutritious forages that are ingested as a high proportion of their diet.

2.4.2.2.6 Stocking density

Changing the density of stock on a pasture can affect the forage selections made by sheep. It has been suggested that changes in social interactions resulting from different stocking density contribute to changing grazing habits (Peyraud *et al.* 1996), but there are few studies that have measured such interactions and related these to grazing behaviour. Differences in diet selection that are related to changes in stocking rate are principally due to differences in overall forage depletion. Rotational grazing at higher stocking rates has been found to reduce grazing selectivity in sheep compared to set-stocking (Sharrow 1983). This was probably mostly due to the increased pasture depletion under rotational grazing. However, Harvey *et al.* (2000) found that by altering the stocking density in a pasture containing two monoculture blocks of pastures, one of perennial ryegrass and one of white clover, the selections made by the sheep changed. As stocking density is increased the time sheep spend grazing on clover is increased and less time is spent grazing on ryegrass (Harvey *et al.* 2000), and this change in diet selection appears to be independent of forage depletion. The implications for our research are that forage depletion, rather than social interactions, are important in relation to stocking density.

2.4.2.2.7 Individuality

Individual sheep vary widely in their preferential grazing behaviour. For example, the voluntary intake among forages has been shown to vary for the proportion of green material selected by sheep (Arnold 1964) and the relative preference of lambs between alfalfa and barley (Scott and Provenza 1999). The wide variability in the preferences of individual sheep makes generalising about their forage preferences difficult. To use knowledge of the preferences of sheep to aid pasture management decisions, generalizations are necessary, and they are useful if accurate. The difficulty is that the plant populations that make up a sward of pasture are affected by the combined interactions of the entire flock, which is effectively the average sheep. However, the importance of individual preference is emphasised by some authors. Scott and Provenza (1999) contend that the characteristics of the ‘average’ animal are over emphasized due to the application of statistics and that this has obscured the importance of variation among individuals. Provenza *et al.* (2003) believe that if all the animals in a group are viewed as having identical needs, that of the ‘average’ animal, this may be detrimental to animal production systems and that the overall herd performance is likely to be improved if the individuals are offered a more diverse diet through which the individual animal’s preference can be exhibited. Essentially, Provenza *et al.* (2003) suggest that animals, given a diverse diet, will select their own optimal diet and there is evidence that this may vary widely from individual to individual (Distel *et al.* 1996). Animals offered limited feeding options suited to the ‘average’ animal may under-perform but food intake may be depressed by “transient food aversions” when food choice is limited to a single feed (Provenza *et al.* 2003). If we accept these viewpoints the variability in the food preference of individuals may have significant implications for animal production, but may not be so important to consider for pasture management.

2.4.2.2.8 Parasitism

Sheep are able to identify fecal material and thereby avoid ingesting parasites from contaminated areas of forage (Hutchings *et al.* 2000a). In addition, sheep that are subject to parasitism have a greater aversion to faecal contamination than those that are not (Hutchings *et al.* 2000a). Furthermore, Hutchings *et al.* (2000a) found evidence that the level of parasitism in sheep is associated with forage selection. Sheep need their sight to avoid parasites as applying blinkers to them can reduce their selection against dung patches (Arnold 1966a).

2.5 Grazing experience and diet selection

The prior grazing experience of herbivores is an important animal factor contributing to diet selection. It is considered separately in this review because of its significance in the study of the selective grazing behaviour of sheep and its importance in this study. The forage preferences of sheep are not static, they are subject to ongoing change in relation to grazing experience and dietary learning (Provenza 1995). Sheep to discriminate between forages from their mother, other sheep and their own grazing experience. As a result the preference of naïve sheep often differs from that of sheep familiar with grazing a particular forage.

The influence of prior experience is integrated with the physical and chemical characteristics of the forage because of the sensory and digestive feedback signals that these characteristics give. Herbivores respond innately to different chemical stimuli and can therefore make immediate or ‘short-term’ grazing decisions based on the chemical characteristics of forage (Laing 1985). However, there is strong evidence that postingestive chemical feedback influences forage preference over the ‘longer-term’ and that this feedback can over ride the initial (short-term) preference (Provenza 1995). So sensory feedback, postingestive feedback, or both may act to increase or decrease preference.

Many other factors complicate the degree to which the animal's previous experience affects its preference for a particular type of forage. For instance, the proportion of the forage in the animal's diet, aversion responses and maternal training of the animal during rearing affect food choice to varying degrees (Distel and Provenza 1991; Provenza 1995). These factors all contribute to the attractiveness of various forages to sheep and can over-ride innate taste or smell-based selection to some degree (Rook *et al.* 1997). Sheep grazing plants with toxic secondary compounds reduce their intake to avoid toxicosis (Provenza 1995). When no alternatives are available the total food intake of the herbivore can be depressed (Burritt and Provenza 2000). The feedback from nutrients and toxins has a complex but powerful influence on the way the individual animal interacts with its potential food and their influence is only beginning to be understood (Provenza *et al.* 2003). The effect of the animals grazing history on food preference is still not widely understood and this field lends itself to many areas of further study. To carry out valid investigations of grazing preferences the strict control and recording of the animal's grazing history is critical to ensure that some previous plant-animal interaction does not bias the result.

2.5.1 *Individual learning*

The history of an individual's diet can have a wide range of effects on the current grazing preferences of that individual (Thorhallsdottir *et al.* 1987). As a result the animal is able to adapt to select a more nutritious and less toxic diet from what is available. In many instances this ability is critical to the animal's survival as it has a diverse and changing range of vegetation from which to meet its nutritional requirements.

2.5.1.1 Postingestive feedback

Metabolic feedback to the animal via the central nervous system is known to play a critical role in modifying the animal's preferential grazing behaviour so that an appropriate balance of nutrients and energy is made available to the animal. Anil *et al.* (1993) and Mbanya *et al.* (1993) cited by Provenza (1995) suggest that various chemo-, osmo- and mechano-receptors in the body may be responsible for linking metabolic signals received during digestion, to the central nervous system, thereby inducing changes in eating behaviour. An example supporting this view is that lambs have been shown to develop an aversion to food containing imbalanced proportions of amino acids (Rogers and Egan 1975).

2.5.1.2 Aversive behaviour in sheep

Food aversions in grazing herbivores are a product of associating specific forages with negative postingestive feedback. Herbivores can develop an aversion within minutes, alternatively this response may also take weeks to develop Garcia (1989) cited by Provenza (1996a). Herbivores can identify forage through sensory mechanisms (taste, smell, touch and sight), and learn to associate the sensations with the metabolic consequences of eating the food (Provenza 1996a). The concept of food aversions was also suggested by McClymont (1967) who recognized the probable avoidance of toxic plants resulting from the herbivore associating ingestion with intoxication. Aversions to forage containing toxins or a nutrient imbalance are the most common. In addition, sheep and cattle frequently choose an alternative food than that previously offered and this preference for a varied diet is thought to be the result of mild short-term aversions to a previous meal (Provenza 1996a). This may be a mechanism by which an animal is motivated to diversify its diet, which has advantages in that it reduces the risk to the animal that a particular forage may not fully meet its nutritional requirements. Animals can develop aversions to feeds of normally high preference. Sheep have been found to develop a strong aversion to barley when they eat a large amount (Phy and Provenza 1998).

There are a wide variety of plants and plant compounds that have been found to induce food aversions in herbivores, some of these are specifically identified by Provenza (1996a). Lithium chloride is perhaps the most well known emetic compound that produces strong aversions in livestock.

Emetic compounds can be used artificially to induce food aversions associated with a particular food, particularly with novel foods (Ralphs 1992). Conditioned food aversions have been investigated in grazing animals as a management tool either to induce animals to avoid palatable but harmful plants or to avoid damaging plants not intended for grazing (e.g. trees). An example of this has been with the poisonous plant *Delphinium occidentale* (tall larkspur), a palatable plant containing diterpenoid alkaloid toxins that grows in rangeland areas of the United States that is acutely toxic to cattle (Pfister *et al.* 1990). However, cattle apparently do not develop natural aversions to tall larkspur (Ralphs 1992). Cattle that had grazed tall larkspur-infested rangeland as calves, were offered fresh cut tall larkspur plants and were administered an emetic compound whenever they ate the larkspur. The emetic was lithium chloride and each dose (100 mg/kg body weight of an 8% isotonic solution) was administered directly into the rumen through a tube running through a blind rumen fistulae. Total aversion to tall larkspur was achieved after 2-3 infusions of lithium chloride and the condition persisted for at least one year (Ralphs 1992).

Artificially induced aversions are attenuated in some circumstances. If animals with a conditioned food aversion graze together with animals without the aversion the aversion can be extinguished (Lane *et al.* 1990). Hunger can reduce the effectiveness on an aversion (Grote and Brown 1973), (Wellman and Boissard 1981) cited by Ralphs (1992). Where an animal has no alternative but to eat the forage for which it has a conditioned aversion the animal is more likely to eat the forage and the aversion may then be attenuated (Kimball *et al.* 2002). Food aversions can be elicited in deeply anesthetized sheep demonstrating that the mechanisms that trigger the aversive response are independent of the animal’s cognitive behaviour (Provenza *et al.* 1994).

2.5.1.3 Familiarity/ Neophobia

Sheep are neophobic; they prefer to eat foods with which they are familiar and their previous experience contributes to their degree of neophobia (Thorhallsdottir *et al.* 1987; Burritt and Provenza 1989). Sheep will usually follow a typical pattern when incorporating a novel feed into their diet where they gradually increase their intake of the food if the gastrointestinal consequences are not adverse (Provenza and Balph 1988). However, many other factors impinge on this model of response to novel foods. Where sheep are offered a pasture containing many novel foods simultaneously they are unlikely to restrict total feed intake until they have learned the postingestive consequences of what they have already eaten. Furthermore, postingestive consequences depend on the quantity of a particular plant eaten, so sheep that have eaten a small amount of a particular forage on one occasion are likely to perceive the forage differently to sheep that have eaten the forage as part of a mixed diet over a long time. In addition, sheep that eat a diet containing one forage type may be motivated to increase the diversity of their diet (see 2.4.2.2.5). Animals reared on a particular pasture (Arnold and Maller 1977), or offered a forage during weaning (Ramos and Tennessen 1992), tend to display a preference for the familiar forage if they are offered it at some later stage.

2.5.2 *Social and maternal (Allelomimetic) learning*

The social transmission between animals in reference to their grazing behaviour has been reviewed by Nicol (1995). The preferences of the offspring are affected through a number of mother-young interactions during their nurture. Even before the young begins to imitate its mother's diet through observational leaning, flavour associations are transferred in utero across the placenta and through the parent's milk (Provenza *et al.* 1992). When the young begin to eat the same foods as their parents they are able to copy preference and avoidance behaviour. Food avoidance in sheep is indirectly mediated by the behaviour of the ewe (Thorhallsdottir *et al.* 1987; Mirza and Provenza 1994).

2.6 Measuring relative preference

A number of methods to determine relative preference have been applied across a range of feeding environments and situations. This partly reflects the difficulty in quantifying the composition of the grazing animal's voluntary intake, but also the diversity of research questions being addressed and the range of environments for which it is useful to determine diet selection. When determining diet selection there is a clear trade-off between achieving precise measurements and disturbing the animal's grazing behaviour. To determine preference usefully the investigator should be aware of the impact of their experimental method on behaviour.

In the many studies where selective grazing behaviour has been investigated there are a number of core issues that have been considered. These are; the environment (physical and social, i.e. access to conspecifics), the type and preparation of the forage to be offered, the method of quantifying intake composition and the method of calculating selectivity and preference from the intake composition. Similarities in methods typically coincide with similarities in the hypotheses being tested, therefore like studies can be usefully grouped by their key attributes.

2.6.1 *Environment*

To determine relative preference the animal's grazing environment is designed to meet a number of objectives. The environment must contain the forage options in a form and quantity useful for measuring diet selection. The animal should resume typical eating behaviour. For example, voluntary food intake ideally should not be reduced as a result of stresses such as changes in physical and social environment or from the method of measurement.

The design of the environmental conditions depends on the research question being addressed. Applied research questions are typically best answered where the environment closely matches a production environment. For example, if the animal's capacity to graze selectively is important the investigator may choose to offer feeds in a mixed sward. However, if the researcher wishes to determine a change in the animal's preference as a result of a treatment, it may be useful to offer separate stands of the forage to enhance the selective ability of the animal and increase the sensitivity of the study to detect a significant difference. Environmental design also depends on the commercial grazing environment of the experimental animals. To address research questions related to rangeland grazing systems, a researcher may determine browsing selection among a number of shrubs.

Indoor diet selection studies, generally with individually penned animals e.g. (Cooper *et al.* 1995), are commonly used to improve the control and statistical validity of preference studies (Dumont and Petit 1995). However, a drawback of indoor studies is that the experimental animal must adapt to atypical surroundings and social structure. Gregarious animals that are kept isolated are predisposed to behavioural inhibition, which may be relieved by the presence of their conspecifics (Clayton 1978) in (Nicol 1995).

2.6.2 *Social structure*

Social interactions influence the diet selection of sheep (Nicol 1995). Therefore the social structure of the animals should be considered in the design of experiments to determine preference. Diet selection has been measured in large groups of animals (≥ 20) (Glimp 1971; Mills 1977) small groups of animals (< 20) (Mills 1977; Scott *et al.* 1996; Penning *et al.* 1997; Shinde *et al.* 1997), paired animals (Ramos and Tennessen 1992) and individually penned animals (Hutson and van Mourik 1981; Kenney and Black 1984; Cooper *et al.* 1995; Provenza *et al.* 1996; Cosgrove *et al.* 1999; Fisher *et al.* 1999; Villalba and Provenza 1999; Burns *et al.* 2001). However, in general, individually penned animals do not graze devoid of social interaction, as other experimental animals are proximate. Individual pens vary widely in their design (e.g. size, elevation, materials, lighting), and these differences are likely to affect the animal's level of anxiety. Diet selection has been reported in animals isolated from their conspecifics (Krueger 1972; Dumont and Petit 1995). No one approach is uniquely valid, but each is likely to have strengths and carry constraints to interpretation.

2.6.3 *Feed preparation*

The preparation of a feed affects its chemical (Colebrook *et al.* 1990) and physical (Kenney and Black 1984) characteristics. Kenney and Black (1984) showed that sheep increased their intake rate of hay or straw when it was cut in shorter lengths, and Villalba and Provenza (1999) have found that sheep preferred whole rather than ground foods. The range of methods of feed preparation cited is partly due to the number of feeds and diversity of methods used to assess preference.

Feeds are generally offered as standing swards in the field or harvested plant material, which can undergo a range of treatments (e.g. drying, milling, pelleting). Alternatively, turfs of pasture may be prepared and offered to animals in indoor studies (Newman *et al.* 1992; Dynes 1993). Sometimes commercially available feed products used in the evaluation of preference. For instance, Hutson and van Mourik (1981) offered sheep a range of commercial foods (breakfast cereals, fruits etc.).

Pasture swards are commonly established to determine preference, usually during the plants growth phase for annual species (Heady and Torell 1959; Arnold *et al.* 1966; Krueger 1972; Guy 1981; Ramos and Tennessen 1992; Orr *et al.* 1997; Penning *et al.* 1997; Shinde *et al.* 1997; Ciavarella *et al.* 1998). Mills (1977) determined grazing preference for ewes and cows in pasture re-growth where the pasture was initially cut to 75 mm and allowed to regenerate. Preference for herbivores grazing rangeland vegetation is typically determined in unimproved native vegetation (e.g. Aldezabal and Garin 2000).

As an alternative to offering growing swards, pasture forage may be collected and prepared, which is generally the case for indoor preference studies. Dunlop (1986), Walker *et al.* (1994) and Cosgrove *et al.* (1999) offered sheep pasture forage that was cut fresh daily. Fisher *et al.* (1999) offered sheep, goats and cattle hays that had been cut previously and kept in undercover storage (length of time not specified). Kenney and Black (1984) offered sheep pasture and crop forage that was cut and oven dried at 50°C. Burns *et al.* (2001) offered sheep 8 cultivars of tall fescue hay which were cut, baled and stored in a building prior to feeding. Colebrook *et al.* (1990) harvested subterranean clover forage from monoculture plots, which were snap frozen with liquid carbon dioxide and maintained at -5°C until freeze-dried. After drying, the material was cut into 25 mm lengths and stored at -5°C until it was used. Studies by MacRae (1970, 1975) indicate that freeze-stored herbage would have had minimal impact on digestive physiology provided the material was fed within 24 hours after thawing. This does not account for the olfactory stimulus, which is an important component of the feed selection process. Siever-Kelly *et al.* (1999) considered that this method might also alter the digestible dry matter intake over time.

Concentrate feeds are commonly prepared in a wide range of forms and offered in preference tests. Scott *et al.* (1996) offered sheep feed-grains (wheat and milo). Provenza *et al.* (1996) offered sheep diets comprised mostly of ground grains and seeds. Glimp (1971) and Cooper *et al.* (1995) offered sheep pelleted feed comprised of varying proportions of feed ingredients. Pelleted feeds have several advantages including reduced selection among the feed components, ease of handling and once made the feed will remain ubiquitous, for example it will not settle by density when it is transferred.

Where preference differences among green pasture plants are being investigated it is preferable that they be offered in the live state. While experimental logistics might mean this is not always possible, Colebrook *et al.* (1990) suggest that the rapid changes that occur in herbage after it is cut may lead to inaccurate representation of the animal's preferences. Therefore, it seems preferable to offer forage in the same form as it would be available under natural or commercial conditions. Where this is not possible, changes in the physical and chemical properties in the feed should be considered when evaluating preference.

2.6.4 ***Methods to determine intake composition***

Quantifying the intake of grazing animals is necessary to determine preference and has wider application in estimating feeding value and to understand better the mechanisms that regulate voluntary food intake. Techniques for estimating the composition of the diet of a grazing animal have typically been inadequate or difficult and laborious to use (Dove 1996). The methods are selected to suit the environment and situation for which diet composition must be determined. In developing methods there is commonly a trade-off between being able to take precise measurements of intake and imposing changes to the animal's normal grazing environment. For example, observational studies reduce disturbance to the animal's behaviour, but are less convincing because direct quantification of intake is not made. Whereas indoor studies may be adequately controlled and allow greater precision in measurements but may interfere with the animal's natural grazing behaviour.

2.6.4.1 Associational observations

An animal's position and eating movements during grazing are indicative of its feeding preferences and are widely used in preference studies. Hutson and van Mourik (1981) offered paired feeds of equal volume (500 cm^3) and measured the number of feeding bouts using a video recorder. Shinde *et al.* (1997) and Penning *et al.* (1997) continuously observed one sheep in a flock to make a visual assessment of its diet selection. Ramos and Tennessen (1992) introduced sheep to a paddock containing alternating strips of white clover and ryegrass and observed the position of the sheep and whether or not the animal was grazing. Dumont and Petit (1995) placed barriers between one feed source while the animal had ready access to an alternative, preference was determined by recording the walking time and length of feeding bouts. Harvey *et al.* (2000) offered sheep two monocultures and recorded the time spent grazing on each sward. Sensors that record jaw movements have been used to estimate time spent eating, ruminating and idling (Penning *et al.* 1997).

Another technique to subjectively measure intake is to identify evidence of grazing on individual plants. Using the point-centered quarter method individual plants along a transect were checked for evidence of recent browsing (Robles 1988) cited by (Aldezabal and Garin 2000). The proportion of browsed leaves and/or twigs on each plant was then recorded.

2.6.4.2 Feed biomass

The estimation of intake by measuring relative feed abundance before and after access to grazing is the most common method used to determine preference. The feed options are offered to the experimental animals either for a specified time or until the desired depletion in the total feed biomass offered is achieved. Due to the wide variety of forms in which feed is offered, feed abundance is measured or estimated by a number of methods.

Walker *et al.* (1994) and Cosgrove *et al.* (1999) offered sheep fresh cut pasture species from paired feeders and estimated intake by weighing refusals. Offering feeds from identical paired feeders creates an equal probability of the experimental animal selecting from either feeder unless the animal exhibits selectivity. Fisher *et al.* (1999) offered sheep, goats and cattle hay from paired feeders; for the cattle a video recorder was used to determine feeding bouts so that rate of intake could be estimated. Glimp (1971) offered groups of sheep four pellet types concurrently from feeders divided into four sections, daily feed refusals were weighed to estimate the total group intake. Cooper *et al.* (1995) offered pelleted feeds to individually penned sheep from two feed troughs.

Kenney and Black (1984) offered paired oven-dried pasture collections to sheep in separate containers for eight one-minute intervals. The amount of feed consumed during each one-minute interval was measured. Colebrook *et al.* (1990) offered paired freeze-dried pasture collections to sheep in separate containers for four one-minute intervals. In both studies the position of the feeders was reversed between each interval. Villalba and Provenza (1999) offered sheep paired feeds for 5 minutes and measured the food consumed and feeding times for each feed. Scott *et al.* (1996) offered sheep grazing pasture two supplements from two feeding troughs and measured the quantity removed from each by weighing the feed refused, in addition to recording the location and activity of the animals at intervals. Provenza *et al.* (1996) offered sheep equal quantities of 3 feeds over 8 days and measured intake, and therefore preference, among the feeds. Burns *et al.* (2001) offered sheep paired hays and measured intake 30 minutes after feeding.

Biomass removed by grazing can also be estimated in the field. Mills (1977) offered cows and ewes plots of grasses and estimated intake by cutting and weighing quadrats of herbage above 75 mm prior to grazing and total herbage above 75 mm after grazing.

2.6.4.3 Intake collection

The surgical installation of fistulas has enabled eaten material to be retrieved and examined to determine diet selection. Oesophageal fistulas have been used to collect plant material eaten by sheep without much disturbance to the animal's grazing habits (Heady and Torell 1959). However, an oesophageal fistula may modify diet selection by not allowing animals to experience rumen fill and digestion (Heady 1975). Preference testing with oesophageal fistulated animals should be interpreted carefully because of the physiological imposition to the animal (Mayes and Dove 2000), for example by removing postingestive feedback signals.

Krueger (1972) collected pasture samples from oesophagelly fistulated sheep and analysed their composition using the microscopic point method under 15x magnification (Van Dyne and Heady 1965). Guy (1981) collected pasture samples from oesophageal fistulated sheep and comparisons were made between the pasture and extrusa composition.

2.6.4.4 Faecal analysis

Faecal analysis methods can be used across a range of situations. However, they are typically applied in situations where the available forage species can be clearly differentiated, preferably with few forage options. Dove (1992) outlined a promising method to determine diet composition from identifying faecal concentrations of the saturated hydrocarbons (n-alkanes) of plant cuticular wax. Containing a distinct profile of alkanes of specific carbon-chain length gives plants their own "alkane fingerprint" (Dove 1996). Therefore, the proportion of each alkane contained within animal faeces can, given suitable experimental conditions, be used to determine diet composition. The proportion C_3 and C_4 plant material eaten in a mixed stand can be estimated from faecal collections using stable carbon isotope ratios (^{13}C:^{12}C) (Mayes and Dove 2000).

2.6.5 *Calculating relative preference*

Relative preference was described by Heady (1964) as "proportional choice amongst two or more foods". This review covers the development of a calculation suitable to measure relative preference among living plant stands of differing relative abundance. Skiles (1984) has provided an extensive review of the methods to calculate animal preferences among feeds and Cock (1978) has provided a more general review of methods for the assessment of preference.

In situations where (i) equal quantities of food are offered and (ii) food intake can be measured on a dry weight basis, relative preference may be determined by simply calculating the amount of a particular forage eaten as a proportion of the animal's total intake. The calculation commonly used (e.g. Kenney and Black 1984; Dunlop 1986; Colebrook *et al.* 1990) for determining relative preference is described by Bell (1959):

$$\text{Preference (\%)} = \frac{\text{amount of forage (a) eaten x 100}}{\text{amount of forage (a) eaten plus the amount of forage (b) eaten}}$$

Where the relative abundance of forage is variable the difficulty of calculating preference increases. The analysis of preference is often calculated incorrectly in situations where disproportionate amounts of forage are available (Chesson 1983). This problem is avoided experimentally if food availability of the different food types is equal. However in a natural grazing situations this is virtually unachievable, and where forage intake is used directly as a measure of preference, conclusions can be misleading.

A measure of relative preference (α) referred to as the Chesson-Manly selection index has been developed, where relative preference does not change with food density (Manly 1985). The index was first described intuitively for use in a predation experiment by Manly *et al.* (1972) and later Chesson (1978) showed that this equation could be mathematically derived as a stochastic model where a consumer has a choice of *m* food types, and the number of type *i* present is n_i, $i = 1,\ldots, m$.

The probability of the next food consumed being type *i* is given by the formula:

$$P_i = \alpha_i n_i \Big/ \sum_{j=1}^{m} \alpha_j n_j$$

(Chesson 1978) (1)

Where the vector 'α' are positive numbers ranging from 0-1 used to measure deviations of the predator from the random sampling of its food types. In this model the animal selects its diet randomly when $\alpha_i = 1/m$. An animal exhibits a relative preference if the diet it chooses differs from what was expected had the animal chosen randomly. If no selectivity is exhibited the animal is expected to select its diet in proportion to what is available. By definition, α is a valid measure of the relative preference of a consumer for a given food type.

This model has been adapted specifically to a foraging model by Chesson (1983) and has the advantage that the calculation of relative preference (α) is not affected by the relative abundance of the food that is available. For a set of conditions where the food densities do not change appreciably during the experiment or observational period, relative preference can be estimated as follows:

$$\alpha_i = r_i n_i \ / \ \sum_{j=1}^{m} r_j n_j \ , \quad i = 1,\ldots, m$$

(Chesson 1983) (2)

Where r_i is the quantity of food type i in the consumers diet and n_i is the number of forage types offered or available.

In many cases the relative abundance of forage available to the grazing animal is depleted during feeding by the consumer. In this situation, Chesson (1983) has derived an approximate moment estimate of relative preference where:

$$\alpha_i = \ln((n_{i0} - r_i) / n_{i0}) \ / \ \sum_{j=1}^{m} \ln((n_{j0} - r_j) / n_{j0}) \ , \quad i = 1,\ldots, m$$

(Chesson 1983) (3)

Where r_i is the quantity of forage type i in the consumers diet and n_{i0} is the quantity of forage type i offered or available initially.

The index value α_i as defined by equation 3 derived from the index developed by Manly *et al.* (1972) is suitable to calculate relative preference between a number of food types differing in initial relative abundance and relative and total abundance as a consequence of forage depletion by consumer exploitation (Cock 1978). The validity of α_i is dependent on two assumptions: (i) that α_i does not vary with time (it is an instantaneous measure of relative preference) and (ii) that it is not dependent on the absolute quantities of forage available (Lascano 2000). Cock (1978) identifies this index for relative preference comparison as one of the few selection indexes developed, along with *C* Murdoch (1969) and the second index presented by Jacobs (1974), which is derived from Ivlev's electivity index, that "do[es] not have major drawbacks".

Conclusion

In this review we examined how the relative preference of sheep affects the diet they select and how this in turn impacts on the botanical composition of annual pastures. From this knowledge we developed a strategy to improve the efficacy of sheep to control weeds of crops in a grazed pasture phase by sowing a legume of low preference relative to the target weed. The experimental chapters 4, 5, 6 and 7 in this thesis report the studies conducted to develop this strategy with reference to the scientific literature reported in this review.

Chapter 3

General Materials and Methods

This chapter describes materials and methods that were used in more than one instance in the conduct of the research for this thesis. Animal ethics approval was sought from, and granted by the University of Western Australia Animal Ethics Committee, for the experimental procedures undertaken during the course of this research.

Determining the relative preference of sheep

Calculating the relative preference of sheep for living forages that are offered as whole plants growing *in situ* is made difficult because selection may be confounded by differences in plant biomass and therefore the relative availability of the forages. The methods commonly used for calculating relative preference (e.g. Bell 1959) are inadequate to determine preference when sheep are offered rows of pasture since the methods rely on equal quantities of each forage type being available for selection.

Typically in biological systems, when an animal selects its diet from more than one alternative, there is an unequal availability of the alternatives. To measure relative preference (α_i) independent of relative availability at any particular time, Chesson (1983) has derived an "approximate moment estimate" of relative preference that is referred to as the Chesson-Manly selection index, where:

$$\alpha_i = \ln((n_{i0} - r_i) / n_{i0}) \;/\; \sum_{j=1}^{m} \ln((n_{j0} - r_j) / n_{j0}) \;, \quad i = 1,\ldots, m$$

(Chesson 1983)

Where r_i is the quantity of forage type i in the consumers diet and n_{i0} is the quantity of forage type i offered or available initially to a herbivore given m choices for selection.

The value of α_I, as defined by this equation, is a measure of the relative preference of sheep between a number of forage types differing in initial relative abundance and relative and total abundance as a consequence of forage depletion by grazing.

Quantity of forage type "i" in the consumers diet

When conducting the 'cafeteria-type' field studies the quantity of the various forages consumed was determined by estimating the quantity of above-ground forage on offer prior to grazing and then subtracting the quantity of above-ground forage on offer after grazing. Forage on offer was measured using quadrat sampling (described below) from pre-determined paired sites (Leps *et al.* 1995) along the rows of the forages offered. The method recommended for determining intake by Chesson (1983) was modified in that they suggest using separate, adjacent plots as ungrazed controls to determine intake. However, due to the scale of the grazing experiments, differences in soil types across paddocks and the relatively short time over which the experiments were conducted, it was more practical to measure the food on offer in the paddocks to be grazed, prior to grazing, and use these results as the ungrazed comparison. Where necessary, the ungrazed forage biomass (used as a control) was adjusted to allow for plant growth during the period of grazing (the calculations are specified for individual experiments). For those sites where the quantity of food consumed was estimated to be negative, it was assumed that food intake was zero.

Pasture collection and biomass determination using quadrats

A site was selected within the pasture and (unless a specific sampling site had been pre-determined) the quadrat was thrown into the pasture so that the sampling site in the plot was selected at random. The forage samples were cut at ground level using a serrated knife and all above-ground plant material was collected from within the quadrat and stored in a paper bag. All samples were weighed fresh then dried in a fan-forced oven at 60°C for 48 hours. The dried samples were then weighed and the dry matter (DM) content was calculated.

Pasture composition

Forage was collected from the pasture site by sampling quadrats as described above. The fresh pasture was sorted into the appropriate class of plant material and then dried for dry matter determination as described above.

Estimating seed set of annual ryegrass

A site was randomly selected within the pasture by throwing quadrats (described above) and all annual ryegrass tillers were collected from within the quadrat using a serrated knife. The number and length of each tiller inflorescence was measured for each sample collected. Annual ryegrass seed production was estimated based on the number of tillers and the length of inflorescence and assuming 4 seeds/cm of inflorescence.

Plant maturity

Since the characteristics of a forage change as the plants mature, assessments of the relative preference of forage were made at three phases of the plants growth during the season. These were referred to as the vegetative, reproductive and senescent phases of plant growth. Vegetative was the growth phase before flowering, reproductive was the flowering and fruit development and senescent was the dry stage where the forage consists of dry stems, leaves, pods and seeds.

Sheep live weight and condition score

The liveweight of the sheep was measured using a crate mounted on Ruddweigh® load cells. At each weighing the body condition score (condition score 1 to 5) of each sheep was recorded as described by Russell (1969).

Chapter 4

Selection by sheep among pasture legumes, crop legumes and annual ryegrass: Field experiment

Part A - Relative preference

Introduction

A strategy to improve the control of weeds of crops in a grazed pasture phase is to sow into the pasture a legume of low preference by sheep. The combined stress on the weeds of increased grazing pressure and plant competition should improve the efficacy of grazing for weed control. Introducing a legume into an annual pasture on the basis that sheep will avoid eating it and thereby disadvantage weeds has not previously been investigated in Western Australia. The aim of this study was to determine whether there was variation in the preference of sheep among lines of annual legumes, and if so identify those of low preference to sheep relative to annual ryegrass, a potential target weed for this grazing management strategy.

It is well recognised that forages differ in their preference by grazing animals, and that less preferred species generally have a better capacity to persist in a regenerating pasture system (e.g. Stobbs 1977). Preference among annual legumes has been frequently reported between cultivars of subterranean clover because of their dominant place in Mediterranean-type pastures (e.g. Francis 1973; Dunlop 1986; Colebrook *et al.* 1990; Dynes 1993). Recently, many new legumes were introduced as cultivars into Australian agriculture for use in annual pasture-livestock systems and phase farming rotations to take advantage of the diverse characteristics of these new lines (e.g. suitability to soil type, rainfall, length of growing season, harvesting suitability and disease and pest resistance). The variation in the physical and chemical characteristics among these legumes should result in differences in their preference for grazing by sheep. However, the traits in a pasture species that determine its preference to grazing animals have not been fully determined, making preference difficult to predict. Hodgson (1982) suggested that the avoidance of individual plant species by herbivores can usually be related to either particular chemical constituents in the plant or the surface characteristics of the plant. Forage with a low potential intake rate is also likely to be avoided (Kenney and Black 1984). The favoured attributes of highly preferred plants are more diverse and difficult to isolate but they include traits such as high nutritive value. For example, a high content of nitrogen and/or water soluble carbohydrates (Edwards *et al.* 1993) and a high potential intake rate (Kenney and Black 1984).

The relative preference of herbivores among forages changes with plant maturity (Heady 1964). For example, steers were found to select legumes in preference to grasses more exclusively as the plants matured (Bohnert *et al.* 1985). The nutritive value of grasses declines more rapidly than legumes as the pasture matures and the selectivity of sheep for nutritious pasture components increases to meet their nutritional requirements (Clark and Ulyatt 1985). The preference rank of sheep for *Erodium botrys* decreased in October relative to other pasture species, probably as a result of the sheep avoiding the spike-like seed heads of the *Erodium* that harden at this time (Arnold 1987). The relative preference of sheep offered cut fresh material of different cultivars of subterranean clover changed between three phases of plant maturity (Dunlop 1986). In this case the sheep were able to discriminate between apparently subtle changes in the plant's characteristics between plant lines of close genetic similarity. This finding was reinforced by Colebrook *et al.* (1990) who also measured the relative preference of sheep for different cultivars of subterranean clover.

In this study we identified gross differences in the relative preference of sheep for annual forage and grain legumes at the vegetative, reproductive and senesced phases of plant growth. We hypothesised that sheep would avoid eating some annual legumes at the vegetative phase, while in contrast annual ryegrass would be eaten readily at the vegetative phase of plant growth. We also hypothesised that the relative preference of sheep among legumes would change with plant maturity. Specifically, that the legumes the sheep avoid eating at the vegetative stage would have a higher relative preference at the senesced phase of plant growth while the relative preference of sheep for ryegrass would decrease with plant maturity.

Methods and Materials

Experiment design

At the 3 phases of plant growth (vegetative, reproductive and senesced), 65, 80 and 80 Merino wether hoggets were grazed on 6 blocks, each containing 20 parallel rows (20 m x 1.2 m) of 19 legumes and annual ryegrass. The wethers had access to all 6 blocks at the same time at each of the 3 phases of plant growth for 60,140 and 80 hours, or when it was visually estimated that 50% of the forage initially available had been removed. Forage intake was estimated as the forage available before grazing minus the forage available after grazing (see below). Relative preference was calculated from forage availability and the estimated forage intake.

Prior to the hoggets being put into the plots to test their relative preference among the forages at each of the three phases, they were offered 3 other plots containing 20 parallel rows (30 m x 1.2 m) of the same forages for 24 hours to familiarise them with the feed options that would be available to them (familiarisation plots).

Compared to the preliminary study (Lindsay, unpublished data) reported in the general introduction, a number of additional legumes were included, replication was increased to 5 and a 24 hour period of familiarisation was used to reduce the incidence of ‘sampling’ by the sheep during the preference test.

Experimental site

This experimental site was established in June, 2000 on a gravely sand soil, pH 4.8 ($CaCl_2$) at Allandale Farm, Wundowie 70 kilometres east of Perth (116.3 E, 31.8 S). The area has a Mediterranean type climate where typically 80% of the 610 mm average rain falls during the months of May to September. A crop of oats was grown on this site in 1999.

Pasture establishment

To provide adequate plant material to assess the preferences of sheep, 18 blocks each consisting of 20 monoculture rows (20 m x 1.2 m) of 19 legume genotypes and annual ryegrass were established in random order (Table 4.1). The 1.2 m monoculture rows comprised six evenly spaced rows. Appropriate seeding rates were estimated on the basis that they would produce pasture rows of similar biomass (i.e. higher rates for larger size seeds). The 18 blocks were divided into 3 groups of 6 blocks and fenced so that fresh plant material could be offered for grazing at 3 stages of plant maturity with 6 replicates at each stage. Rows of plants in adjacent blocks were aligned perpendicularly to each other.

A further three blocks (20 x 30 m x 1.2 m) of monoculture rows of the same 20 genotypes were established in another fenced area adjacent to the other 18 blocks to serve as the familiarisation plots.

Table 4.1. Common and scientific names, the rate of seeding (g/m^2) and group of inoculum applied to the seed for each of the 20 plant genotypes grown

Common name	Scientific name	Seeding rate (g/m^2)	Inoculum Group
Annual ryegrass cv. Wimmera	*Lolium rigidum* cv. Wimmera	2.0	no inoculum
Arrowleaf clover cv. Cefalu	*Trifolium vesiculosum* cv. Cefalu	1.5	C
Balansa clover cv. Paradana	*Trifolium michelianum* cv. Paradana	1.5	C
Biserrula cv. Casbah	*Biserrula pelecinus* cv. Casbah	1.5	WSM 1497
Burr medic cv. Santiago	*Medicago polymorpha* cv. Santiago	1.5	AM
Chickpea cv. Tyson	*Cicer arietinum* cv. Tyson	6.0	N
Crimson clover cv. Caprera	*Trifolium incarnatum* cv. Caprera	1.5	C
Dwarf chickling cv. Chalus	*Lathyrus cicera* cv. Chalus	5.0	F
French serradella cv. Cadiz	*Ornithopus sativus* cv. Cadiz	3.0	S
Gland clover	*Trifolium glanduliferum* acc. CPI 87182	1.5	C
Grasspea	*Lathyrus sativus* acc. BIO L254	5.0	F
Lotus	*Lotus ornithopodioides* acc. SA33851/SA33845	1.5	Special
Rose clover cv. Kondinin	*Trifolium hirtum* cv. Kondinin	1.5	C
Snail medic cv. Kelson	*Medicago scutellata* cv. Kelson	2.5	AM
Snail medic cv. Sava	*Medicago scutellata* cv. Sava	2.5	AM
Bladder clover	*Trifolium spumosum* acc. 88TUR593SPU	1.5	C
Sub. clover cv. Dalkeith	*Trifolium subterraneum* cv. Dalkeith	2.0	C
Sub. clover cv. Dinninup	*Trifolium subterraneum* cv. Dinninup	4.0	C
Trigonella	*Trigonella balansae* acc. SA5045	1.5	Special
Vetch cv. Languedoc	*Vicia sativa* cv. Languedoc	5.0	E

Seasonal conditions

A total of 615 mm of rain was recorded at Allandale Farm in 2000. The 2000 growing season was unusually short as most of the rain for the season began in June and finished in early September. From mid-September to the end of December 2000, less than 15 mm of rain was recorded.

Table 4.2. Monthly rain (mm) recorded at Allandale Farm in 2000

Month	Rain (mm)
January	112.5
February	0.0
March	14.6
April	23.1
May	21.2
June	107.5
July	154.0
August	136.4
September	34.9
October	3.3
November	8.0
December	0.0

Site management

Two fertiliser applications were made. Prior to seeding, 100 kg/ha of superphosphate with Cu, Zn and Mo was applied. During seeding, 100 kg/ha of muriate of potash (KCl) was applied.

Weeds that had germinated before seeding were killed with an application of Roundup® 100 herbicide at 2 L/ha prior to seeding. Grass weeds that emerged post sowing were controlled by a single application of 400 mL/ha of Fusilade®, a group A selective herbicide, 5 weeks after initial plant germination. The rows of ryegrass were protected from damage by the Fusilade® application by covering them with black plastic sheeting during application. The red-legged earth mites observed 4 weeks after sowing were controlled by an application of Lemat® insecticide at 150 mL/ha.

Plant maturity and flowering time

The plant species studied had different characteristics of growth and maturity but were compared simultaneously to measure their relative preference by the sheep. The three phases of plant growth at which relative preferences were measured was based on the following criteria. The vegetative phase (81 days post seeding) was based on providing sufficient plant biomass for the test prior to flowering. Dispite this intention, several of the medics had begun flowering when the preference test was conducted at the vegetative-phase.

The reproductive phase (110 days post seeding) was based on the time when all legumes had commenced flowering. The maturity of the different plant genotypes at the reproductive phase ranged from early flowering (e.g. arrowleaf clover) to the soft seed stage (e.g. vetch). The senesced stage (180 days post seeding) was assessed when all plants had dried off. At this stage, maturity was also variable because some of the early maturing plants had begun to shed seed pods, while the later maturing genotypes still had pods intact. Flowering time was measured as the time from seeding until approximately 50% of the plants of the particular genotype had flowered, and these observations are reported in Part B.

Animals

The 225 Merino wether hoggets used in this experiment were all from the same commercial flock at the University of Western Australia Allandale farm, Wundowie. They weighed between 40 and 50 kg and were about 14, 15 and 17 months of age when grazing the plots during the vegetative, reproductive and senesced phases of plant growth. The number of sheep was increased from 65 to 80 for the reproductive and senesced phases to improve the rate at which forage was consumed to account for the higher biomass available at these phases. At all times during the experiment, the sheep had water available *ad libitum*.

Forage intake and availability

The forage biomass available and the estimated forage intake of each plant type was determined by cutting paired quadrat samples (30 cm x 100 cm), one per row, before and after grazing, from a site within the row that was visually assessed as having a uniform biomass (see General Methods, pasture sampling with a quadrat). Paired quadrats were taken for each of the 20 forages in 5 out of the 6 blocks offered to the sheep. The sampling site was marked with a peg before grazing to allow the position of the post-grazing site to be located.

In the preliminary experiment (General Introduction) it was assumed, for simplicity, that the pasture grown between the forage sampling measurements made before [A_0] and after [A(t)] grazing was negligible. This assumption may under-predict the actual amount of forage available in the vegetative and reproductive phases because of plant growth while the sheep were grazing the plots. Therefore, to improve the accuracy of the measure of preference, the growth rate of each plant type was estimated at the vegetative and reproductive stages and the biomass of forage available was adjusted accordingly. Exponential growth was assumed. The biomass (g DM/quadrat) of each plant type was calculated using the equation:

$$A(t) = A_0e^{kt}$$

Where:

A_0 is the biomass (g DM/quadrat) at the vegetative stage before grazing
$A(t)$ is the biomass (g DM/quadrat) t days from A_0
k is the coefficient for the sward growth rate

There was a uniform variation in biomass between the vegetative and reproductive sites, perhaps due to differences in soil type, and the biomass measurements that were used to predict plant growth rate were adjusted to account for this.

Familiarisation with forages

Most of the forages offered to the wether hoggets were novel plant cultivars, and because of the age of the sheep they would have had little, if any, prior experience grazing any of the forages used in this experiment. Immediately prior to being put in the testing plots the wethers were offered all forages in large plots for 24 hours. The aim of this was to give the wethers olfactory experience with all of the forages to reduce indiscriminate sampling during the preference test.

Determining relative preference

To determine the extent to which sheep exhibited selectivity among the forages they were offered, their relative preference (α), was calculated using an adaptation of the Chesson-Manly selection index (Chapter 3) using measures of forage availability and estimated forage intake of the sheep.

Plant chemical analyses

Plant material was collected for chemical analyses (Chapter 4, Part B) at each of the three stages of plant maturity (vegetative, reproductive and senesced), immediately prior to the preference test. At the vegetative and reproductive stages, whole, above-ground plant material was taken and immediately cut into 2 cm lengths, wrapped in alfoil and snap frozen in liquid nitrogen. These samples were kept frozen (with dry ice in an insulated box) then immediately dried in a freeze-drier. In addition to these samples, representative sub-samples were taken for measurement of oven dry matter from the first of the paired samples that were collected to determine forage preferences.

Statistical analysis

Analysis of variance and LSD (Fisher's protected LSD) were used to determine the statistical significance of the results. These statistical analyses were conducted using the GenStat® (Version - 6.1.0.200) statistical package.

Results

Relative preference (α)

The hoggets showed clear differences in their relative preference for the 19 legumes and annual ryegrass at each phase of plant growth and their ranking differed according to the phase of plant growth (Table 4.3). At the vegetative phase, values for $\alpha \leq 0.019$ were significantly lower than neutral preference and these forages were considered to be avoided while values of $\alpha \geq 0.081$ were higher than neutral preference and these forages were considered to be preferred by the sheep. At the reproductive and senesced phases the range of neutral preference was found to be $0.020 \geq \alpha \geq 0.080$.

Six legumes, vetch, lotus, the two snail medics, chickpea and biserrula were avoided by the hoggets, while grasspea, annual ryegrass, rose clover and crimson clover were preferred over others at the vegetative phase (Table 4.3). At the reproductive phase, the snail medics and the subterranean clovers were avoided, while arrowleaf clover, crimson clover, bladder clover and grasspea were preferred. At the senesced phase, Dalkeith subterranean clover, annual ryegrass, gland clover, French serradella, biserrula and burr medic were avoided while chickpea, dwarf chickling, grasspea, vetch, arrowleaf clover and trigonella were preferred. All other forages were within the range of neutral preference for each growth phase.

The four crop legumes; chickpea, dwarf chickling, grasspea and vetch had the highest relative preference of the forages at the senesced phase (0.130, 0.112, 0.108 and 0.104; Table 4.3). The high relative preference for both chickpea and vetch at the senesced phase was in marked contrast to their very low relative preference at the vegetative stage (0.130 *v* 0.009 and 0.104 *v* 0.000; Table 4.3). Three of four pasture legumes that had low relative preference at the vegetative phase had a neutral relative preference at the senesced phase. The exception was biserrula, which had a low relative preference at senescence. The relative preference of biserrula was neutral at the reproductive phase (Table 4.3).

Table 4.3. Relative preference (α) for each of 20 different forages offered to Merino wether hoggets at the vegetative, reproductive and senesced phases of plant growth

Forage	**Phase of plant growth**		
	Vegetative	Reproductive	Senesced
Arrowleaf clover cv. Cefalu	0.073 ± 0.012	0.108 ± 0.017	0.081 ± 0.012
Balansa cv. Paradana	0.069 ± 0.005	0.049 ± 0.013	0.059 ± 0.014
Biserrula cv. Casbah	0.015 ± 0.007	0.059 ± 0.010	0.017 ± 0.006
Burr medic cv. Santiago	0.074 ± 0.005	0.065 ± 0.006	0.015 ± 0.004
Crimson clover cv. Caprera	0.083 ± 0.015	0.095 ± 0.010	0.074 ± 0.006
Subterranean clover cv. Dalkeith	0.060 ± 0.014	0.019 ± 0.004	0.008 ± 0.004
Subterranean clover cv. Dinninup	0.043 ± 0.012	0.018 ± 0.007	0.069 ± 0.010
Gland clover acc. CPI 87182	0.032 ± 0.006	0.021 ± 0.003	0.013 ± 0.004
Snail medic cv. Kelson	0.007 ± 0.003	0.004 ± 0.002	0.023 ± 0.009
Lotus acc. SA33851/845	0.006 ± 0.003	0.021 ± 0.010	0.049 ± 0.013
Rose clover cv. Kondinin	0.088 ± 0.013	0.062 ± 0.025	0.037 ± 0.006
Snail medic cv. Sava	0.009 ± 0.003	0.009 ± 0.005	0.026 ± 0.010
French serradella cv. Cadiz	0.046 ± 0.004	0.052 ± 0.003	0.016 ± 0.006
Bladder clover acc. 88TUR593SPU	0.078 ± 0.012	0.093 ± 0.012	0.061 ± 0.010
Trigonella acc. SA5045	0.060 ± 0.013	0.051 ± 0.007	0.083 ± 0.015
Chickpea cv. Tyson	0.009 ± 0.006	0.026 ± 0.007	0.130 ± 0.016
Dwarf chickling cv. Chalus	0.066 ± 0.017	0.071 ± 0.007	0.112 ± 0.015
Grasspea acc. BIO L254	0.116 ± 0.019	0.080 ± 0.012	0.108 ± 0.008
Vetch cv. Languedoc	0.000 ± 0.000	0.072 ± 0.007	0.104 ± 0.015
Annual ryegrass cv. Wimmera	0.092 ± 0.009	0.028 ± 0.002	0.011 ± 0.002
neutral preference	0.05	0.05	0.05
LSD (P = 0.05)	0.031	0.030	0.030
P-value	< 0.001	< 0.001	< 0.001

Error terms are standard errors of the means (n = 5).

Pasture growth

The forage genotypes differed in their biomass production. The biomass of the forages ranged from 36 – 208 g DM/m^2 at the vegetative phase, 58 – 384 g DM/m^2 at the reproductive phase and 27 – 285 g DM/m^2 at the senesced phase (Table 4.4). The highest producing genotypes across the three growth phases were vetch cv. Languedoc, crimson clover cv. Caprera, gland clover, subterranean clover cv. Dalkeith and burr medic cv. Santiago. The lowest producing genotypes across the three growth phases were lotus, trigonella, chickpea cv. Tyson, grasspea and spumosum. The mean amount of forage available was higher at the reproductive stage than at the senesced stage (224 *v* 122 g

DM/m^2; Table 4.4). This was probably due to lower biomass production in the senesced plot compared to the reproductive plot, probably related to soil fertility rather than a loss in plant biomass between the reproductive and senesced phases.

Table 4.4. Biomass (g DM/m^2) of plots of 20 different forages at the vegetative, reproductive and senesced phases of plant growth

Forage	**Phase of plant growth**		
	Vegetative	Reproductive	Senesced
Arrowleaf clover cv. Cefalu	89 ± 17.1	242 ± 13.9	137 ± 16.9
Balansa cv. Paradana	156 ± 11.2	252 ± 29.0	113 ± 16.1
Biserrula cv. Casbah	115 ± 13.6	292 ± 35.3	140 ± 9.1
Burr medic cv. Santiago	212 ± 18.0	363 ± 31.7	77 ± 9.7
Crimson clover cv. Caprera	190 ± 37.5	306 ± 17.8	223 ± 5.9
Subterranean clover cv. Dalkeith	175 ± 29.6	384 ± 28.0	121 ± 10.5
Subterranean clover cv. Dinninup	124 ± 17.0	187 ± 46.5	101 ± 15.6
Gland clover acc. CPI 87182	208 ± 24.4	299 ± 16.3	175 ± 4.2
Snail medic cv. Kelson	140 ± 15.9	187 ± 7.5	117 ± 17.7
Lotus acc. SA33851/845	49 ± 11.8	58 ± 14.6	31 ± 2.5
Rose clover cv. Kondinin	67 ± 4.8	245 ± 37.9	121 ± 13.9
Snail medic cv. Sava	172 ± 23.5	270 ± 31.6	123 ± 29.7
French serradella cv. Cadiz	113 ± 11.6	317 ± 35.6	123 ± 11.2
Bladder clover acc. 88TUR593SPU	59 ± 9.2	106 ± 15.7	46 ± 9.3
Trigonella acc. SA5045	61 ± 9.6	62 ± 7.0	27 ± 3.5
Chickpea cv. Tyson	36 ± 5.0	77 ± 13.5	53 ± 8.9
Dwarf chickling cv. Chalus	85 ± 11.9	175 ± 14.9	176 ± 21.1
Grasspea acc. BIO L254	39 ± 4.1	75 ± 10.2	65 ± 17.8
Vetch cv. Languedoc	118 ± 11.0	358 ± 25.4	285 ± 11.3
Annual ryegrass cv. Wimmera	114 ± 7.6	226 ± 9.4	196 ± 15.0
Mean	116	224	122
LSD	47	68	39
P-value	< 0.001	< 0.001	< 0.001

Error terms are standard errors of the means (n = 5).

Forage intake

The voluntary intake of the hoggets varied among the plant genotypes and decreased between the vegetative, reproductive and senesced phases of plant growth (46, 34 and 30 g DM/sheep.day; Table 4.5).

Table 4.5. Daily intake by Merino wether hoggets (g DM/sheep.day) offered monoculture plots of 20 different forages at the vegetative, reproductive and senesced phases of plant growth

Forage	**Phase of plant growth**		
	Vegetative	Reproductive	Senesced
Arrowleaf clover cv. Cefalu	52 ± 12.0	50 ± 2.2	51 ± 7.5
Balansa cv. Paradana	90 ± 6.1	43 ± 11.4	35 ± 6.5
Biserrula cv. Casbah	21 ± 8.2	49 ± 8.3	18 ± 7.3
Burr medic cv. Santiago	128 ± 14.2	70 ± 5.5	9 ± 3.7
Crimson clover cv. Caprera	123 ± 32.7	70 ± 6.7	80 ± 2.4
Subterranean clover cv. Dalkeith	92 ± 27.9	33 ± 8.7	4 ± 7.3
Subterranean clover cv. Dinninup	45 ± 17.9	16 ± 10.0	30 ± 10.8
Gland clover acc. CPI 87182	80 ± 21.0	30 ± 5.8	16 ± 4.9
Snail medic cv. Kelson	10 ± 6.0	3 ± 1.9	19 ± 7.9
Lotus acc. SA33851/845	0 ± 3.2	6 ± 4.2	5 ± 3.4
Rose clover cv. Kondinin	31 ± 9.2	34 ± 13.1	27 ± 5.0
Snail medic cv. Sava	22 ± 6.6	11 ± 4.3	22 ± 9.0
French serradella cv. Cadiz	51 ± 9.1	50 ± 5.8	14 ± 5.0
Bladder clover acc. 88TUR593SPU	35 ± 5.6	23 ± 4.0	16 ± 5.0
Trigonella acc. SA5045	34 ± 8.3	12 ± 1.2	10 ± 1.6
Chickpea cv. Tyson	4 ± 2.9	11 ± 3.2	25 ± 4.5
Dwarf chickling cv. Chalus	42 ± 10.0	42 ± 5.3	77 ± 13.4
Grasspea acc. BIO L254	26 ± 3.5	19 ± 2.8	12 ± 11.8
Vetch cv. Languedoc	0 ± 0.0	85 ± 5.3	119 ± 7.9
Annual ryegrass cv. Wimmera	74 ± 8.5	26 ± 3.1	18 ± 4.7
Mean	46	34	30
Total	928	673	603
LSD	37	18	19
P-value	< 0.001	< 0.001	< 0.001

Error terms are standard errors of the means (n = 5).

The amount of each forage type consumed by the hoggets was positively related to the amount of each forage type available ($P < 0.001$); Figure 4.1. The strength of the positive relationship remained consistent as the forage matured (R^2 = 0.55, 0.55 and 0.56 at the vegetative, reproductive and senesced phases).

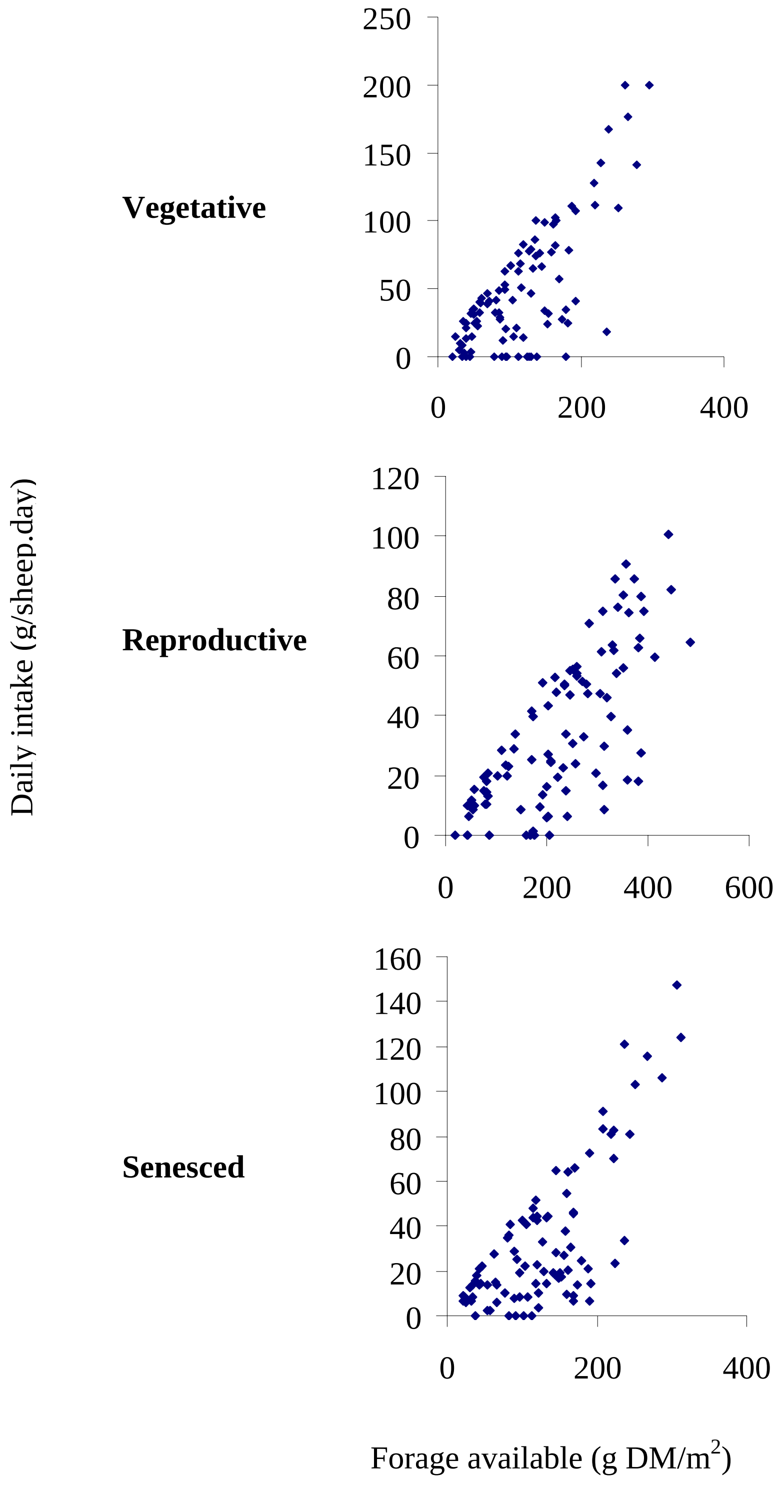

Figure 4.1. Relationship between the amount of forage available and the amount consumed at the vegetative, reproductive and senesced phases of plant growth.

Discussion

The two hypotheses of this experiment were supported. We were able to identify six legumes (vetch, lotus, Kelson and Sava snail medic, chickpea and biserrula) that sheep showed a low relative preference for at the vegetative phase. With the exception of Casbah biserrula, all of the legumes with a low relative preference at the vegetative phase had either a neutral or high relative preference at the senesced phase. The marked differences in the relative preference of the sheep for the forages between the three stages of plant growth generally followed one of three patterns. Either relative preference was high and decreased as the plant matured (e.g. annual ryegrass), was low and increased as the plant matured (e.g. vetch) or was high at all phases of plant growth (e.g. crimson clover).

Annual ryegrass had a high relative preference at the vegetative phase and this decreased at each successive phase of plant maturity. Grasses are usually found to be readily eaten by sheep during early stages of growth because of their high nutritive value and they also generally do not produce unpalatable secondary compounds (Cooper and Owen-Smith 1986). The high relative preference for the grain legumes at the senesced phase of growth was probably associated with the high proportion of grain in these forages and consequently their high nutritive value (Allden and Geytenbeek 1980).

The selectivity exhibited by the sheep in this study agrees with the widely held view that grazing ruminants exercise a high degree of selection from their range of potential food (McClymont 1967). The variability in preference by sheep among these annual legumes suggests there is potential to find suitable candidates for a grazing strategy to help reduce the ryegrass component of the pasture. However, the relative preferences of sheep measured under this set of experimental conditions may not necessarily transfer to a grazed pasture. The selection of pasture components by sheep from mixed pastures is much more complex and includes factors such as postingestive experience, accessibility, patch grazing and the location and availability of watering points. However, identifying trends in the relative preference of sheep may provide a screening tool to identify legumes that may be useful in a pasture phase, which is designed to increase grazing pressure on target weeds of crops, such as annual ryegrass.

The methods employed in this experiment allowed us to investigate the innate grazing behaviour of sheep, rather than the factors that influence grazing behaviour through learning. In this study the forages offered were assumed to be novel to the sheep, although a few may have been present at low densities in the pastures previously grazed by the sheep. Therefore the sheep were able to exhibit a high degree of selectivity among the forages independent of them having previously experienced any postingestive consequences from eating the forages. Similarly, conditioned responses to the forages offered are unlikely to have occurred within the experiment as the sheep had only a short period of time to become familiar with the 20 different forages.

When there is a diverse range of forages offered, the animals are more likely to rely on “trial and error” learning because the risk of error is lower than where there is less variability between the forage offered (Provenza and Balph 1988). Postingestive feedback mechanisms that trigger learned responses generally depend on the concentration of specific secondary compounds (Kyriazakis *et al.* 1999; Villalba *et al.* 2002). With the wide diversity of forages to select from in this experiment, it is unlikely that the sheep would have eaten enough of any particular forage to experience an aversion response, and even if they did, it is unlikely that they could associate a response with a specific forage given the number of forages that were available at the one time. Among the novel forages that were presented to the sheep in the experiment, some were highly preferred and others were avoided almost totally, so the novelty value of the test species did not have a consistent influence.

Furthermore, it was apparent that the less preferred forages were not absolutely rejected by the sheep. When the sheep were left in the pasture for an extended period they did eventually eat more of the less preferred forages as the alternative forages became exhausted. However, this behaviour is not always the case, as livestock are known not to eat some forage types irrespective of how scarce the alternative source of food becomes (Ngwa *et al.* 2000).

Part B - Chemical and physical forage components

Introduction

The chemical and physical composition of forages has a strong influence on diet selection in sheep. The net result of this selectivity is that the diet consumed is more nutritious and less toxic than the average of the available forage (Provenza 1995; Nolan *et al.* 1996). However, due to the complexity of the decisions that need to be made by grazing animals and the many chemical components that are contained in forages, linking specific chemical attributes to herbivore preference is difficult and the results are variable. The large diversity of chemical and physical components in forage necessitates that relationships between an animal's preference and a single plant component should be viewed with caution, and further investigations of the interactions and environment are necessary before its significance to diet selection by the animal can be interpreted. Arnold (1981) states that a lack of correlation between preference and a plant component does not prove that this component is unimportant, and neither does a correlation offer proof that the particular component measured influences preference. However, such correlations do offer evidence of chemical and physical plant characteristics that are linked to preferential grazing and as such are important for building an understanding of the pathways by which plant characteristics influence preference.

Many single plant chemical and physical characteristics affect selection for and particularly against certain types of forage. Examples of aversive plant characteristics include thorns, secondary plant compounds and low nutritive value. Examples of attractive plant characteristics are green, dense, high intake rate and high nutritional value. It should be noted that at very high concentrations compounds that are commonly measured to be attractants can become aversive (Arnold 1981). This statement supports the view of Provenza (1995) that energy and nutrient balance are important and when consumed in excess or deficit the animal receives metabolic signals to modify its diet (refer to Figure 2.3 in Chapter 2).

Nitrogen content, water soluble carbohydrates, fibre and digestibility have been linked to diet selection in sheep. Lambs have been found to select for a specific protein intake

(Kyriazakis and Oldham 1993). Sheep and cattle prefer grass that is higher in nitrogen (Cosgrove *et al.* 2002). The forage preference of sheep for grasses can be increased by the application of a nitrogen fertiliser (calcium ammonium nitrate), thereby increasing plant nitrogen (Edwards *et al.* 1993). However, if nitrogen intake is excessive, sheep may then select forage that is lower in nitrogen to balance their nitrogen intake (Kyriazakis and Oldham 1993). Sheep were found to have reversed their preference from clover to grass when infused with ammonia in an indoor study (Cosgrove *et al.* 1999), although the severity of the treatment meant that only two of the four sheep in the treatment group, were in a suitable state to complete the experiment. This suggests that sheep may have the capacity to regulate protein intake to avoid excessive levels of rumen ammonia since the clover was of much higher protein content than the grass. However, this result was not repeated in the field where ammonia decreased voluntary food intake but had little effect on diet selection (Cosgrove *et al.* 1999). Generally, we would expect sheep to select for feeds of high nitrogen content when the average nitrogen content of the plant material available is lower than its requirement or if the sheep had been eating a diet deficient in nitrogen.

Sheep preferred *Phalaris aquatica* L. forage with higher levels of water soluble carbohydrates (126 *v* 62 mg/g DM) in unshaded *v* shaded pastures that differed significantly in only water soluble carbohydrate concentration (Ciavarella *et al.* 2000). Sheep preferred to eat an annual ryegrass-silver grass pasture after it was sprayed with the herbicide glyphosate, which led to an increase in nitrogen and water soluble carbohydrate and an increase in digestibility. However, it is unclear whether one or a combination of these nutritive factors increased its preference by sheep (Siever-Kelly *et al.* 1999). Sheep exhibited increasing preference for pastures as the rate of phosphorus application was increased (Ozanne and Howes 1971).

Compression and shear energy may influence diet selection in sheep. Higher intrinsic shear strength (Baker *et al.* 1994; Inoue *et al.* 1994; Henry *et al.* 1996), grinding energy (Henry *et al.* 1996) and compression energy (Wang 1997) are associated with lower intake rates in sheep and sheep show less preference for forage with a low potential intake rate (Kenney and Black 1984).

Sheep generally do not prefer to eat forage that is of low digestibility and consequently a poor source of energy (Provenza 1995). A number of studies report a strong negative relationship between dry matter intake and fibre (Minson 1982).

Sheep are known to become more selective as the pasture increases in maturity (Arnold 1960; Ozanne and Howes 1971; Jung and Sahlu 1986). The nutritive value of annual pastures in a Mediterranean environment declines rapidly as the pastures mature in late spring and the rain events subside. The structural plant compounds such as fibre and lignin increase as plants mature (Theander and Westerlund 1993) and protein declines (Coleman and Henry 2002). However, the magnitude of these changes varies widely among plant genotypes (Tamminga 1993).

In general terms, sheep avoid forage that is of low nutritive value, especially when the average diet consumed does not meet their nutrient requirements. In this experiment we hypothesised that sheep would select forages that were of higher nutritive value, and show a preference for forages of higher nitrogen and water soluble carbohydrate content, higher digestibility and lower fibre and shear and compression energy. Therefore it was expected that selection by sheep, on the basis of plant nutritive value, would be less important earlier in the growing season as the forage quality is higher and more likely to meet the animal's nutritional requirements. At this time sheep may demonstrate less selectivity, or selection for other attributes such as eating those that can be consumed faster, since the amount of food on offer may be limiting at this time.

Materials and Methods

Plant material from each of the forages grown for the preference experiment (Part A) was collected immediately prior to grazing at each phase of plant growth. A sample of approximately 100 g DW of entire above-ground plant material was oven-dried (60 ºC for 72 hours) at the vegetative and reproductive phases. Additionally, approximately 50 g of entire above-ground plant material was snap-frozen by immersion in liquid nitrogen and later freeze-dried. Senesced plant material was collected when it had dried to an acceptable moisture content *in situ* and no additional drying was required, pre-analysis. Approximately 50 g of the dried and all of the freeze-dried samples were ground to pass through a 1 mm screen using a Tecator Cyclone© mill, and 50 grams of each sample was retained unground. Ground, oven-dried plant samples were analysed for nitrogen (N), sulphur (S), neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* digestibility (IVD) and NIR reflectance. Unground, oven-dried plant samples were analysed for shear energy and compression energy. Ground, freeze-dried samples were analysed for water soluble carbohydrates (WSC).

Total nitrogen and sulphur

Total nitrogen and total sulphur were determined using a Leco FP-428 Nitrogen Analyser (Sweeney 1989). The analyses were repeated and the two sets of results (rep 1 and rep 2) were combined.

Neutral detergent fibre and acid detergent fibre

NDF was measured (Goering and Van Soest 1970) using an Ankom 200/220 Fibre analyser in accordance with the operating instructions for this equipment (Ankom ® Tech. Co., Fairport, NY, USA). Sub-samples of the dried and ground material were digested for 60 minutes in the analyser using a neutral detergent solution. This procedure dissolved the readily soluble pectins and plant cell components of proteins, sugars and lipids, leaving the fibrous residue of cellulose, hemicellulose and lignin.

ADF was measured using an Ankom 200/220 Fibre analyser in accordance with the operating instructions for this equipment (Ankom® Tech. Co., Fairport, NY, USA). ADF was determined sequentially on the samples previously used for the measurement of NDF.

The samples were digested in an acidified quaternary detergent solution to dissolve the remaining soluble cell components, hemicellulose and soluble minerals. The entire dissolution process was performed in an ANKOM 200/220 Fiber Analyzer over 60 minutes. Available standards were included to check that the equipment was operating correctly for each NDF and ADF run.

In vitro digestibility - pepsin-cellulase digestion

Samples were digested as described by Klein and Baker (1993) using a modified pepsin-cellulase procedure to estimate (*in vitro*) the amount of feed that would be digested by a ruminant (expressed as a percentage of its intake).

Pepsin-cellulase digestion of the dry matter (P-CDDM) (an estimation of the digestibility of the whole sample) was calculated as follows:

$$\text{P-CDDM} = \frac{\text{dry matter in the sample} - \text{dry matter in the residue}}{\text{dry matter in the sample}}$$

Dry matter digestibility

An appropriate range of calibration standards were included in each *in vitro* run so that the *in vivo* digestibility (DMD) was able to be predicted.

Shear and compression energy (kJ/kg DM)

Shear and compression energy were measured on five replicates of each forage at each of the three phases of growth. Forage was cut into approximately 0.5 – 1.0 cm lengths, then analysed for compression and shear energy using the Instron Materials Testing Machine (Baker *et al.* 1993).

Water soluble carbohydrates

The concentration of the total water soluble carbohydrates (WSC) in each of the forages was measured by the anthrone method (Yemm and Willis 1954; Thomas 1977).

Near Infrared Reflectance Spectroscopy (NIRS)

NIR Spectroscopy (Givens and Deaville 1999) was carried out on oven-dried samples of the forages to obtain the reflectance spectra from 400 to 2500 nm at 2 nm intervals using a Foss NIRSystems Model 6500 instrument.

Results

Plant composition (Table 4.6, Table 4.7 and Table 4.8)

The physical and chemical characteristics of the 20 forages clearly differed within and between the three phases of plant growth. The dry matter content (DM) of the forages at the reproductive phase was nearly double that at the vegetative phase (means of 33.6% *v* 17.0%). Forage nitrogen decreased between successive phases of growth with mean values of 3.7%, 2.2% and 2.0%. The greatest decrease in nitrogen content was between the vegetative and reproductive phases. Similarly, the sulphur concentration at the reproductive phase was almost half that at the vegetative phase (0.12% *v* 0.21%). Sulfamethazine standards were used in each of the repeated analyses (Rep 1: S, 11.66 N, 20.64 Rep 2: S, 11.48, 20.14)

The ADF and NDF content increased between the phases of plant growth with the largest increase between the reproductive and senesced phases (mean NDF of 27%, 32% and 45%, mean ADF of 18%, 20% and 30%). The DMD decreased in line with the increase for fibre content for the three phases of plant growth with the largest decrease between the reproductive and senesced phases (mean DMD of 77%, 72% and 61%). The WSC content for the legumes increased between the vegetative and reproductive phases and was lowest at the senesced phase (11.9%, 15.7% and 4.6%). The WSC content for the annual ryegrass was approximately double that of the legumes at each phase of plant growth. Both shear and compression energy increased with plant maturity. Shear energy increased between the vegetative and reproductive phases, but not the senesced phase (6.8, 9.6 and 10 .1 kJ/kg DM) while compression energy increased only between the reproductive and senesced phases (3.5, 3.4 and 5.0 kJ/kg DM).

Dwarf chickling, arrowleaf clover, burr medic and biserrula had the highest nitrogen content at the vegetative phase (N > 4.5%). These four legumes and crimson clover, but not burr medic, had the highest nitrogen content at the reproductive phase (N > 2.5%). At the senesced phase crimson clover and the four pulses had the highest nitrogen content. There was a consistent positive relationship for nitrogen content between the vegetative and reproductive phases (R^2 = 0.66) and between the reproductive and senesced phases (R^2 = 0.51). Gland clover was consistently low in nitrogen content at all phases of growth, while the snail and burr medics were of relatively low nitrogen content at the reproductive and senesced phases.

Gland clover, Dalkeith subterranean clover, snail medic and annual ryegrass generally had the highest levels of NDF across the three phases of plant growth. There was a consistent positive relationship for NDF between the vegetative and reproductive phases ($R^2 = 0.32$; $P < 0.05$) which strengthened between the reproductive and senesced phases ($R^2 = 0.60$; $P < 0.05$). The NDF of Dalkeith subterranean clover was about 7% higher than Dinninup subterranean clover across all phases of plant growth. The trends for ADF among the forages were similar to NDF. Hemicellulose was markedly higher in annual ryegrass compared to the legumes at each stage of plant growth and the hemicellulose content of the pulses was high compared to the pasture legumes at the senesced phase of growth (Table 4.8).

DMD was highest in annual ryegrass, arrowleaf clover, biserrula, bladder clover and trigonella at the vegetative phase (DMD > 80%) and lowest in the subterranean clovers, vetch, burr medic and lotus (DMD < 73%). Arrowleaf clover, bladder clover, crimson clover, annual ryegrass, balansa clover, chickpea, dwarf chickling and biserrula had the highest DMD (DMD > 75%) at the reproductive phase and Sava snail medic, gland clover, burr medic and Dalkeith subterranean clover had the lowest DMD (DMD < 68%). At the senesced phase the pulses, balansa clover, bladder clover, arrowleaf clover, Dinninup subterranean clover, trigonella and biserrula had the highest DMD (DMD > 60%).

Gland clover, the snail medics, burr medic and ryegrass generally had the highest shear and compression energy across the three phases of plant growth, while the clovers generally had the lowest shear and compression energy.

The number of days from seeding to flowering ranged from 67 days for burr medic to 110 days for arrowleaf clover, with the medics and pulses flowering earliest, and the aerial seeding clovers (arrowleaf, crimson, bladder, balansa and rose clovers) were the last to start flowering.

Table 4.6. Physical and chemical components of 15 pasture legumes, 4 grain legumes and annual ryegrass at the vegetative phase of plant growth

Forage type	**DM (%)**	**N**	**S**	**NDF**	**ADF**	**Hemicel.**	**DMD**	**WSC**	**Comp.**	**Shear**	**Flowering**
Arrowleaf clover cv. Cefalu	15.1	4.8	0.26	23.0	15.4	7.6	81.0	14.5	3.30	6.2	110
Balansa cv. Paradana	13.0	4.2	0.17	26.0	16.6	9.4	76.9	17.2	3.24	6.6	99
Biserrula cv. Casbah	17.0	4.5	0.25	21.9	14.5	7.4	80.7	12.7	3.59	5.0	98
Burr medic cv. Santiago	16.0	4.6	0.20	22.9	16.4	6.5	72.6	13.9	3.66	6.2	67
Crimson clover cv. Caprera	14.4	4.4	0.22	27.9	17.0	10.9	75.8	10.7	3.29	6.0	105
Subterranean clover cv. Dalkeith	16.2	3.7	0.21	33.9	22.1	11.8	69.1	15.0	3.29	7.5	84
Subterranean clover cv. Dinninup	18.3	3.4	0.19	28.1	19.7	8.4	72.6	13.7	3.32	6.1	88
Gland clover acc. CPI 87182	12.8	2.9	0.18	34.3	26.0	8.3	76.3	6.8	3.84	6.2	88
Snail medic cv. Kelson	18.2	4.2	0.20	21.7	14.5	7.3	79.5	15.7	3.71	6.0	80
Lotus acc. SA33851/845	18.7	3.1	0.21	26.1	17.4	8.8	72.9	8.0	3.68	6.9	92
Rose clover cv. Kondinin	19.1	3.9	0.22	27.8	18.3	9.5	75.1	7.5	3.28	7.9	98
Snail medic cv. Sava	20.2	2.9	0.17	26.4	18.2	8.2	76.6	12.9	3.75	6.4	72
French serradella cv. Cadiz	12.1	3.7	0.22	24.4	16.6	7.7	75.8	6.2	3.27	7.9	91
Bladder clover acc. 88TUR593SPU	16.2	3.2	0.18	21.4	14.5	7.0	80.0	8.7	3.34	7.0	104
Trigonella acc. SA5045	18.7	3.1	0.27	20.2	12.1	8.0	84.4	7.5	3.60	3.6	85
Chickpea cv. Tyson	21.8	3.4	0.18	27.3	18.6	8.7	75.1	9.8	3.68	7.1	90
Dwarf chickling cv. Chalus	17.2	5.2	0.23	29.4	21.0	8.4	76.5	13.6	3.50	7.4	85
Grasspea acc. BIO L254	16.6	3.8	0.18	24.9	17.6	7.3	76.0	18.7	3.76	9.2	82
Vetch cv. Languedoc	17.4	4.3	0.22	30.3	20.1	10.1	72.5	12.1	3.67	9.3	78
Annual ryegrass cv. Wimmera	20.4	1.3	0.17	33.6	16.4	17.3	87.3	36.3	3.32	9.0	100
Mean	17.0	3.7	0.21	26.6	17.6	8.9	76.8	13.1	3.50	6.9	90
SE	0.6	0.2	0.01	0.9	0.7	0.5	1.0	1.5	0.05	0.3	3

N, S, NDF, ADF, Hemicellulose, DMD and WSC are all expressed on a dry matter basis.
Compression (Comp.) and Shear energy are expressed as kJ/kg DM.
Flowering refers to the number of days from sowing until approximately 50% of the plants are flowering.

Table 4.7. Physical and chemical components of 15 pasture legumes, 4 grain legumes and annual ryegrass at the reproductive phase of plant growth

Forage type	**DM (%)**	**N**	**S**	**NDF**	**ADF**	**Hemicel.**	**DMD**	**WSC**	**Comp.**	**Shear**	**Flowering**
Arrowleaf clover cv. Cefalu	32.0	2.88	0.14	23.2	14.9	8.3	81.3	16.5	2.79	6.8	110
Balansa cv. Paradana	33.2	2.44	0.12	26.1	17.6	8.5	76.6	21.1	2.94	6.3	99
Biserrula cv. Casbah	33.6	2.65	0.15	24.5	15.9	8.7	75.7	14.6	3.16	7.5	98
Burr medic cv. Santiago	38.6	2.34	0.12	38.9	25.1	13.8	65.3	10.7	4.07	12.1	67
Crimson clover cv. Caprera	33.5	2.67	0.12	29.8	17.4	12.4	76.5	16.5	2.81	7.7	105
Subterranean clover cv. Dalkeith	33.6	1.89	0.12	36.9	25.2	11.7	67.7	19.2	3.12	10.4	84
Subterranean clover cv. Dinninup	35.7	1.98	0.12	29.5	19.4	10.1	73.1	15.4	3.08	9.1	88
Gland clover acc. CPI 87182	41.5	1.70	0.10	39.8	28.9	10.9	64.4	11.8	4.33	12.8	88
Snail medic cv. Kelson	31.4	1.65	0.08	34.8	23.3	11.5	72.0	16.2	3.36	4.5	80
Lotus acc. SA33851/845	36.1	2.24	0.11	27.9	18.3	9.6	69.5	11.2	-	-	92
Rose clover cv. Kondinin	36.3	2.13	0.11	35.2	22.1	13.1	67.5	15.8	3.29	10.7	98
Snail medic cv. Sava	36.0	1.88	0.09	41.5	27.2	14.4	62.8	12.2	4.13	18.6	72
French serradella cv. Cadiz	30.5	2.56	0.13	32.3	21.9	10.4	68.5	18.0	3.37	11.8	91
Bladder clover acc. 88TUR593SPU	32.8	2.02	0.14	29.3	19.2	10.1	78.8	18.8	3.23	7.3	104
Trigonella acc. SA5045	32.2	2.46	0.17	25.8	16.4	9.4	73.3	19.9	3.43	8.4	85
Chickpea cv. Tyson	30.6	2.32	0.11	34.0	22.0	12.0	75.8	13.3	3.72	10.3	90
Dwarf chickling cv. Chalus	30.8	3.12	0.11	29.4	17.2	12.2	75.5	11.8	3.51	7.5	85
Grasspea acc. BIO L254	31.0	2.47	0.11	27.9	16.3	11.7	74.1	17.0	4.2	11.5	82
Vetch cv. Languedoc	30.4	2.37	0.11	32.4	18.4	14.0	73.3	17.9	3.26	9.7	78
Annual ryegrass cv. Wimmera	32.2	0.79	0.12	44.0	21.4	22.6	76.3	36.6	3.16	10.1	100
Mean	33.6	2.23	0.12	32.2	20.4	11.8	72.4	16.7	3.42	9.63	90
SE	0.7	0.11	0.00	1.3	0.9	0.7	1.1	1.2	0.10	0.69	3

N, S, NDF, ADF, Hemicellulose, DMD and WSC are all expressed on a dry matter basis.
Compression (Comp.) and Shear energy are expressed as kJ/kg DM.
Flowering refers to the number of days from sowing until approximately 50% of the plants are flowering.

Table 4.8. Physical and chemical components of 15 pasture legumes, 4 grain legumes and annual ryegrass at the senesced phase of plant growth

Forage type	DM (%)	N	S	NDF	ADF	Hemicel.	DMD	WSC	Comp.	Shear	Flowering
Arrowleaf clover cv. Cefalu	93.5	1.66	0.08	38.8	27.0	11.8	64.1	6.2	4.13	5.7	110
Balansa cv. Paradana	94.0	1.89	0.10	37.6	27.6	10.0	68.1	5.2	4.54	5.4	99
Biserrula cv. Casbah	93.8	2.13	0.12	44.9	30.0	15.0	62.9	6.3	5.3	11.8	98
Burr medic cv. Santiago	94.4	1.28	0.10	45.3	32.7	12.6	58.9	2.9	6.41	15.5	67
Crimson clover cv. Caprera	94.1	2.87	0.13	38.6	24.7	13.9	63.2	4.5	5.16	12.0	105
Subterranean clover cv. Dalkeith	95.0	1.76	0.13	47.7	35.8	11.9	57.9	4.8	4.59	6.1	84
Subterranean clover cv. Dinninup	94.2	1.98	0.12	41.0	28.9	12.2	63.7	6.6	4.75	10.6	88
Gland clover acc. CPI 87182	93.0	1.42	0.10	54.7	40.8	14.0	51.0	4.3	6.16	16.8	88
Snail medic cv. Kelson	93.6	1.53	0.10	49.8	35.5	14.3	56.0	2.9	5.16	14.7	80
Lotus acc. SA33851/845	92.8	1.73	0.10	48.5	32.7	15.7	57.1	-	4.38	6.2	92
Rose clover cv. Kondinin	92.8	2.17	0.11	44.7	29.1	15.6	59.7	4.4	4.12	5.4	98
Snail medic cv. Sava	93.4	1.63	0.09	51.8	37.1	14.7	53.4	3.5	4.98	8.0	72
French serradella cv. Cadiz	94.5	1.77	0.10	48.6	33.6	15.0	54.3	4.1	5.58	17.4	91
Bladder clover acc. 88TUR593SPU	93.8	2.31	0.13	39.9	28.3	11.6	64.2	3.8	4.50	4.8	104
Trigonella acc. SA5045	93.1	2.46	0.13	41.8	28.0	13.9	63.1	5.0	5.41	11.9	85
Chickpea cv. Tyson	93.6	2.68	0.16	44.4	24.4	20.0	63.4	3.1	4.64	9.1	90
Dwarf chickling cv. Chalus	92.5	3.20	0.11	42.7	20.2	22.5	69.7	5.2	4.05	7.4	85
Grasspea acc. BIO L254	92.7	2.42	0.10	39.6	21.7	17.9	70.1	4.9	4.46	4.4	82
Vetch cv. Languedoc	92.9	2.46	0.10	39.5	20.1	19.4	64.1	5.0	5.09	14.0	78
Annual ryegrass cv. Wimmera	93.6	0.63	0.08	62.4	33.2	29.2	54.2	9.0	5.65	14.7	100
Mean	93.6	2.00	0.11	45.1	29.6	15.6	60.9	4.8	4.95	10.1	90
SE	0.2	0.13	0.00	1.4	1.3	1.0	1.2	0.3	0.15	0.98	3

N, S, NDF, ADF, Hemicellulose, DMD and WSC are all expressed on a dry matter basis
Compression (Comp.) and Shear energy are expressed as kJ/kg DM
Flowering refers to the number of days from sowing until approximately 50% of the plants are flowering

Relationships among plant chemical and physical components (Table 4.9, Table 4.10 and Table 4.11)

At each phase of plant growth the fibre measurements; ADF, NDF and Hemicellulose were, in general, strongly and positively correlated. ADF and NDF were negatively related to nitrogen and sulphur content, which were positively correlated, and these positive relationships became stronger as the forage matured. ADF and NDF were negatively related to DMD at all phases of plant growth. ADF was negatively related to WSC, and this was significant at the reproductive and senesced phases. ADF was positively related to shear energy, although this was only significant at the reproductive phase. ADF and NDF were positively related to compression energy at the reproductive and senesced phases (Table 4.10 and Table 4.11). ADF and NDF were negatively related to flowering time at the reproductive and senesced phases.

Shear and compression energy were positively correlated at the reproductive and senesced phases, but there was no relationship between the vegetative and other phases. There was a negative relationship between both compression and shear energy and flowering time across the three phases of plant growth, but not between shear energy and flowering time at the vegetative phase. Compression energy was negatively related to nitrogen content at the reproductive phase. WSC was positively correlated to nitrogen at the vegetative phase, but there was no relationship between WSC and nitrogen at the reproductive and senesced phases.

Figure 4.2, Figure 4.3 and Figure 4.4 are the principal component analysis biplots showing the chemical and physical forage components at the vegetative, reproductive and senesced phases of plant growth and grouped into pasture legumes, grain legume or annual ryegrass. Both grain and pasture legumes were located within the same grouping of data points, while annual ryegrass was placed as an outlying data point in this analysis for all phases. Grain legumes tended to be clustered together within the grouping of legumes. At the vegetative phase, the groups of similar forage components are less distinct compared to the reproductive and senesced phases. This is in agreement with a lower overall correlation between the

Table 4.9. Correlation coefficients (R) between chemical and physical components of annual pasture legumes grazed by Merino wether hoggets at the vegetative phase of plant growth (n=15)

Component	**ADF**	**Comp.**	**DM**	**DMD**	**Flower.**	**Hemicel.**	**N**	**NDF**	**S**	**Shear**
Compression	0.12									
DM	-0.26	0.36								
DMD	$^{+}$-0.65**	0.15	0.07							
Flowering	-0.17	-0.61*	-0.35	0.32						
Hemicellulose	0.44$^{\#}$	-0.43	-0.10	0.51$^{\#}$	0.21					
N	-0.35	-0.36	-0.30	0.07	0.27	-0.04				
NDF	0.95***	-0.05	-0.24	-0.69**	-0.06	0.70**	-0.29			
S	-0.48$^{\#}$	-0.13	0.12	0.46$^{\#}$	0.31	-0.05	0.36	-0.40		
Shear	0.42	-0.42	-0.26	-0.63	0.11	0.29	-0.02	0.43	-0.45$^{\#}$	
WSC	-0.10	-0.11	0.06	-0.13	-0.17	0.09	0.53*	-0.05	-0.20	-0.12

Significance of differences: $^{\#}P < 0.1$; $*P < 0.05$; $** P < 0.01$ and $***P < 0.001$.

$^{+}$ An outlying data point for gland clover was excluded

Table 4.10. Correlation coefficients (R) between chemical and physical components of annual pasture legumes grazed by Merino wether hoggets at the reproductive phase of plant growth (n=15)

Component	ADF	Comp.	DM	DMD	Flowering	Hemicel.	N	NDF	S	Shear
Compression	0.81***									
DM	0.61*	0.69**								
DMD	-0.90***	-0.82***	-0.61*							
Flowering	-0.70**	-0.73**	-0.38	0.79***						
Hemicellulose	0.70**	0.55*	0.47#	-0.74**	-0.61*					
N	-0.76**	-0.52*	-0.38	0.55*	0.52*	0.44#				
NDF	0.97***	0.78***	0.61*	-0.91***	-0.72**	0.85***	-0.71**			
S	-0.72**	-0.39	-0.34	0.54*	0.41	-0.61*	0.70**	-0.73**		
Shear	0.69**	0.73**	0.57*	-0.81***	-0.56*	0.65**	-0.26	0.73**	-0.32	
WSC	-0.55*	-0.67**	-0.74**	0.55*	0.48#	-0.57*	0.24	-0.59*	0.47#	-0.57*

Significance of differences: #P < 0.1; *P < 0.05; ** P < 0.01 and ***P < 0.001.

Table 4.11. Correlation coefficients (R) between chemical and physical components of annual pasture legumes grazed by Merino wether hoggets at the senesced phase of plant growth (n=15)

Component	ADF	Comp.	DMD	Flowering	Hemicel.	N	NDF	S	Shear
Compression	0.48#								
DMD	-0.90***	-0.50#							
Flowering	-0.65**	-0.59*	0.58*						
Hemicellulose	0.29	0.29	-0.58*	-0.20					
N	-0.74**	-0.35	0.57*	0.58*	0.08				
NDF	0.95***	0.50#	-0.96***	-0.63*	0.56*	-0.62*			
S	-0.33	-0.06	0.34	0.16	-0.03	0.72**	-0.30		
Shear	0.43	0.88***	-0.55*	-0.44#	0.47#	-0.28	0.51*	-0.11	
WSC	-0.49#	-0.41	0.57*	0.48#	-0.28	0.38	-0.51*	0.24	-0.32

Significance of differences: #P < 0.1; *P < 0.05; ** P < 0.01 and ***P < 0.001.

components at the vegetative phase. However, at the vegetative phase NDF and shear energy grouped together as did DMD and flowering time.

At the reproductive phase ADF, shear energy, compression energy and DM are grouped together and opposite to DMD and flowering. Similarly at the senesced phase ADF, shear energy and compression energy are grouped together opposite DMD and nitrogen. At all three phases hemicellulose and WSC are grouped together.

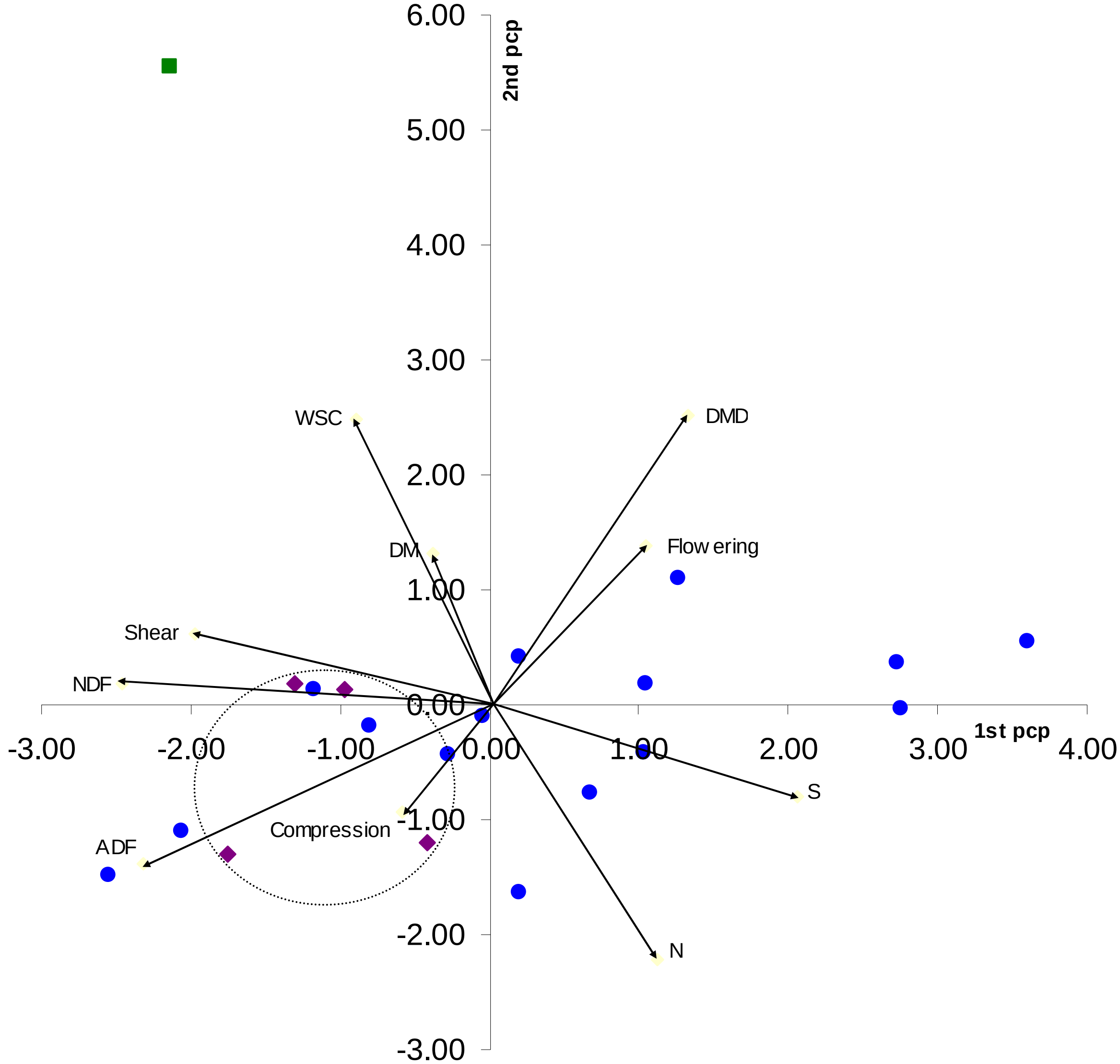

Figure 4.2. Principal component analysis biplot of the chemical and physical characteristics for annual legumes (●), grain legumes (◆) and annual ryegrass (■) at the vegetative phase of plant growth. The dotted line is drawn around the grain legumes.

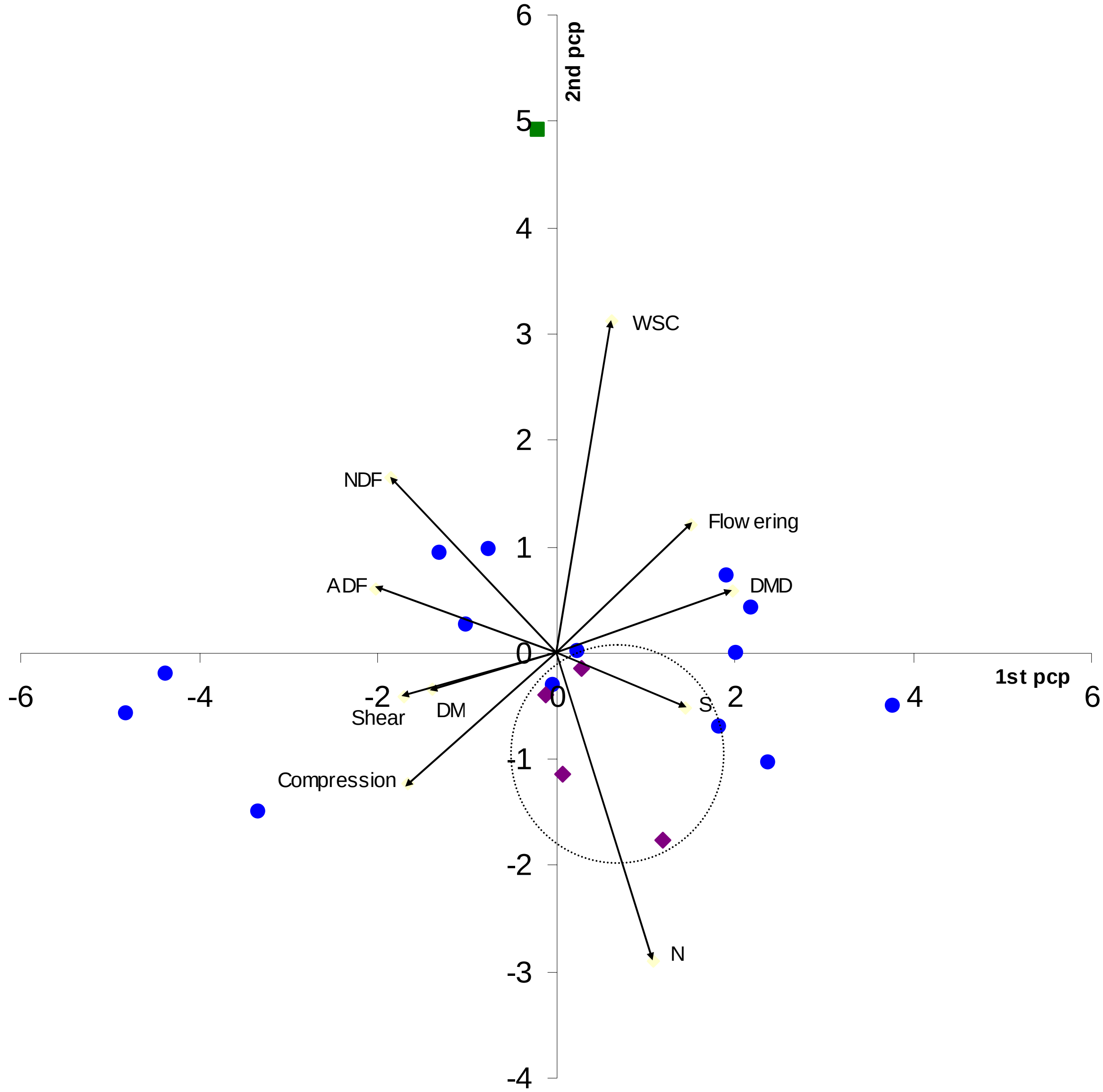

Figure 4.3. Principal component analysis biplot of the chemical and physical characteristics for annual legumes (●), grain legumes (◆) and annual ryegrass (■) at the reproductive phase of plant growth. The dotted line is drawn around the grain legumes.

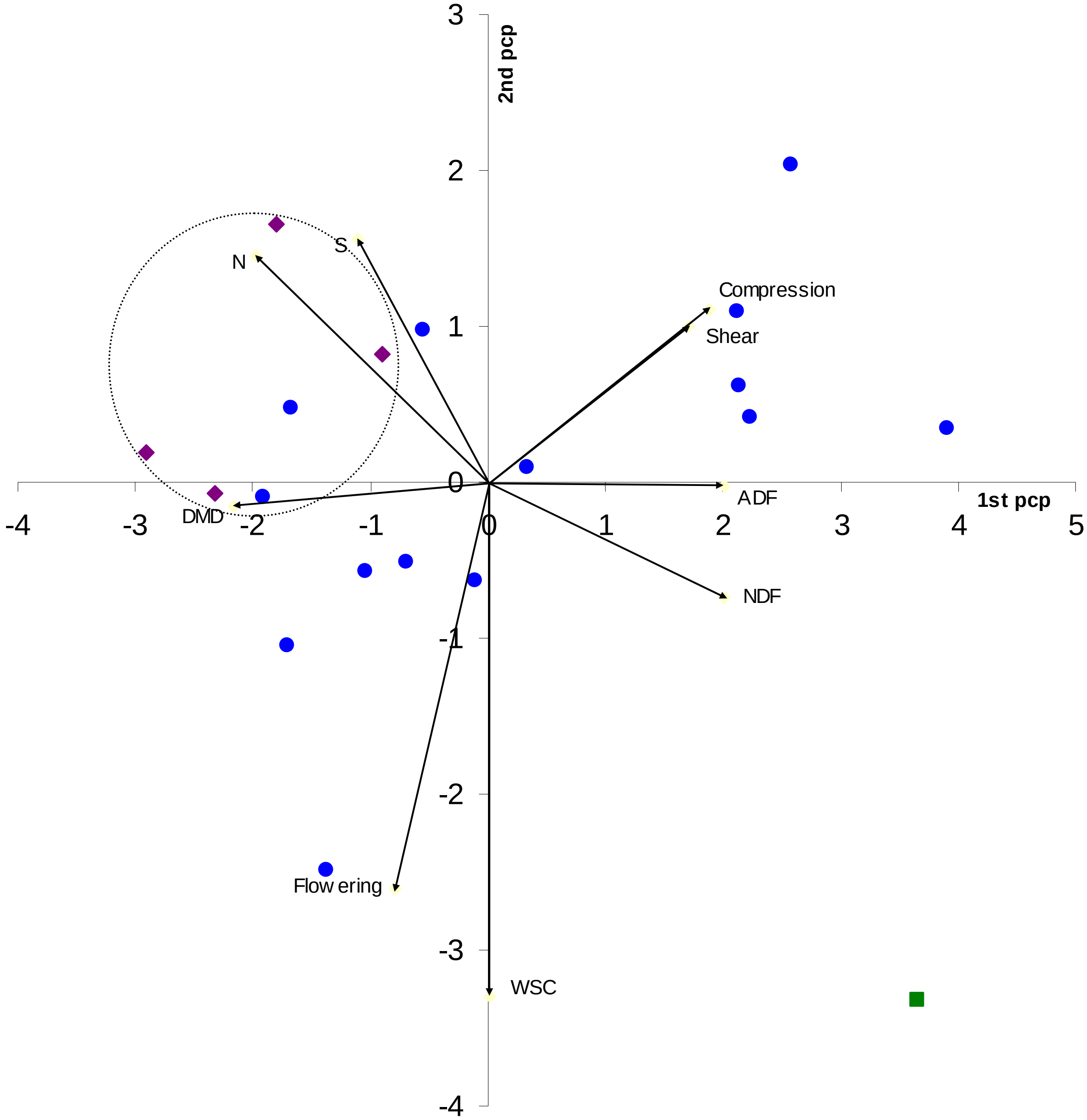

Figure 4.4. Principal component analysis biplot of the chemical and physical characteristics for annual legumes (●), grain legumes (◆) and annual ryegrass (■) at the senesced phase of plant growth. The dotted line is drawn around the grain legumes.

Relationships among plant characteristics and the relative preference of sheep (Table 4.12)

Many of the plant chemical and physical characteristics measured were related to relative preference and the relationships differed between the three phases of plant growth. At the vegetative phase compression energy was negatively related to relative preference (R = -0.71, P < 0.01) and no other relationships were significant at this phase. Compression energy was also negatively related to relative preference at the reproductive and senesced phases of plant growth. Shear energy was negatively related to relative preference at the senesced phase. ADF and NDF were negatively related to relative preference at the reproductive and senesced phases. Nitrogen, DMD and flowering time were positively related to relative preference at the reproductive and senesced phases as was WSC.

Table 4.12. Correlation coefficients (R) among chemical and physical components of annual pasture legumes and their relative preference by Merino wether hoggets (n = 15)

	Vegetative	**Reproductive**	**Senesced**
ADF	-0.10	-0.64*	-0.81***
Compression energy	-0.71**	-0.47#	-0.52*
DM	-0.34	-0.19	n/a
DMD	-0.03	0.63*	0.74**
Flowering time	0.41	0.67**	0.57*
Hemicellulose	0.26	-0.26	-0.36
Nitrogen	0.31	0.76**	0.64*
NDF	0.01	-0.56*	-0.81***
Sulphur	0.13	0.57*	0.19
Shear energy	0.23	-0.33	-0.45#
WSC	-0.08	0.17	0.55*

Significance of differences: #P < 0.1; *P < 0.05; **P < 0.01 and ***P < 0.001.

The physical and chemical characteristics of the forage were compared with relative preference using principal component analysis. Annual ryegrass was excluded from these analyses because its physical and chemical characteristics differed markedly from those of the legumes. Figure 4.5, Figure 4.6 and Figure 4.7 are the principal component analysis biplots showing chemical and physical forage components at the vegetative, reproductive and senesced phases of plant growth and grouped as high, neutral or low relative preference by sheep. Relative preference rankings were determined as those either significantly higher, the same, or significantly lower than neutral preference. At the vegetative phase the relative preference of the forage did not appear to be related to physical and chemical plant characteristics measured (Figure 4.5). At the reproductive and senesced phases the relative preference of the sheep among the forages was related to the physical and chemical plant characteristics, and the grouping was the strongest at the senesced phase of plant growth (Figure 4.6 and Figure 4.7). At these phases ADF and NDF and to a lesser extent shear and compression were associated with forages of low relative preference. On the other hand, nitrogen, sulphur, DMD and to a lesser extent WSC were associated with forages of high relative preference.

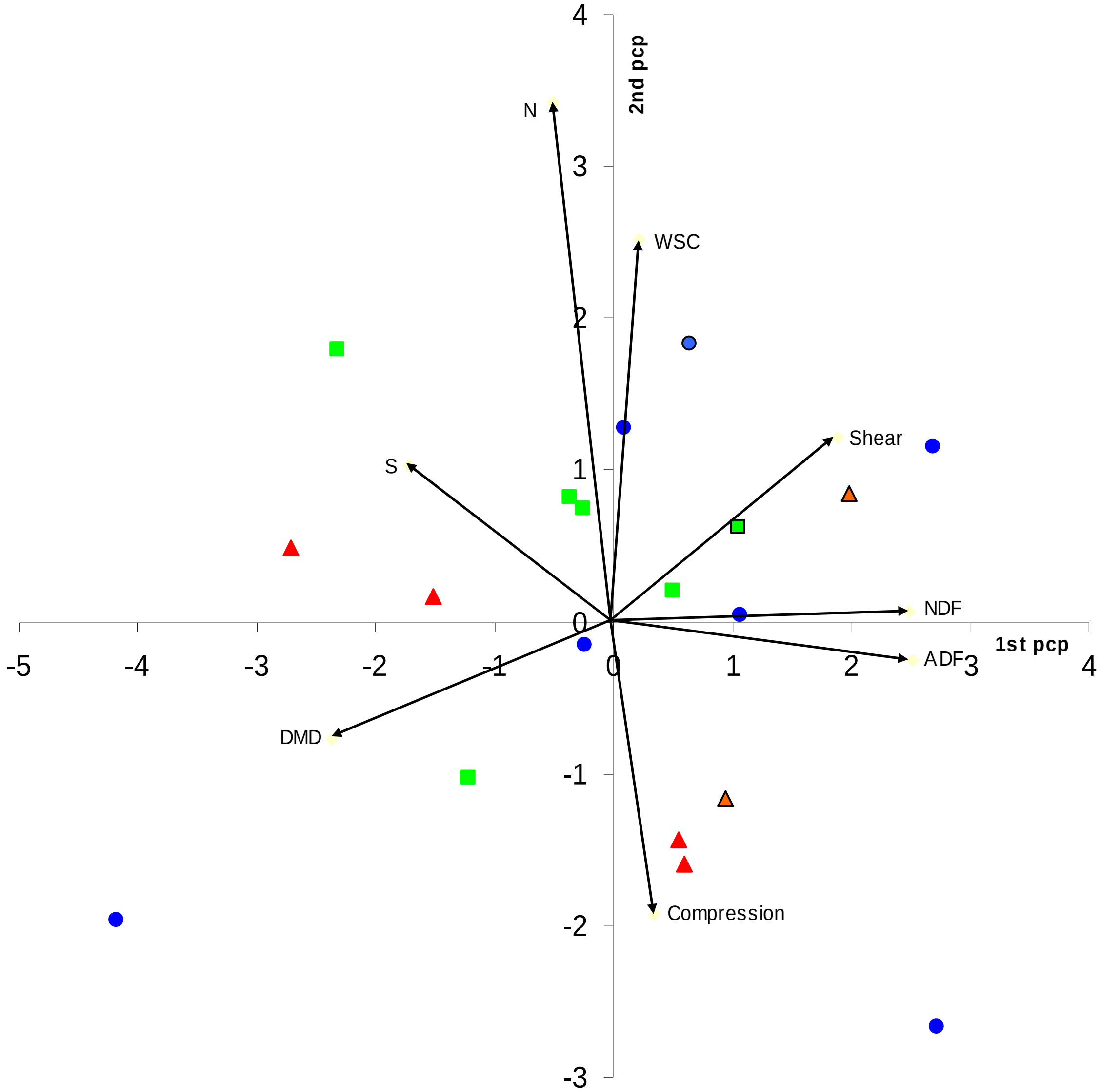

Figure 4.5. Principal component analysis biplot of chemical and physical characteristics of annual legumes of high (■), neutral (●) and low (▲) relative preference by sheep at the vegetative phase of plant growth. Symbols with a bold border represent grain legumes and unbordered symbols represent pasture legumes.

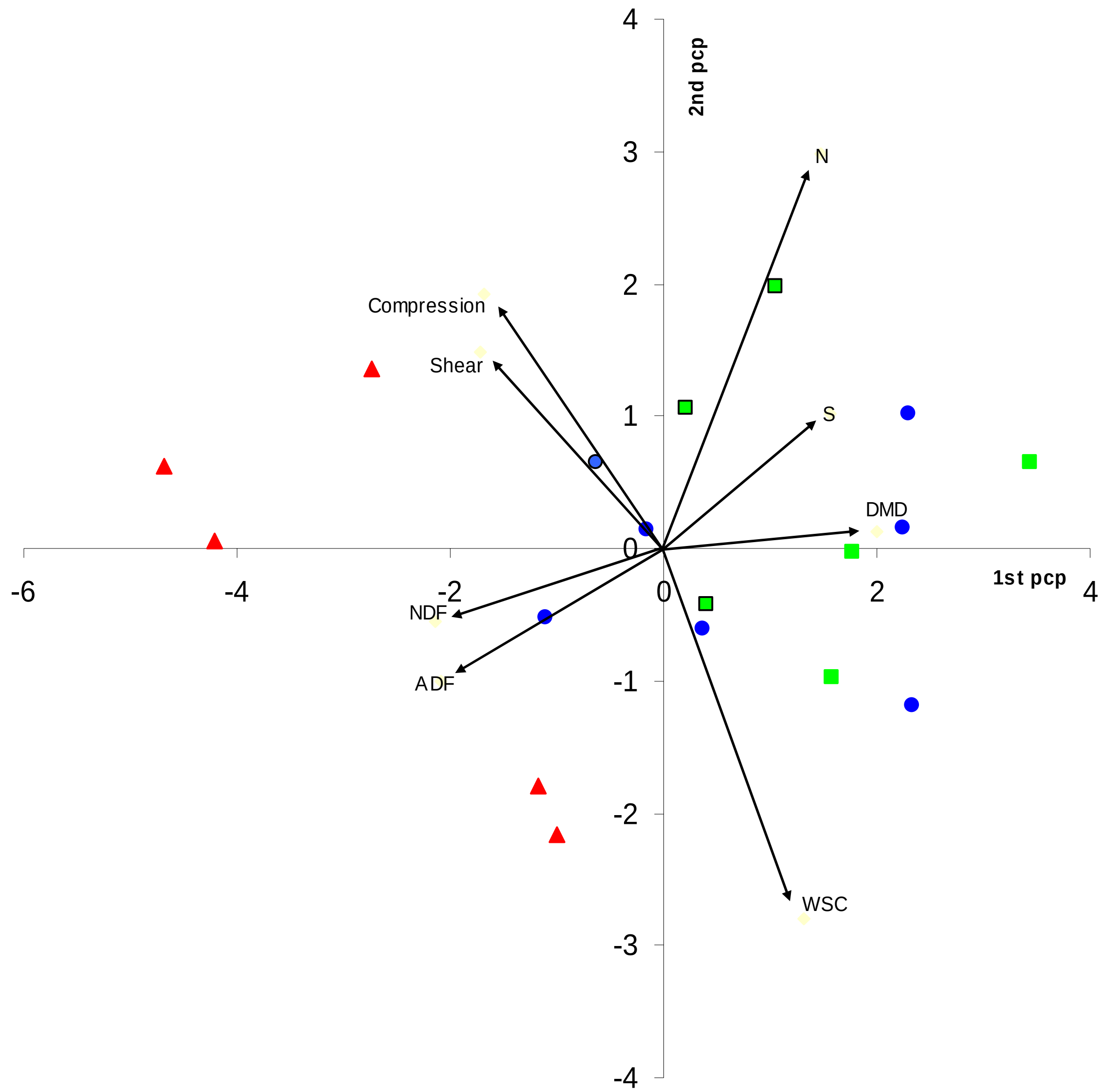

Figure 4.6. Principal component analysis biplot of chemical and physical characteristics of annual legumes of high (■), neutral (●) and low (▲) relative preference by sheep at the reproductive phase of plant growth. Symbols with a bold border represent grain legumes and unbordered symbols represent pasture legumes.

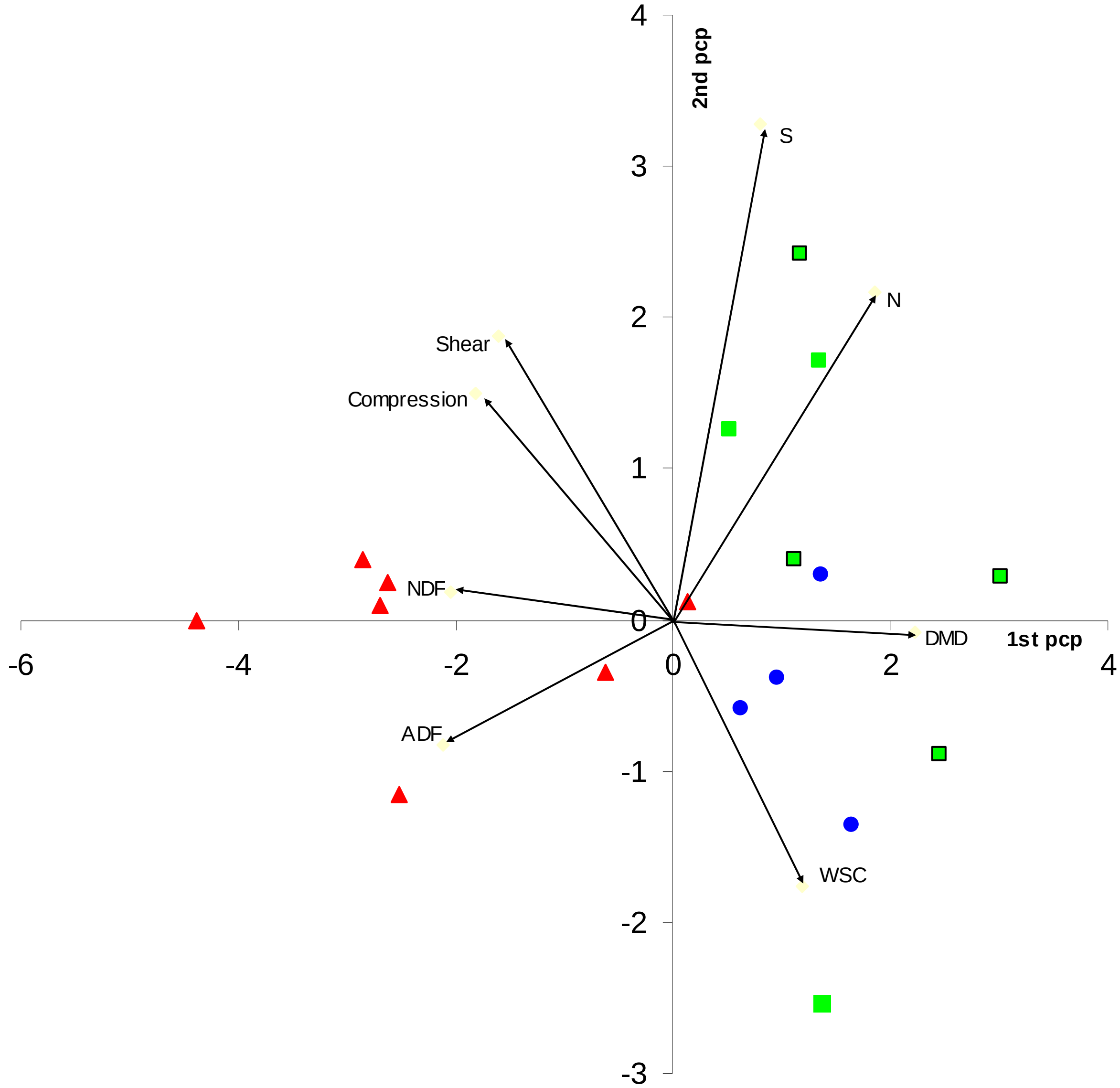

Figure 4.7. Principal component analysis biplot of chemical and physical characteristics of annual legumes of high (■), neutral (●) and low (▲) relative preference by sheep at the senesced phase of plant growth. Symbols with a bold border represent grain legumes and unbordered symbols represent pasture legumes.

Near Infrared Reflectance Spectroscopy (NIRS)

Near Infrared Absorbance (1/NIR) was correlated with plant physical and chemical characteristics (Table 4.13). Nitrogen was found to be well predicted by NIRS at all phases of plant growth, but the closeness of fit between NIRS predicted and the levels measured for other characteristics varied between the different phases of plant growth.

Several wavelengths (λ) were found to correlate with relative preference and, except for the one outlying value for grasspea, the preference values predicted from the NIR spectrum related strongly to actual relative preference values at the vegetative, reproductive and senesced phases of growth (R^2 = 0.82, 0.66, 0.92; $P < 0.001$); Figure 4.8. Relative preference could be predicted using wavelengths 1724 and 2228 at the vegetative phase, 2236 at the reproductive phase and 1724, 1788 and 2244 at the senesced phase.

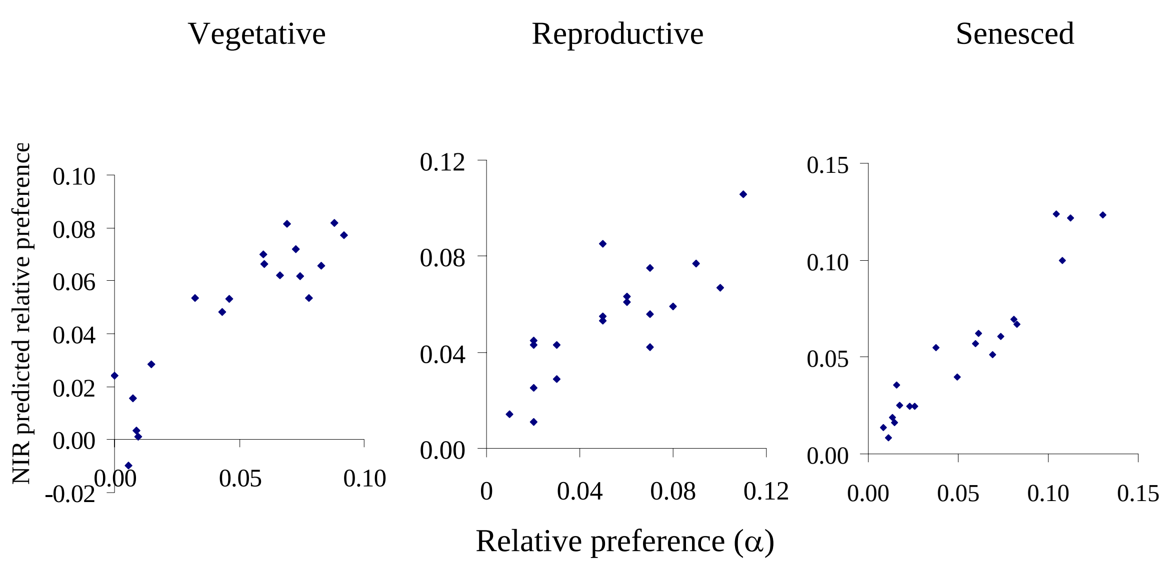

Figure 4.8. Relationship between relative preference and NIRS predicted relative preference at the vegetative (n = 19, one outlier removed; grasspea), reproductive (n = 20) and senesced phases (n = 20).

Table 4.13. Correlation coefficients (R^2) among chemical and physical components of annual pasture legumes, relative preference (α) and their NIRS predicted value (n = 20)

	Correlation coefficient (R^2)			Prediction points (number of wavelengths)		
	Vegetative	**Reproductive**	**Senesced**	**Vegetative**	**Reproductive**	**Senesced**
α	0.82***	0.66***	0.92***	2	1	3
ADF	0.68***	0.66***	0.94***	3	2	2
Compression energy	0.88***	0.72***	0.62***	3	2	3
DM	0.40**	0.63***	n/a	1	2	n/a
DMD	0.66***	0.40**	0.85***	2	1	2
Flowering time	0.01	0.25***	0.76***	1	1	3
Hemicellulose	0.82***	0.81***	0.63***	3	2	1
Nitrogen	0.96***	0.94***	0.84***	3	3	2
NDF	0.76***	0.68***	0.77***	3	1	3
Sulphur	0.60***	0.45**	0.54***	2	1	2
Shear energy	0.75***	0.40**	0.56***	3	1	2
WSC	0.78***	0.65***	0.39**	2	3	2

Significance of differences: *P < 0.05; **P < 0.01 and ***P < 0.001.

Discussion

Our hypothesis that sheep would select forages of higher nutritive value was supported at the reproductive and senesced phases of plant growth. At these phases sheep preferred forages of higher nitrogen content and digestibility, and lower fibre than the average of that available, which is in agreement with most literature (Provenza 1995). Selection for higher nutritive value was not exhibited at the vegetative phase, which was not surprising given that the average nutritional components of the forages was well in excess of the animals requirements. At this phase only compression energy was significantly related (negatively) to relative preference, supporting the view that sheep may select for forage with a higher potential intake rate at the vegetative phase. Shear energy was not significantly related to relative preference at the vegetative and reproductive phases and had only a weak, negative relationship at the senesced phase. The shear energy values measured for the pasture and grain legumes were low relative to cereal hays and may not have been high enough to influence preference (Dynes, pers. comm.).

Although the nutritive value of the legumes decreased substantially between the vegetative and reproductive phases the animal's nutrient intake at the reproductive phase is still likely to have exceeded their requirements. Despite this, sheep showed a clear change in their selectivity for nutritive value between the two phases by selecting forages of higher nitrogen and lower fibre content at the reproductive phase but not at the vegetative phase. It is possible that the pasture eaten prior to the sheep being tested was less than adequate and so they increased their selection of plants of higher nutritive value as has been demonstrated by (Villalba and Provenza 1999). Prior to the experiment the sheep were grazed on pasture containing a high proportion of non-legume plants and this may have become nutritionally limiting by the reproductive phase (mid-October).

The relative preference (α) of the sheep for senesced forage had a strong negative linear relationship to ADF (R = -0.87; including the grain legumes). Cilliers and van der Merwe (1993) have also reported a strong negative relationship between ADF and dry matter intake for sheep (R = -0.924) and cattle (R = -0.922) grazing *Cymbopogon* and *Themeda* summer veld herbage. In our study the relative preference (α) of sheep among senesced legumes can be predicted by the following linear equation:

$$\alpha_{senesced} = -0.0061 \times ADF + 0.235 \; (R^2 = 0.76)$$

Where neutral preference is 0.05, estimated ADF for neutral preference is 30.3%. Given that the range of neutral preference for senesced material in this study was $0.020 \geq \alpha \geq 0.080$, we can predict that senesced material $25.4 \geq ADF \geq 35.2$ will be of neutral preference and forage with an ADF below this range will be preferred and forage above will be avoided.

The high variation in ADF provides an explanation for the very low preference of Dalkeith subterranean clover at the senesced phase, while Dinninup subterranean clover had a neutral relative preference (ADF%: 35.8 *v* 28.9, α: 0.01 *v* 0.07). Higher ADF and NDF in Dalkeith compared to Dinninup subterranean clover was also reported by Wang (1997). Baker and Dynes (1999) suggest that digestibility could be used as a parameter to select legumes of improved feeding value because IVD accounts for 60% of the variability in voluntary food intake. Similarly, ADF may be a useful selection parameter for plant breeders to improve the feeding value of annual legumes at senescence. The sensory mechanisms that sheep use to preferentially select forages of low ADF are unclear. However, the results from this experiment suggest that neither the lower shear nor compression force, nor a higher digestibility of the forage, accounts for their preference. Other plant attributes such as taste and texture may also be of importance.

The strong relationship between ADF and the relative preference of sheep found in this study seems contrary to the view of Arnold (1964) who suggests that because of the diversity of chemical compounds found in plants that sheep detect through taste, smell and touch it is unlikely that any single compound would individually contribute to relative preference among plants. Although ADF is not a single compound it does demonstrate that, in some cases, a single plant characteristic can have a dominant relationship with diet selection. However, the diversity of chemical compounds may also be reduced in senesced plants making it more likely to isolate individual plant characteristics that contribute to animal preferences. Although ADF does not account for all of the variability in relative preference among the 19 senesced annual legumes in this experiment, the strength of this relationship links this component closely to the selectivity of sheep at the senesced phase. The strength of this relationship probably contributes to the high prediction accuracy of relative preference by the NIR spectrum at the senesced phase.

The significance of protein and fibre as factors influencing the preference of sheep may be linked to the differences in the accumulation and loss of these plant components, respectively, as pasture forage matures. What is evident in the change in plant composition is that protein decreases most between the vegetative and reproductive phases of growth while ADF and NDF increase most during the reproductive and senesced phases of growth. Protein was positively related to preference and fibre was negatively related to preference at both the reproductive and senesced phases of plant growth, but at the reproductive phase protein had a stronger correlation with relative preference than fibre (R = 0.76 *v* -0.64). At the senesced phase fibre had a stronger correlation with relative preference than nitrogen (R = -0.81 *v* 0.64). The sharp drop in protein between the vegetative and reproductive phases may explain its high correlation with preference at this phase. There is evidence in the literature to suggest that sheep can select foods to meet their protein requirements (Cropper *et al.* 1986; Kyriazakis and Oldham 1993).

In our study compression energy had a stronger relationship with relative preference than did shear energy. Compression energy may be more closely linked to potential intake rate than shear energy due to a greater effect on the animal's communition time. Additionally, the shear energy of the legumes in this study may have been lower than the level at which plant strength inhibits intake. So although the relationship between shear energy and preference was consistently negative across the three phases of growth, this may not have influenced preference greatly. A low, but significant correlation between shear strength and preference has been reported in cattle among different forage grasses (MacAdam and Mayland 2003). Similarly, Bergman *et al.* (2001) show evidence that bison forage to minimize grazing time rather than to maximise their energy intake. The significance of shear and compression in influencing preference may also be confounded by their relationship to plant chemical attributes (Villalba and Provenza 1999). Shear and compression energy were more variable at senescence and more variable among pulse varieties. The variability among the pulses may have been due to the hardness of their large seeds and the number of seeds included in each replication of the shear and compression measurement may have varied considerably.

In addition to differences in plant physical and chemical components among different plant genotypes it is well established that the components of different plant parts differ markedly (e.g. Allden and Geytenbeek 1980; Ru and Fortune 2000). For example, the proportion of stem and leaf differs among grasses and sheep tend to avoid grasses with a higher proportion of stem (O'Reagain 1993). However, the magnitude of the differences in physical and chemical characteristics among plant parts varies among plant genotypes. For example, the fibre content in the leaf and stem of plant A might be very similar, whereas in plant B the fibre content might be much higher in the stem compared to the leaf. So, it is likely that variation between the plant parts in each monoculture sward will have significant interactions in diet selection, although in the present study we have considered the entire above-ground plant material in determining preference.

Dry matter content is reported to affect diet selection (Kenney *et al.* 1984). In our study DM was not correlated with relative preference. Sheep have been reported to reduce their daily dry matter consumption when grazing forage that contains less than 16% dry matter (Lloyd Davies 1962) in (Purser 1981). A possible explanation for the lack of effect of DM is that, by testing relative preference among a wide range of forages varying in many physical and chemical characteristics, factors with greater effect on the sheep’s preference may reduce the correlation of some factors that may contribute to preference but to a lesser extent.

The potential to utilise NIR Spectroscopy (NIRS) in evaluating the short term relative preference of sheep among legumes was demonstrated in this study. Not only does NIRS offer a simple method of measuring plant nutrients that may relate to animal preference, but it can cover a much wider range of chemical entities and may therefore be used to detect relationships between preference and compounds that are not commonly measured, for instance plant secondary compounds. Although the chemical composition of forage is commonly reported to influence the animal's preference, attempts to relate chemical composition to preference may not be conclusive unless a very specific treatment is imposed e.g. (Ciavarella *et al.* 2000). This is probably due to both the complex and variable nature of plant chemical components and the plasticity in the grazing behaviour of sheep because of the animal factors contributing to preference. In this experiment, the physical and chemical characteristics of the forages correlated particularly well with their relative preference to sheep at the senesced phase, but at the vegetative phase the relative preference of the forage was not well related to its chemical characteristics. NIR allows coverage of a much broader range of chemical interactions simultaneously and may therefore reduce variability in measures of preference due to the complex chemical composition of forages, which would be a large step in our understanding of preferential grazing behaviour. This view is supported by the improvement we see in prediction of preference at the vegetative phase by NIR compared to the plant components that we measured. Conversely, at the senesced phase the plant components that we measured had greater relevance to the sheep's preference and preference could be predicted equally well using NIR or plant chemical attributes.

Chapter 5

Selection by sheep among pasture legumes, crop legumes, annual ryegrass and wild radish: Animal House experiment

Introduction

Sheep choose their diet from pastures by actively selecting their preferred forage components. They exhibit preferences between plant patches, plant species and plant parts and their choice is related to differences in the physical and chemical characteristics of the forage modified by the many behavioural responses of the individual (Arnold 1981). The physical and chemical characteristics of pasture plants change constantly during each season as plants complete their life cycle and the preference of sheep between plant genotypes changes accordingly.

In the two previous experiments (Chapter 2 and Chapter 4) the short-term relative preference of sheep between a wide range of annual legumes and annual ryegrass was examined. Hoggets grazed selectively at three separate phases of plant growth, vegetative, reproductive and senesced and their preference between plant genotypes differed between the three phases. The legumes that were preferred and those that were avoided by sheep were identified, but restrictions in the methodology of testing in the field were identified. In this chapter we examined a novel indoor method for evaluating the short-term relative preference of sheep between pasture plants, with the aim of overcoming some of the limitations of the field method.

There are at least four major restrictions in the methodology when using field studies. First, residual weeds in the pasture are difficult to control. Chemical herbicides may be required, which is not desirable because these may also affect the growth and development of the experimental plants. Second, plant growth in the field depends on seasonal conditions, so results are likely to vary between seasons. Dumont *et al.* (1995) cited the improved control of the experimental conditions as an important reason to develop an indoor method to test feeding preferences. Third, site variability affects plant growth and therefore increases variability in the estimation of relative preference. In addition to these experimental constraints, a fourth problem in setting up a field experiment is that they are expensive in both time and resources. The requirement for fencing equipment, large numbers of stock and land meant that carrying out the field study was expensive. We believed that it would be possible to design an indoor study to test the same hypotheses as those originally tested in the field study.

In this test we sought a wide diversity of preference options; so we chose those species that were known to contrast greatly one from the other from the results of our short-term preliminary study into relative preferences. The field study showed that Caprera crimson clover was highly preferred at the vegetative, reproductive and senesced phases of plant growth so this plant was selected in this experiment as a highly preferred species. Snail medic had a low relative preference to hoggets at these three phases so was used as a universally unattractive species. By comparison with these two species, the relative preference of hoggets for chickpea is low at the vegetative phase, neutral at the reproductive phase and high at the senesced phase. This pattern was also found in Languedoc vetch, another grain legume, so we considered it important to test whether this large shift in relative preference could be identified in the indoor study. In contrast to the two grain legumes, the relative preference of hoggets for Dalkeith subterranean clover was found to decrease with maturity and because of the importance of subterranean clovers to West Australian pastures this was also included for comparison. The relative preference of hoggets for Casbah biserrula increased between the vegetative and reproductive phases and then decreased at the senesced stage. French serradella had a neutral short-term relative preference at the vegetative and reproductive phases and a low relative preference at the senesced phase, so was chosen as a neutral comparison and this seemed an appropriate species because of the increasing interest of graziers for this line of serradella that has been developed more recently. Our hypothesis was that the same trends in short-term relative preference reported above could be measured by offering sheep combinations of these forages grown and presented in pots. It should be emphasised that it was not our aim to replicate the field study in an indoor study, but to compare between the patterns of relative preference exhibited by sheep under the two different experimental conditions.

In our study we also tested the hypothesis that the relative preference exhibited by the sheep for the plants offered would differ between the individual sheep tested. In addition to variability in the preference of sheep between forage types, individual sheep from the same flock may vary widely in their diet selection. A study by Arnold (1964) showed that the proportion of green grass in the diet of sheep offered a mixture of dry grass and dry clover with a "pick" of green grass varied from 10% to 80% of the total forage intake between individuals. The consistency of the proportion of green grass selected by individual sheep in Arnold's experiment also varied widely. Of the 20 replicates the 6 most consistent sheep had a range of < 10% in the green grass selected in their diet while the 8 least consistent (possibly the least selective) had a range > 40% in the proportion of green grass selected in their diet (Arnold 1964). Similarly Scott and Provenza (1999) found that the relative preference of lambs for alfalfa and barley is highly variable, with one lamb selecting a 94% barley diet, while another selected a 70% alfalfa diet.

The preference exhibited by sheep between forage types is related to forage accessibility and the spatial characteristics of the forage, especially its height (O'Reagain 1993; Hutchings *et al.* 2000b) and potential intake rate (Kenney and Black 1984). Because of this, we hypothesised that the morphological characteristics (height, density and width) of the forage offered would affect the short-term relative preference of sheep between legumes in the indoor study.

We anticipated that potential intake rate would be reduced by grazing due to the expected depletion of the most edible plant parts in the preferred forages and so we hypothesised that relative preference for the initially less preferred plants would increase over time.

Materials and Methods

Experimental Design

Sheep were offered combinations of 3 different plant genotypes from separate pots presented in a purpose-built feeding trolley in a series of 3-way preference tests. The 3 genotypes in each combination (treatment) comprised one of six annual legumes along with annual ryegrass and wild radish. The six treatments were offered to each of six sheep according to a 6x6 Latin-square design balanced for residual effects (Cochran and Cox 1957).

Beginning at 9:00 am each day, a pre-determined treatment combination (see *Forage combinations*) was offered, and re-offered, to the same sheep six times in consecutive 1-minute periods, each 30 minutes apart. This was repeated for each of the six forage combinations. The three pots in each treatment combination were presented to each sheep simultaneously, but the position of each pot was assigned alternately for each of the six times the forage combination was offered. The pots were weighed before and after each feeding period. Each day, for 6 days, each sheep was offered a different treatment for 6 1-minute intervals (as previously described). Treatments offered on day 1 was re-offered (with fresh plants) on day 9. At other times, sheep were given free access to oaten chaff and lupins (discussed below) *ad libitum*, except when they were denied from one hour prior to the start of experimentation each day.

The experiment was conducted in three sections so that the relative preference was determined for the different plant genotypes at three phases of plant growth; vegetative (Day 69), reproductive (Day 119) and senesced (Day 228).

Relative preference (α)

Whether or not sheep exhibited selectivity in their choice between the six legumes, relative to annual ryegrass and wild radish, and the magnitude of these preferences, was determined using an adaptation of the Chesson-Manly selection index (see General Experimental Materials and Methods), to estimate relative preference (α). Relative preference was calculated using the amount of food available and the forage intake of the sheep for the first and second of the six 1-minute periods. By the third period the intake rate of the sheep had decreased significantly at the vegetative and senesced phases, so the relative preference determined after this could not be used to make a valid comparison of real preferences between genotypes as the plant material being offered was incomplete.

Forage intake and availability

The forage intake (g fresh weight/sheep.minute) of the sheep was determined by weighing the pots of forage before and after they were offered for each 1-minute period. The plant material that remained after the preference test was cut at ground level using a serrated knife and the pot was re-weighed to obtain an estimate of total fresh forage biomass that had been offered to the animals. Calculations of intake made on a fresh weight basis among forage plants have been found to be similar to those made on a dry weight basis (Walker *et al.* 1994).

Forage types, their management and preparation

Six legumes; biserrula (*Biserrula pelecinus* cv. Casbah), chickpea (*Cicer arietinum* cv. Tyson), crimson clover (*Trifolium incarnatum* cv. Caprera), French serradella (*Ornithopus sativus* cv. Cadiz), snail medic (*Medicago scutellata* cv. Sava), subterranean clover (*Trifolium subterranean* cv. Dalkeith) and two weeds of crops; annual ryegrass (*Lolium rigidum* cv. Wimmera) and wild radish (*Raphanus raphanistrum*) (Table 5.1) were grown outdoors, under shade-cloth, in 25 cm diameter black plastic pots. A total of 130 pots of annual ryegrass, 130 pots of wild radish and 24 pots of each of the six legume genotypes were sown using inoculated and lime pelleted seed at an appropriate seeding rate on day 0 (Table 5.1). The pots were then placed on plastic sheeting to prevent the plants roots from growing into the soil and watered as required to optimise plant growth.

The six legumes, annual ryegrass and wild radish were sown into pots containing potting mix on May 1st 2001 (Day 0) and 5 g of Osmocote® fertiliser. Six kilograms of potting mix was used to fill each pot to 90% of volume. This comprised a mixture of pinebark, sawdust, riversand and peat to which were added nutrients and lime to correct for pH (Peter Skinner, El-Wassat Nursery, Orange Grove, WA). Folimat 450® insecticide was applied at 2 ml/L on Day 36. 500 ml of 2 g/L Thrive® fertiliser was applied to each pot on Day 78.

Table 5.1. Common and scientific names, the rate of seeding (g/pot) and group of inoculum applied to seed for the 8 plant genotypes grown

Common name	**Scientific name**	**Seeding rate (g/pot)**	**Inoculum Group**
Annual ryegrass cv. Wimmera	*Lolium rigidum* cv. Wimmera	2.0	no inoculum
Wild radish	*Raphanus raphanistrum*	1.5	no inoculum
Biserrula cv. Casbah	*Biserrula pelecinus* cv. Casbah	1.5	Biserrula
Chickpea cv. Tyson	*Cicer arietinum* cv. Tyson	6.0	N
Crimson clover cv. Caprera	*Trifolium incarnatum* cv. Caprera	1.5	C
French serradella cv. Cadiz	*Ornithopus sativus* cv. Cadiz	3.0	S
Snail medic cv. Sava	*Medicago scutellata* cv. Sava	2.5	AM
Subterranean clover cv. Dalkeith	*Trifolium subterraneum* cv. Dalkeith	2.0	C

At the vegetative and reproductive phases of growth, one pot of each legume genotype, 6 pots of annual ryegrass and 6 pots of wild radish were randomly selected and transported to the sheep preference testing area. Any loose soil was removed from the pots and care was taken not to handle the plant foliage. Evapo-transpiration from the pots containing each of the plant genotypes was measured on Day 63 by taking consecutive measurements of the weight of the pots every 5 minutes for 30 minutes. At the senesced stage of plant growth the plant material was thought to be too fragile to be offered to the sheep as entire plants and so it was cut into lengths of about 2 cm and offered in 25 cm diameter black plastic pots, double lined so that material would not fall through the drainage holes.

At the vegetative and reproductive stages of the experiment the height, width and density of the plant material offered was assessed. Height was measured by recording the distance between the soil and the highest point of the plant. Density was visually assessed by looking down on the pots and using a 0 – 5 ranking scale, where 0 was given for a very light forage distribution with a lot of soil visible and 5 for dense forage with no soil visible in the pot. Width was measured as the distance across the pot where the forage plants were of maximum diameter.

Animals, housing and diet

Three groups of ten hogget Merino wethers were selected from a commercial flock at The University of Western Australia's research farm at Wundowie (Allandale Farm). Hoggets were chosen because it was considered that their relative naïvity to different pasture genotypes would create less bias in their preference between the forages. The three groups of wethers were transported to the UWA animal house at Shenton Park in Perth two weeks prior to the commencement of each phase of preference testing (June, August and December 2001) and quarantined over this period. The sheep were housed in individual pens, but were able to see all other experimental sheep. The sheep were weighed and the mean weights of each group of wethers was 43 ± 1.5 kg (vegetative phase), 54 ± 1.4 kg (reproductive phase), 50 ± 1.7 kg (senesced phase).

The wethers were put into individual pens and each was initially offered 800 g of 80% oaten chaff, 18% lupins and 2% of a complete mineral mix for 7 days. Following this their diet was increased daily by 200 g increments until the sheep were eating *ad libitum* and this was maintained for the duration of the experiment. Perth metropolitan drinking water was provided *ad libitum*.

Training to the experimental procedure

Each day, for three days prior to the commencement of preference testing, the hoggets were offered approximately 10 g of lupins in the feeding trolley used in the experiment, which was left at each pen until the animal had eaten the lupins (generally less than 5 minutes). For two days prior to testing all 8 forage types were offered to all of the sheep for a short interval (until the animal had started sampling). After this six of the initial 10 sheep were selected for the main experiment based on how well they adapted to the training and experimental procedures.

Offering the forage

The pots of forage were offered to sheep from a custom built feed trolley that was designed to hold three pots securely positioned while the sheep selected their diet. The size of the positioning ring was determined so that pots would be held 2/3 of the way up the side of the pot (the pots were tapered, decreasing in diameter from top to bottom). The feed trolley was a standard trolley with a purpose-built frame with three rings (20 cm diameter) into which the pots were inserted (Figure 5.1). The direction the pots were offered was such that pots A and C were closest to the sheep.

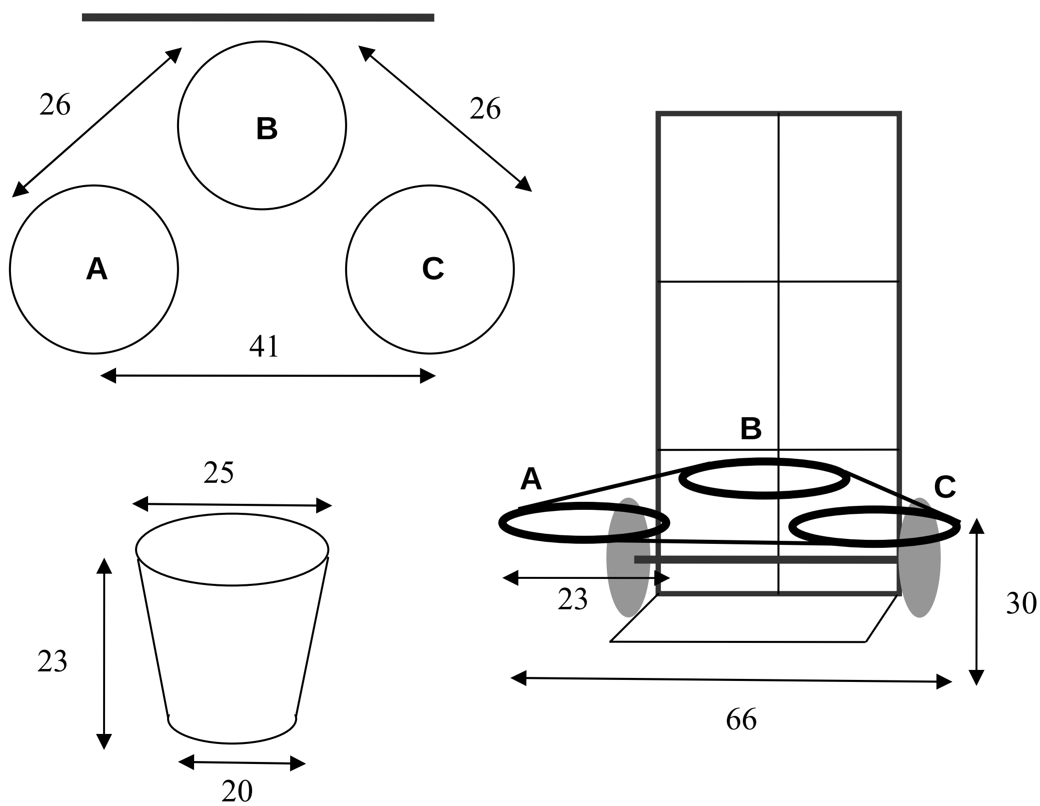

Figure 5.1. Dimensions (cm) of the pot-holding trolley and the pots. Pot positions A, B and C.

Forage combinations and statistical analysis

We considered that the forage offered from each of the three positions in the feeding trolley may have a disproportionate chance of being selected. There are six possible combinations that the pots of forage could be arranged in the feeding trolley, ABC, ACB, BAC, BCA, CAB, CBA. Since we had six replicates, each replicate was assigned one of the combinations when it was initially offered. Additionally, the forage was re-offered to the sheep over six 1-minute periods, each 30 minutes apart, and one of these forage combinations was assigned to each period that the three forages were offered. Analysis of variance and Fisher's protected LSD were used to determine statistical differences between treatment means. These analyses were conducted using the GenStat® (Version - 6.1.0.200) statistical package.

Results

Relative preference (α) of sheep for annual ryegrass, wild radish and six legumes (Table 5.2)

Clear differences in relative preference (α) were expressed by the Merino hoggets for the six legumes when they were offered with the two weeds of crops and their ranking differed according to the phase of plant growth. The hoggets had a high relative preference for crimson clover compared to the other legumes at all phases of plant growth. By contrast, snail medic, subterranean clover and chickpea were the least preferred legumes at the vegetative phase but, of these, only snail medic remained relatively unattractive at all phases of growth.

At the vegetative phase, the hoggets selected annual ryegrass in preference to wild radish ($\alpha = 0.51$ *v* 0.24, $P < 0.01$) and annual ryegrass was eaten in preference to all legumes except crimson clover. The relative preference of sheep for annual ryegrass decreased as the plants matured and was lowest at senescence ($\alpha = 0.20$). Annual ryegrass was generally the most accepted forage when a legume of low relative preference was offered at the vegetative phase (Table 5.5). This is consistent with the high relative preference shown by sheep for annual ryegrass at this phase of plant growth. However, at the reproductive and senesced phases of growth the preference of the sheep for annual ryegrass and wild radish was similar.

The hoggets exhibited selectivity ($\alpha \neq 1$/(number of forage types available) (Chesson 1983); $P < 0.05$) in their forage choice between all forage combinations at the vegetative and reproductive phases, with the exception of biserrula where selectivity was not significant ($p = 0.08$ and 0.11). At senescence the hoggets were not selective ($P > 0.05$) in their choice between any forage combinations except for French serradella ($P = 0.03$); this was eaten in preference to the two weeds (Table 5.2). However, there was also a trend to prefer crimson clover and chickpea at this phase ($P = 0.12$ and 0.07). The relative preference of the sheep varied most at the senesced phase.

Table 5.2. Relative preference (α) of Merino hoggets offered one of six legumes together with wild radish and annual ryegrass at three phases of growth

Phase of growth		Legume	Comparison weed: Wild radish	Comparison weed: Annual ryegrass	LSD (5%)	P-value
Vegetative	Biserrula	$0.30^{b} \pm 0.06$	0.23 ± 0.07	0.47 ± 0.08	ns	0.08
	Chickpea	$0.15^{a} \pm 0.03$	0.23 ± 0.06	0.62 ± 0.06	0.16	0.00
	Crimson clover	$0.49^{c} \pm 0.04$	0.17 ± 0.05	0.34 ± 0.05	0.14	0.00
	Subterranean clover	$0.15^{a} \pm 0.05$	0.27 ± 0.08	0.58 ± 0.09	0.22	0.00
	Snail medic	$0.13^{a} \pm 0.04$	0.30 ± 0.09	0.57 ± 0.10	0.25	0.01
	Serradella	$0.29^{b} \pm 0.05$	0.20 ± 0.06	0.51 ± 0.09	0.20	0.02
	Mean	0.25	0.24	0.51		
	P-value	0.00	0.80	0.18		
Reproductive	Biserrula	$0.47^{b} \pm 0.09$	$0.25^{abc} \pm 0.04$	$0.28^{ab} \pm 0.08$	ns	0.11
	Chickpea	$0.48^{b} \pm 0.08$	$0.18^{a} \pm 0.05$	$0.33^{bc} \pm 0.05$	0.18	0.01
	Crimson clover	$0.71^{c} \pm 0.07$	$0.12^{a} \pm 0.05$	$0.17^{a} \pm 0.03$	0.15	0.00
	Subterranean clover	$0.49^{b} \pm 0.08$	$0.23^{ab} \pm 0.03$	$0.28^{ab} \pm 0.08$	0.21	0.04
	Snail medic	$0.20^{a} \pm 0.04$	$0.35^{bc} \pm 0.06$	$0.45^{c} \pm 0.05$	0.15	0.01
	Serradella	$0.45^{b} \pm 0.07$	$0.39^{c} \pm 0.06$	$0.15^{a} \pm 0.03$	0.18	0.01
	Mean	0.47	0.26	0.28		
	P-value	0.00	0.01	0.01		
Senesced	Biserrula	0.39 ± 0.14	0.43 ± 0.11	0.18 ± 0.08	ns	0.26
	Chickpea	0.63 ± 0.19	0.26 ± 0.15	0.11 ± 0.09	ns	0.07
	Crimson clover	0.64 ± 0.20	0.19 ± 0.15	0.18 ± 0.16	ns	0.12
	Subterranean clover	0.54 ± 0.18	0.21 ± 0.11	0.25 ± 0.16	ns	0.27
	Snail medic	0.22 ± 0.09	0.45 ± 0.16	0.33 ± 0.14	ns	0.48
	Serradella	0.64 ± 0.15	0.21 ± 0.14	0.15 ± 0.10	0.39	0.03
	Mean	0.51	0.29	0.20		
	P-value	0.35	0.57	0.85		
	Neutral Preference	0.33	0.33	0.33		

Errors terms are standard errors of the means (n = 6). Values within columns for each phase of growth with different superscripts are different (P < 0.05).

Day of experiment and relative preference (α) (Figure 5.2)

There was a change in the relative preference of the hoggets between each day of the experiment. The relative preference of sheep for annual ryegrass increased from 0.36 on day 1 to 0.57 on day 4 before reaching a plateau (Figure 5.2). The relative preference of sheep for wild radish decreased in a similar pattern from 0.34 on day 1 to 0.18 on day 4 and remained constant thereafter. The overall relative preference for the legumes offered appeared to decrease, although this was not significant, between day 1 and 2 and then remained constant. The sheep retained this behaviour during a break of 3 days, after which the relative preference test was repeated. However, there may have been a small shift toward their preference on day 1, but this was not significant.

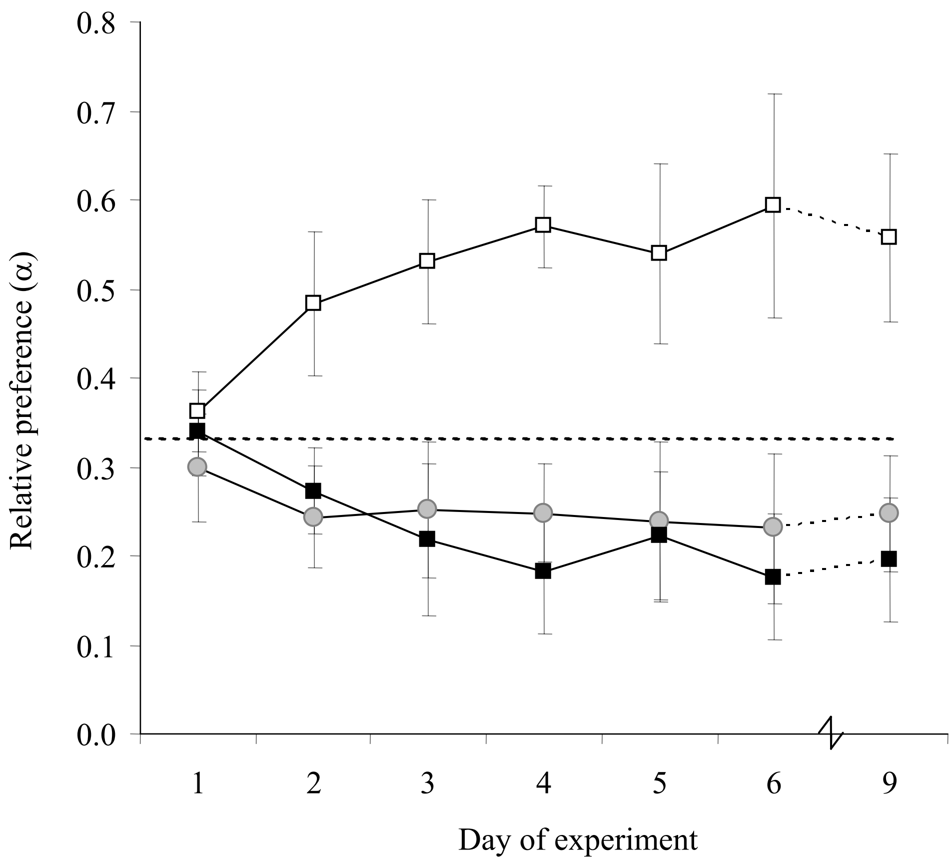

Figure 5.2. Relative preference (α) of Merino hoggets offered annual ryegrass (□), wild radish (■) and legumes (●) for two 1-minute periods on six consecutive days, and on day nine, at the vegetative phase of plant growth. Neutral preference ($\alpha = 0.33$) line (----). Error bars are standard errors of the means ($n = 6$).

Individual sheep and relative preference (α) (Figure 5.3)

Individual sheep had clearly different forage preferences. At the vegetative phase the relative preference for annual ryegrass ranged from 0.28 to 0.70 ($P < 0.01$), wild radish from 0.09 to 0.42 ($P < 0.01$), while the overall relative preference for the legumes offered did not differ between sheep ($P = 0.48$) (Figure 5.3; Vegetative). In contrast, at the reproductive phase relative preference for the legume component ranged from 0.34 to 0.68 ($P = 0.06$), annual ryegrass from 0.13 to 0.41 ($P = 0.02$), while the relative preference for wild radish did not differ between individual sheep ($P = 0.81$) (Figure 5.3; Reproductive). Preferences may have differed between individual sheep at the senesced phase, but the Coefficient of Variation (CoV) in relative preference was higher for the senesced forage (CoV = 112%) than at the vegetative (CoV = 58%) and the reproductive phases (CoV = 55%) and consequently no differences were significant at the senesced phase (Figure 5.3; Senesced).

Forage depletion and relative preference (α) (Figure 5.4)

The relative preference of the sheep for the three different types of forage offered changed markedly in response to depletion of the preferred forage. This was particularly obvious at the vegetative phase when the three legumes with a low relative preference were offered to the hoggets. The relative preference (α) of the hoggets for annual ryegrass dropped rapidly, typically from ~ 0.7 to ~ 0.33 (neutral) in the first three 1-minute periods, while the preference for the initially less preferred legume increased gradually over the six 1-minute periods that the forage was re-offered.

Forage morphology and relative preference (α) (Figure 5.5)

The relationship between relative preference (α) and the height, width and density of the forage was analysed using a linear regression model for the vegetative and reproductive phases of plant growth. At the vegetative phase there was a weak positive linear relationship ($P < 0.01$; $R^2 = 0.23$) between forage density and relative preference. At the reproductive phase there was weak positive linear relationship ($P < 0.01$; $R^2 = 0.30$) between forage density and relative preference (Figure 5.5). There was no relationship between forage height or width and relative preference.

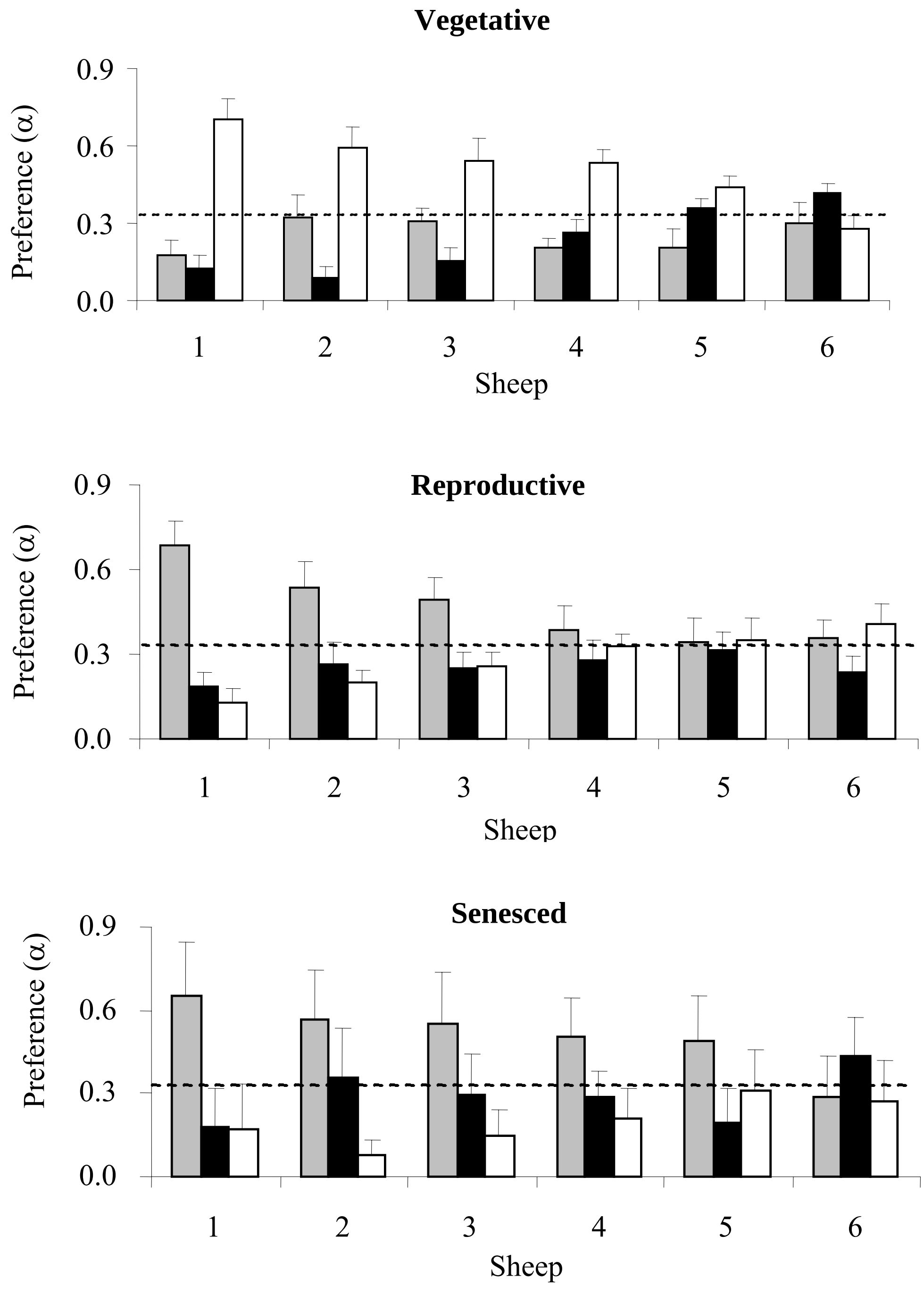

Figure 5.3. Mean individual relative preference (α) of six individual Merino hoggets offered annual ryegrass (☐), wild radish (■) and a legume (■) for two 1-minute periods on six consecutive days at the vegetative, reproductive and senesced phases of plant growth. Error bars are standard errors of the means (n = 6). Neutral preference (α = 0.33) line (----).

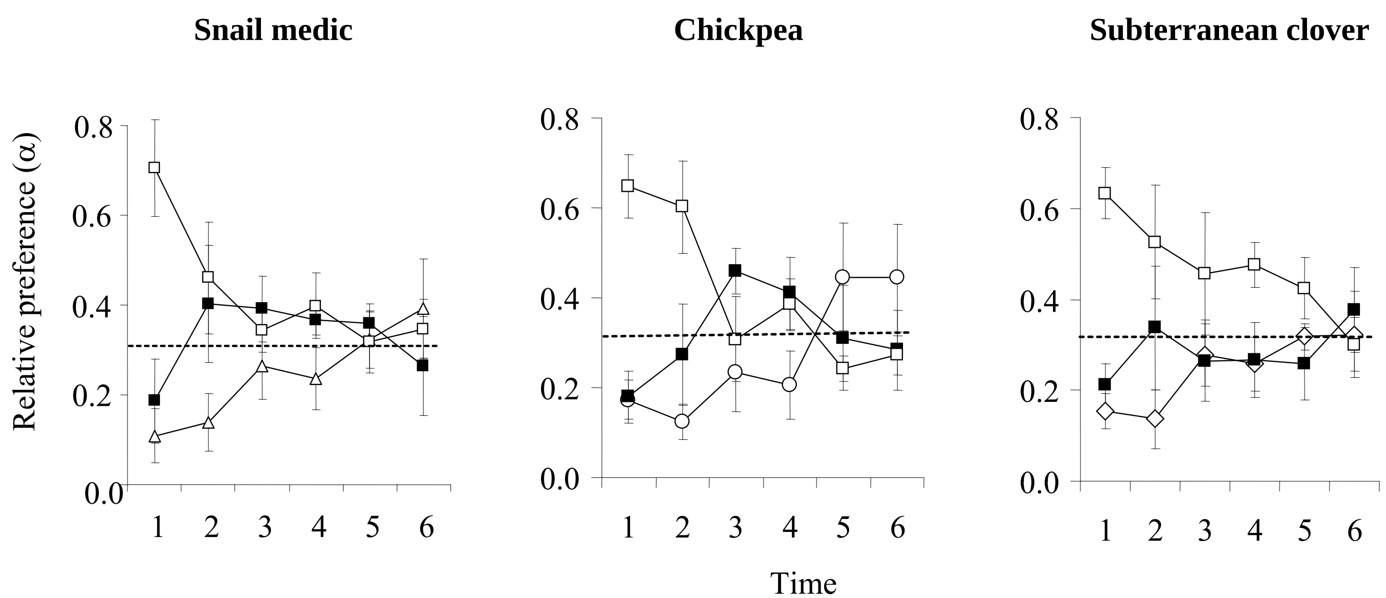

Figure 5.4. Relative preference (α) of six Merino wether hoggets offered wild radish (■) and annual ryegrass (□) with snail medic (△) chickpea (○) or subterranean clover (◇) over six 1-minute periods, each 30 minutes apart, at the vegetative phase of plant growth. Neutral preference line (----) (α = 0.33). Error bars are standard errors of the means (n = 6).

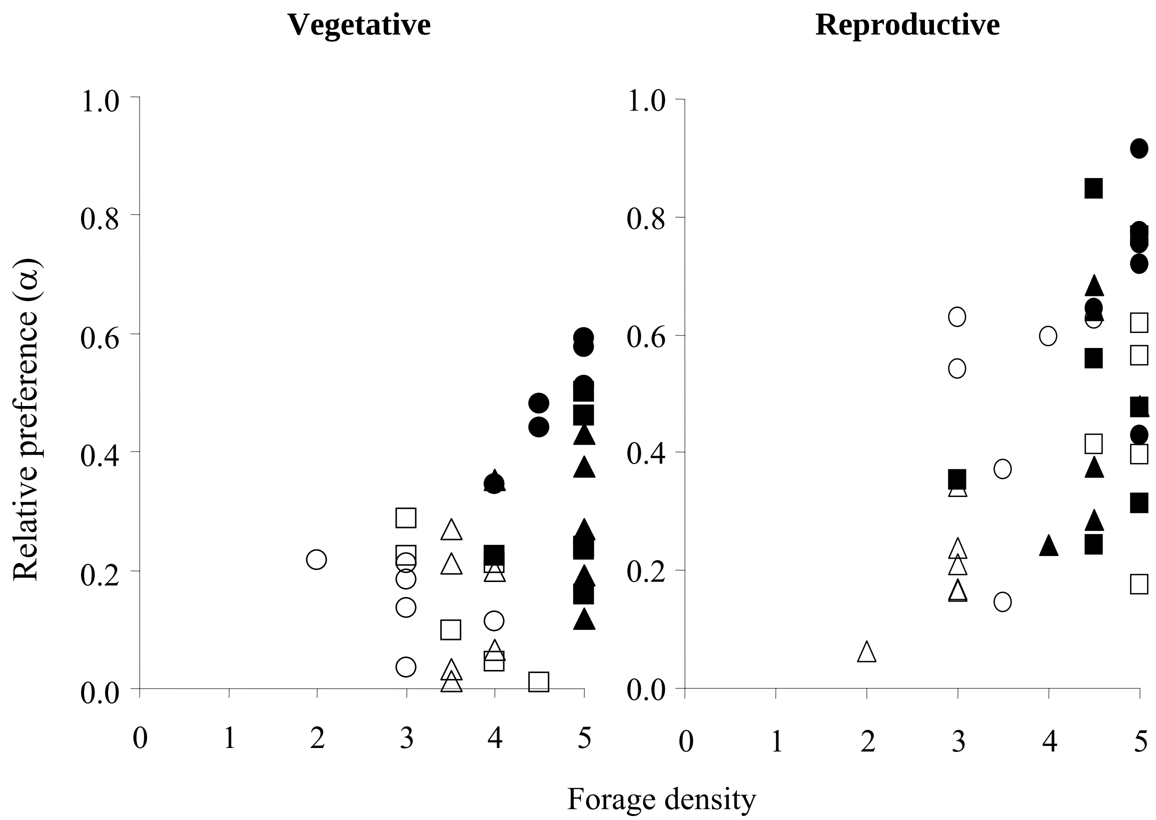

Figure 5.5. Relationship between forage density (0 - 5) and relative preference (α) of Merino hoggets offered combinations of either biserrula (■), chickpea (○), crimson clover (●), subterranean clover (□), snail medic (△) or serradella (▲), with both annual ryegrass and wild radish at the vegetative and reproductive phases of plant growth.

Forage availability and morphology (Table 5.3 and Table 5.4)

The quantity of forage offered to the sheep differed between the six legumes, while the quantity of annual ryegrass and wild radish was the same for each treatment combination, at each of the three phases of plant growth (Table 5.3).

The biomass of each of the three forage types offered within treatments was different for the chickpea, crimson clover, subterranean clover and snail medic treatments at the vegetative phase. At the reproductive and senesced phases only subterranean clover and biserrula differed in the biomass of each forage type offered (Table 5.3).

Table 5.3. Forage biomass (g/sheep, fresh weight) offered to Merino wethers, comprising one of six legumes together with wild radish and annual ryegrass

Phase of growth	Legume		Comparison weed		LSD (5%)	P-value
			Wild radish	Annual ryegrass		
Vegetative	Biserrula	$94^{cd} \pm 6.7$	123 ± 20.1	124 ± 6.4	ns	0.20
	Chickpea	$50^{a} \pm 4.9$	102 ± 9.4	120 ± 7.1	22.2	0.00
	Crimson clover	$80^{c} \pm 4.8$	134 ± 21.3	123 ± 12.7	43.9	0.04
	Subterranean clover	$52^{ab} \pm 2.0$	108 ± 9.0	116 ± 12.4	26.8	0.00
	Snail medic	$74^{bc} \pm 10.6$	133 ± 18.4	116 ± 6.8	38.8	0.02
	Serradella	$105^{d} \pm 12.7$	121 ± 16.8	123 ± 13.8	ns	0.65
	Mean	77	120	120		
	P-value	0.00	0.68	0.98		
Reproductive	Biserrula	$157^{b} \pm 17.1$	137 ± 15.8	110 ± 22.2	ns	0.23
	Chickpea	$105^{a} \pm 11.9$	119 ± 17.7	121 ± 15.3	ns	0.72
	Crimson clover	$155^{b} \pm 8.7$	124 ± 11.5	123 ± 13.3	ns	0.11
	Subterranean clover	$114^{a} \pm 6.8$	172 ± 23.0	107 ± 13.0	47.5	0.02
	Snail medic	$91^{a} \pm 15.5$	127 ± 8.3	112 ± 17.1	ns	0.22
	Serradella	$159^{b} \pm 12.1$	128 ± 18.8	127 ± 10.8	ns	0.24
	Mean	130	135	117		
	P-value	0.00	0.27	0.93		
Senesced	Biserrula	$48^{b} \pm 5.6$	25 ± 5.1	31 ± 4.2	15.1	0.02
	Chickpea	$30^{a} \pm 4.3$	21 ± 3.9	28 ± 5.3	ns	0.37
	Crimson clover	$29^{a} \pm 5.7$	26 ± 2.7	26 ± 5.2	ns	0.88
	Subterranean clover	$28^{a} \pm 3.0$	23 ± 2.7	37 ± 5.8	ns	0.08
	Snail medic	$29^{a} \pm 3.9$	25 ± 2.4	28 ± 2.9	ns	0.53
	Serradella	$39^{ab} \pm 4.2$	25 ± 4.3	29 ± 4.1	ns	0.08
	Mean	34	24	30		
	P-value	0.02	0.95	0.68		

Error terms are standard errors of the means (n = 6).Values within columns for each phase of growth with different superscripts are different ($P < 0.05$).

The total quantity of forage available followed a consistent pattern of decline for each 1-minute period that it was offered, but the trend varied between each of the three phases of plant growth (Figure 5.6). These patterns of decline are well represented by the quadratic functions shown below where:

F(t) = Total forage biomass offered for the 1-minute period (t)

Vegetative phase: $F(t) = 3.6607t^2 - 70.482t + 383.5$ $R^2 = 0.999$

Reproductive phase: $F(t) = 1.9107t^2 - 68.061t + 448.9$ $R^2 = 0.999$

Senesced phase: $F(t) = 0.6786t^2 - 9.0357t + 96$ $R^2 = 0.998$

At the reproductive stage we can predict that there will be no forage available when t = 8.7 or F (8.7) = 0. For the vegetative and senesced phases we can predict that some remaining forage will be refused before it has been fully depleted. This is calculated to occur at t = 9.6 for the vegetative phase and t = 6.6 for the senesced phase where the forage biomass remaining is 44 g and 66 g, where $F'(t) = 0$. Based on the function for the cumulative proportion of forage removed, 100% of the forage is predicted to be depleted at the reproductive phase, while maximum depletion is predicted to be 89% for the vegetative phase and 25% for the senesced phase.

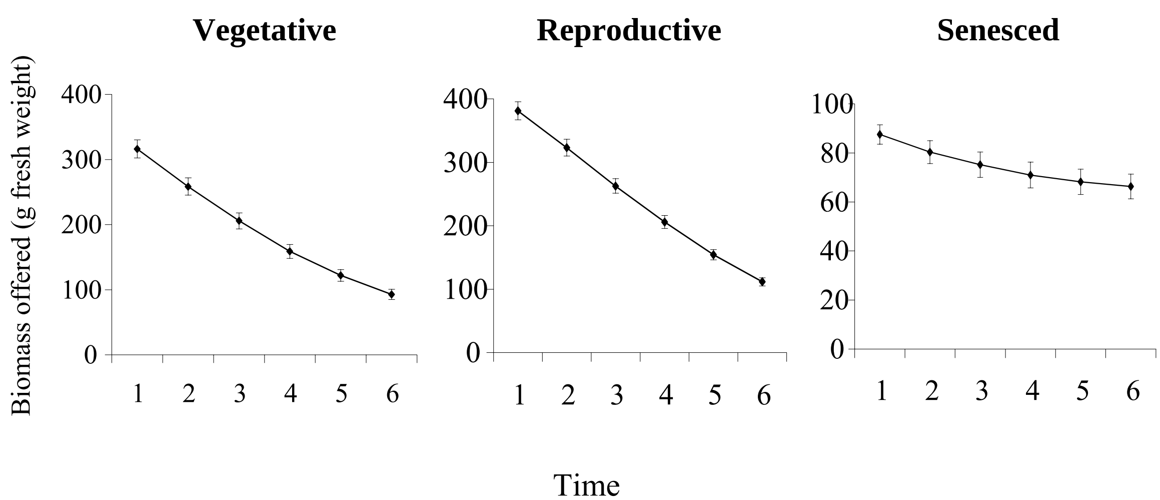

Figure 5.6. Total forage biomass (g fresh weight/sheep) for combinations of one of six legumes, wild radish and ryegrass offered to Merino hoggets over six 1-minute periods, each 30 minutes apart, at the vegetative, reproductive and senesced phases of plant growth. Error bars are standard errors of the means (n = 6).

The height, width and density of the different forage types differed at both the vegetative and reproductive phases of plant growth (Table 5.4). Subterranean clover was the most prostrate at both the vegetative and reproductive phases with heights of 9.8 ± 0.5 and 19.7 ± 0.8 cm. Chickpea was the highest at the vegetative and wild radish the highest at the reproductive phase.

Table 5.4. Forage height (cm), width (cm) and density of six legumes, wild radish and annual ryegrass that were offered to hogget Merino wethers at the vegetative and reproductive phases of growth

Phase of Growth	Forage type	Height (cm)	Width (cm)	Density (0-5)
Vegetative	Biserrula (n = 6)	$15.2^{b} \pm 0.9$	$30.2^{ab} \pm 1.6$	$4.8^{c} \pm 0.2$
	Chickpea (n = 6)	$25.8^{d} \pm 1.1$	$30.0^{ab} \pm 1.5$	$3.0^{a} \pm 0.3$
	Crimson clover (n = 6)	$13.0^{ab} \pm 0.5$	$29.5^{ab} \pm 1.0$	$4.7^{c} \pm 0.2$
	Subterranean clover (n = 6)	$9.8^{a} \pm 0.5$	$27.3^{a} \pm 0.8$	$3.7^{b} \pm 0.2$
	Snail medic (n = 6)	$15.2^{b} \pm 0.5$	$31.8^{abc} \pm 2.4$	$3.7^{b} \pm 0.1$
	Serradella (n = 6)	$16.8^{bc} \pm 0.9$	$31.7^{abc} \pm 2.3$	$4.8^{c} \pm 0.2$
	Annual ryegrass (n = 36)	$20.2^{c} \pm 0.6$	$34.6^{bc} \pm 0.5$	$4.6^{c} \pm 0.1$
	Wild radish (n = 36)	$20.8^{c} \pm 0.9$	$35.8^{c} \pm 1.0$	$3.6^{b} \pm 0.1$
	Mean	17.1	31.4	4.1
	P-value	0.00	0.00	0.00
Reproductive	Biserrula (n = 6)	$30.2^{ab} \pm 2.0$	$57.2^{cd} \pm 4.8$	$4.4^{c} \pm 0.3$
	Chickpea (n = 6)	$42.2^{c} \pm 0.9$	$51.3^{c} \pm 2.5$	$3.6^{b} \pm 0.2$
	Crimson clover (n = 6)	$23.7^{ab} \pm 2.2$	$37.5^{a} \pm 2.6$	$4.9^{c} \pm 0.1$
	Subterranean clover (n = 6)	$19.7^{a} \pm 0.8$	$35.0^{a} \pm 0.8$	$4.9^{c} \pm 0.1$
	Snail medic (n = 6)	$30.0^{ab} \pm 1.2$	$67.2^{d} \pm 5.5$	$2.8^{a} \pm 0.2$
	Serradella (n = 6)	$32.3^{bc} \pm 1.3$	$49.0^{b} \pm 1.8$	4.5 ± 0.1
	Annual ryegrass (n = 36)	$29.0^{ab} \pm 1.2$	$38.6^{ab} \pm 0.8$	$4.8^{c} \pm 0.1$
	Wild radish (n = 36)	$67.9^{d} \pm 2.4$	$56.0^{c} \pm 2.1$	$3.6^{b} \pm 0.1$
	Mean	34.4	49.0	4.2
	P-value	0.00	0.00	0.00

Error terms are standard errors of the means. Values within columns for each phase of growth with different superscripts are statistically different ($P < 0.05$).

Intake rate of sheep offered plants growing in pots (Table 5.5)

The intake rate (g fresh weight/sheep.minute) was 55.2, 59.3 and 6.2 at the vegetative, reproductive and senesced phases of plant growth (n = 6). Intake rate at the senesced phase was nearly only one tenth of that at the vegetative and reproductive phases, where intake rate was the similar.

The rate of intake differed between annual ryegrass, wild radish and the legumes for chickpea, subterranean clover, snail medic and the serradella treatment combinations at the vegetative phase; crimson clover, snail medic and serradella at the reproductive phase; and chickpea, crimson clover and serradella at the senesced phase (Table 5.5).

The intake rate differed between the different legumes that were offered in each treatment combination, but not for annual ryegrass or wild radish where intake did not differ in relation to the legume that was offered concurrently (Table 5.5). The intake rate of all plant material that was consumed differed in relation to which legume was offered at the senesced phase, but not at the vegetative or reproductive phases of plant growth (Table 5.6).

The rate of forage intake (g fresh weight/sheep.minute) decreased as the forage treatments were re-offered to the sheep over the consecutive six 1-minute periods (Figure 5.7). The change in rate of intake between periods differed at each phase of plant growth (Figure 5.7). At the vegetative phase, forage intake had decreased significantly by the third 1-minute period (57.8 *v* 47.0 g fresh weight/sheep.minute; $P < 0.01$). At the reproductive phase forage intake had decreased significantly from the maximum intake rate, which was during the second 1-minute period, by the fourth 1-minute period (60.3 *v* 51.8 g fresh weight/sheep.minute; $P = 0.02$). At the senesced phase forage intake had decreased significantly by the third 1-minute period (7.3 *v* 4.2 g fresh weight/sheep.minute; $P < 0.01$).

The first two 1-minute periods were used to calculate relative preference because the rate of intake of the hoggets was not significantly depressed by the reduced amount of forage available at these times (Figure 5.7).

Table 5.5. Intake rate (g fresh weight/sheep.minute) of Merino hoggets offered one of six legumes together with wild radish and annual ryegrass at three phases of growth

Phase of growth	Legume		Comparison weed: Wild radish	Comparison weed: Annual ryegrass	LSD (5%)	P-value
Vegetative	Biserrula	$13.5^{b} \pm 2.1$	15.3 ± 4.5	25.4 ± 4.6	ns	0.10
	Chickpea	$4.4^{a} \pm 1.0$	13.5 ± 4.0	33.2 ± 3.7	9.6	0.00
	Crimson clover	$21.9^{c} \pm 2.2$	13.5 ± 4.2	25.1 ± 3.5	ns	0.07
	Subterranean clover	$4.1^{a} \pm 1.1$	17.4 ± 5.2	31.6 ± 6.3	14.4	0.00
	Snail medic	$5.6^{a} \pm 1.9$	23.3 ± 7.3	28.2 ± 3.3	14.3	0.01
	Serradella	$15.8^{b} \pm 3.0$	12.7 ± 4.0	27.0 ± 3.5	10.6	0.03
	Mean	10.9	15.9	28.4		
	P-value	0.00	0.68	0.70		
Reproductive	Biserrula	$30.9^{b} \pm 8.2$	17.0 ± 4.4	12.3 ± 4.0	ns	0.10
	Chickpea	$25.3^{b} \pm 5.1$	13.8 ± 4.9	22.3 ± 5.3	ns	0.29
	Crimson clover	$46.7^{c} \pm 6.0$	7.8 ± 3.0	12.3 ± 2.4	12.3	0.00
	Subterranean clover	$23.8^{ab} \pm 2.6$	20.8 ± 4.0	15.2 ± 4.0	ns	0.26
	Snail medic	$8.8^{a} \pm 2.7$	19.5 ± 3.1	20.2 ± 2.2	8.1	0.01
	Serradella	$28.8^{b} \pm 4.7$	21.3 ± 3.9	9.6 ± 2.1	11.2	0.01
	Mean	27.4	16.7	15.3		
	P-value	0.00	0.15	0.11		
Senesced	Biserrula	$2.1^{ab} \pm 0.9$	1.6 ± 0.8	0.8 ± 0.3	ns	0.47
	Chickpea	$5.3^{bc} \pm 1.4$	2.1 ± 1.2	1.0 ± 0.6	3.4	0.04
	Crimson clover	$6.3^{c} \pm 1.9$	2.1 ± 1.2	1.2 ± 0.9	4.2	0.05
	Subterranean clover	$2.3^{ab} \pm 1.1$	1.1 ± 0.5	1.6 ± 0.8	ns	0.58
	Snail medic	$0.5^{a} \pm 0.1$	1.8 ± 0.8	0.8 ± 0.5	ns	0.29
	Serradella	$4.8^{bc} \pm 1.1$	0.8 ± 0.4	1.2 ± 0.9	2.5	0.01
	Mean	3.5	1.6	1.1		
	P-value	0.02	0.87	0.97		

Error terms are standard errors of the means (n = 6). Values within columns for each phase of growth with different superscripts are statistically different ($P < 0.05$).

Table 5.6. Intake rate of total forage (g fresh weight/sheep.minute) of Merino hogget wethers offered one of six legumes together with wild radish and annual ryegrass at three phases of plant growth

	Phase of growth		
Treatment	**Vegetative**	**Reproductive**	**Senesced**
Biserrula	54.2 ± 6.9	60.3 ± 8.2	4.4^{ab} ± 0.8
Chickpea	51.1 ± 3.9	61.2 ± 6.2	8.3^{b} ± 0.7
Crimson clover	60.5 ± 3.2	66.7 ± 4.5	9.5^{b} ± 1.3
Subterranean clover	53.1 ± 3.8	59.7 ± 4.4	5.0^{ab} ± 1.3
Snail medic	57.0 ± 5.8	48.4 ± 3.2	3.1^{a} ± 0.7
Serradella	55.4 ± 3.1	59.8 ± 3.2	6.8^{b} ± 1.5
Mean	55.2	59.3	6.2
P-value	0.78	0.30	0.00

Error terms are standard errors of the means (n = 6). Values within columns with different superscripts are different ($P < 0.05$).

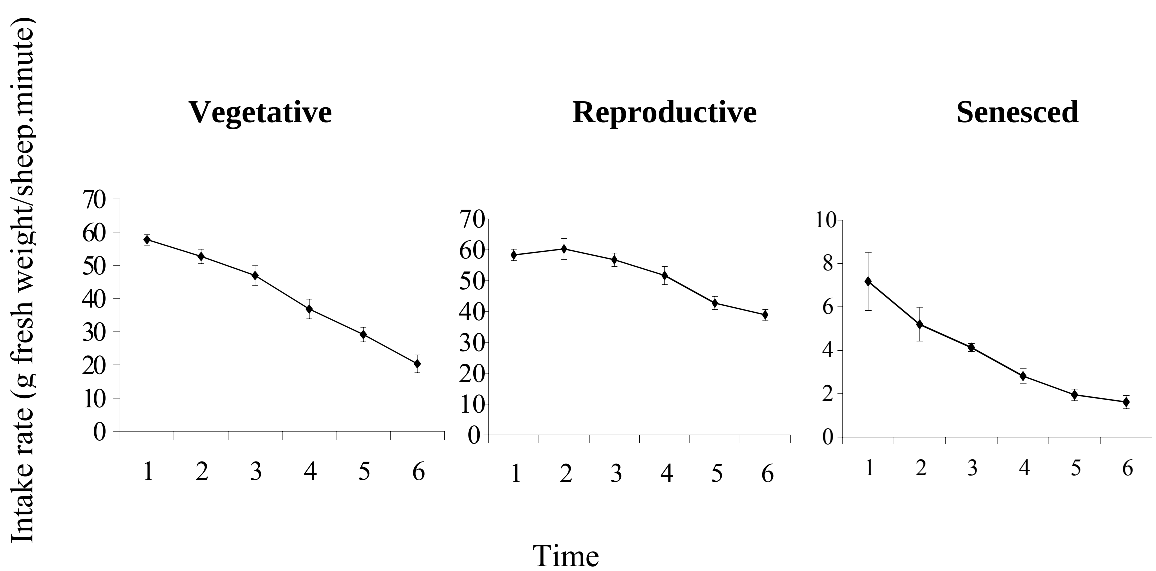

Figure 5.7. Forage intake rate (g fresh weight/sheep.minute) of Merino wether hoggets offered and re-offered combinations of one of six legumes, wild radish and ryegrass for six consecutive 1-minute periods at intervals of 30 minutes. Error bars are standard errors of the means (n = 6).

The relationship between the forage biomass offered and the rate of intake of the sheep was analysed using a linear regression model for each phase of plant growth. At the reproductive phase there was a positive linear relationship ($P < 0.01$; $R^2 = 0.67$) between forage biomass offered and rate of intake, but there was no trend at the vegetative and senesced phases (P > 0.05; Figure 5.8).

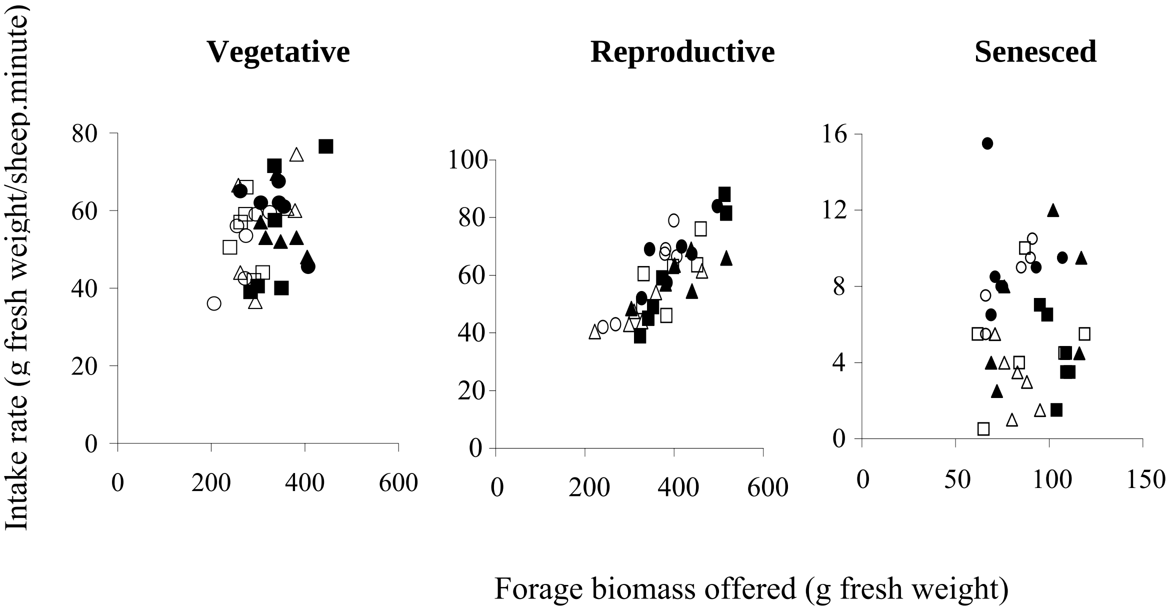

Figure 5.8. Relationship between forage biomass offered (g fresh weight) and intake rate (g fresh weight/sheep.minute) of Merino wether hoggets offered combinations of either biserrula (■), chickpea (○), crimson clover (●), subterranean clover (□), snail medic (Δ) or serradella (◆), with both annual ryegrass and wild radish.

Evapo-transpiration from pots (Table 5.7)

The moisture lost due to evapo-transpiration from each pot at the vegetative phase of plant growth (Day 63) differed between plant genotypes (Table 5.7). Biserrula had the highest moisture loss while subterranean clover had the lowest moisture loss (0.90 *v* 0.14 g/pot.minute; P < 0.01). The mean weight of moisture lost was 0.41 g/pot.minute, while the mean rate of forage intake per pot by the sheep was 18.4 g/pot.minute. Thus, loss of moisture was only about 2% of the total loss in weight during preference testing.

Bias due to the position of the pot (Table 5.8)

The rate of forage intake and the relative preference of the sheep was the same for forages offered from each of the three positions on the feed trolley at each phase of plant growth

(Table 5.8). Although there appeared to be some bias at the reproductive and senesced phases, this was not significant.

Table 5.7. Loss of moisture due to evapo-transpiration (g/pot.min) from pots containing different plant genotypes at the vegetative phase of plant growth

Genotype	**Moisture loss (g/pot.min)**
Biserrula	$0.90^{a} \pm 0.096$
Wild radish	$0.56^{b} \pm 0.041$
Snail medic	$0.42^{bc} \pm 0.040$
Chickpea	$0.34^{c} \pm 0.098$
Crimson clover	$0.26^{cd} \pm 0.041$
Annual ryegrass	$0.23^{cd} \pm 0.026$
Subterranean clover	$0.14^{d} \pm 0.067$

Error terms are standard errors of the means (n = 6). Values with different superscripts are different (P < 0.05).

Table 5.8. Relative preference (α) and intake rate (g fresh weight/sheep.minute) of Merino wether hoggets offered feed from positions A, B or C on the feed trolley (see Figure 5.1)

	Pot position			
Growth phase	**A**	**B**	**C**	**P-value**
		Relative preference (α)		
Vegetative	0.338 ± 0.030	0.328 ± 0.034	0.334 ± 0.033	0.98
Reproductive	0.343 ± 0.027	0.352 ± 0.028	0.304 ± 0.030	0.43
Senesced	0.307 ± 0.049	0.297 ± 0.039	0.396 ± 0.055	0.28
		Intake rate (g fresh weight/sheep.minute)		
Vegetative	37.9 ± 4.2	36.6 ± 4.2	36.0 ± 3.9	0.95
Reproductive	44.9 ± 4.8	38.1 ± 3.0	35.6 ± 3.9	0.24
Senesced	3.9 ± 0.7	3.5 ± 0.6	4.9 ± 0.9	0.40

Error terms are standard errors of the means (n = 36). Values within rows with different superscripts are different (P < 0.05).

Discussion

The indoor method tested in this experiment showed that the short-term relative preference of sheep for six annual legumes was quite similar to that exhibited in the field studies. The hypothesis that sheep would display consistent trends in preference for forage grown and presented in pots compared to forage grown in the field was supported. From reviewing the literature, this is the first time that the preference of sheep for a diverse group of annual legumes and weeds of crops offered in pots has been tested and compared to field results.

The consistent trends in the short-term relative preference of hoggets between the indoor and previous field evaluations demonstrate that sheep exhibit predictable selective behaviour when offered forage in markedly different environments (Table 5.9). In particular, Caprera crimson clover had a high relative preference at all phases of plant growth in both studies. On the other hand, Sava snail medic had a low relative preference at all phases of growth compared to most other legumes, but at the senesced phase preference for snail medic increased relative to annual ryegrass. The relative preference of sheep for annual ryegrass was consistently high when vegetative, but its relative preference declined as the plant matured. This attribute of grasses and other non-legume annual pasture species has been well established by other workers and is thought to be linked to low nutritive value in mature grasses compared to legumes (Arnold 1962; Weston and Hogan 1968; Weston and Hogan 1968). In both the field and indoor experiments relative preference changed markedly between plant species between phases of growth (Table 5.9).

A number of other trends in preference emerged for the two methods. The short-term relative preference of sheep for green biserrula and serradella was shown to be moderate in both methods except that sheep showed a low preference for vegetative biserrula in the field study. The short-term relative preference of sheep for crop legumes has generally been found to follow a consistent pattern of low relative preference at the vegetative phase, moderate relative preference at the reproductive phase and high relative preference at the senescent phase of plant growth. Sheep had an initial low relative preference for Chickpea, which increased as the plant matured in both the indoor and field experiments. A similar trend was found for vetch and subterranean vetch in the field studies and has also been

Table 5.9. Short-term relative preference (α) of Merino hoggets for six legumes offered in three experiments (Field: Chapters 1 and 4; Indoor: the current Chapter 5)

Phase of growth	**Legume**	**Preference experiment** **Indoor (Ch.5)**	**Field (Ch.4)**	**Preliminary (Ch.1)**
Vegetative	Biserrula	0.30 ± 0.06	0.012^{l} ± 0.010	0.06
	Chickpea	0.15^{l} ± 0.03	0.000^{l} ± 0.009	
	Crimson clover	0.49^{h} ± 0.04	0.086^{h} ± 0.016	0.24^{h}
	Subterranean clover	0.15^{l} ± 0.05	0.060 ± 0.014	0.14^{h}
	Snail medic	0.13^{l} ± 0.04	0.008^{l} ± 0.004	0.03^{l}
	Serradella	0.29 ± 0.05	0.046 ± 0.005	
	LSD (P = 0.10)	0.11	0.027	0.05
Reproductive	Biserrula	0.47 ± 0.09	0.062 ± 0.011	0.08
	Chickpea	0.48 ± 0.08	0.024^{l} ± 0.009	
	Crimson clover	0.71^{h} ± 0.07	0.098^{h} ± 0.010	0.16^{h}
	Subterranean clover	0.49 ± 0.08	0.018^{l} ± 0.004	0.05
	Snail medic	0.20 ± 0.04	0.008^{l} ± 0.006	0.01^{l}
	Serradella	0.45 ± 0.07	0.054 ± 0.002	
	LSD (P = 0.10)	0.18	0.025	0.07
Senesced	Biserrula	0.39 ± 0.14	0.016^{l} ± 0.007	0.03
	Chickpea	0.63 ± 0.19	0.134^{h} ± 0.015	
	Crimson clover	0.64 ± 0.20	0.076^{h} ± 0.007	0.09
	Subterranean clover	0.54 ± 0.18	0.000^{l} ± 0.009	0.06
	Snail medic	0.22 ± 0.09	0.024^{l} ± 0.011	0.11
	Serradella	0.64 ± 0.15	0.016^{l} ± 0.005	
	LSD (P = 0.10)	0.38	0.025	0.08
	Neutral Preference	0.33	0.05	0.08

^{l} relative preference (α) is significantly lower than neutral (P < 0.10).
^{h} relative preference (α) is significantly higher than neutral (P < 0.10).

observed for coriander, faba been and narbon bean (Penfold and Miyan 1997). In the field study reported in Chapter 4 *Lathyrus* spp. did not have a low relative preference at the vegetative phase of growth, but was highly preferred by sheep at all phases of plant growth.

One of the clear differences exhibited between the two methods was that the preference for Dalkeith subterranean clover was low at the vegetative phase in the indoor experiment. This also contrasts with other studies where green Dalkeith subterranean clover was eaten readily by sheep. Wang (1997) found that Dalkeith subterranean clover grown in wooden boxes, when offered *in situ*, was readily eaten compared to several other cultivars of subterranean clover. The low relative preference of sheep for Dalkeith subterranean clover in our experiment may have been a result of its prostrate growth and possibly low accessibility in the pots. In our experiment the Dalkeith subterranean clover forage was less than 10 cm in height, which was the lowest of the legume forages. Sheep have been found to have a lower preference for forage if it is poorly accessible (Weston 1996) and has a low potential rate of intake (Kenney and Black 1984). Nevertheless, Caprera crimson clover was also short but was highly preferred. This may have been associated with the petioles of crimson clover being erect and appearing to be accessible to the sheep. The petioles of Dalkeith grew flat against the soil surface and were probably less accessible. The relative preference shown by sheep for Dalkeith subterranean clover increased from 0.15 to 0.49 between the vegetative and reproductive phases. The Dalkeith subterranean clover plants had doubled in height over this time, which may have improved their accessibility and accounted for some of the improvement in preference. Some of this change was probably also associated with the decline in the relative preference of sheep for annual ryegrass between the vegetative and reproductive phases of growth.

Some of the differences in relative preference may also have resulted from of the different alternative forages that were available in the indoor and field experiments. The type and abundance of alternative forage is known to be critical in determining the herbivore’s preference for a particular forage (Parsons *et al.* 1994). By definition, the relative preference of sheep for a forage depends on the available alternatives. In the field study the preference of sheep for each legume was assessed relative to 18 other legumes and annual ryegrass, but in the indoor experiment the preference of sheep for a legume was assessed relative to only two other species, neither being a legume. The implications of this can be seen when preferences were assessed at the senesced phase of plant growth. Here some of the legumes (e.g. serradella and sub clover) that were avoided in the field study, had a moderate relative preference in the indoor study. This indicates that the low acceptability of the two non-legume weeds in the indoor study may have increased the preference for the legume that was offered with the weeds.

An aim of this study was to evaluate the indoor method as an alternative to 'cafeteria' type field studies to investigate relative preference of sheep between pasture plants *in situ*. The results for the two methods were as consistent as might be expected and we can say that either method is equally valid for testing relative preference. Therefore, we now have confidence to use the indoor method to test other hypotheses where the relative preference of herbivores between several pasture species is to be examined. Perhaps the most significant advantage with the indoor method is that all aspects of the experimental procedure can be carried out in a controlled environment. The rearing of the animals, the growing of the plants and the experimental procedure can all potentially be conducted in a controlled environment. The use of individual animals is also considered an advantage by Peterson and Renaud (1989) who noted that using two or more animals may bias preference because of the social interactions between the animals. In the field study the gregarious nature of sheep may have restricted the ability of the sheep to exhibit their preferences between the rows of forage since the 'site' selection of the sheep is altered considerably by the flock. Parsons *et al.* (1994) consider the inherent preference of sheep for forage to be validly measured where food is accessible with a minimum of physical constraints. To reduce the effect of social interactions Peterson and Renaud (1989) suggest that ideally "the potential foods are presented simultaneously to the consumer within a single experimental arena", a concept that we were careful to adhere to in this experiment.

The results from a number of recent studies of grazing behaviour have demonstrated that the food preferences of sheep are being constantly modified by the herbivore's grazing experience (Provenza 1995; Distel *et al.* 1996). That the diet selected by an animal given an identical choice may vary depending on the animal's prior experience raises cause for caution when drawing conclusions about forage preferences from 'cafeteria' type experiments. For this reason, the prior grazing experience of the sheep was carefully considered in this experiment. The sheep selected for this experiment were chosen as 'naïve' sheep to be offered 'novel' forage. However, it was difficult to verify this for all the forages studied, especially given that annual ryegrass and wild radish are commonly present in pastures. With the possible exception of Dalkeith subterranean clover, we were confident that the sheep were not familiar with the annual legumes that were used in this experiment. More work is needed to investigate how the preferences of sheep are affected by different levels of prior experience.

Sheep are neophobic, they prefer to eat foods with which they are familiar and their level of familiarity with a particular forage affects the degree of neophobia (Thorhallsdottir *et al.* 1987). To avoid potential problems associated with neophobic behaviour we briefly familiarised the sheep with the forages prior to testing them. However, the familiarity of sheep with eating a particular plant or its grazing experience must be considered at a number of levels. Sheep that have eaten a small amount of a particular forage on one occasion probably perceive the forage differently to sheep that have eaten the forage as part of a mixed diet over a long period of time. While sheep that eat a diet comprised of mostly a single forage type, for an extended period of time, have a different experience with, and perception of, the forage. Animals are known to develop "transient food aversions" when their food choice is limited to a single diet (Provenza *et al.* 2003) and the strength of aversion may vary depending on the forage offered in the single diet. Strict control and recording of the animal's grazing history is critical to ensure relative preference is not biased by an unknown previous animal-plant interaction. Ideally, part of this method should include managing the diet of the experimental sheep from weaning, or to measure the food available within their grazing range to ensure the results are described in terms of the previous grazing experience of the sheep.

A change in the nature of the preferential grazing drive of sheep could be clearly seen in the results of this short-term study. A consequence of progressively offering sheep forage over a period of six days was that by day six the experience of the sheep had influenced its capacity to identify and select the preferred forage, or combination of forage more precisely than on day one. In this experiment the preference of sheep for annual ryegrass and wild radish diverged from day 1 to day 4 with the sheep adopting an increasing preference for annual ryegrass and increasingly avoiding wild radish (Figure 5.2). This justified our view that it was necessary to use a Latin-square design that was adjusted for residual effects so that the clear pattern of learned behaviour by the sheep did not bias the experiment.

In addition to the effect prior experience has on relative preference, the appetite or the degree of hunger or satiety has a strong influence on the forage selected by herbivores. Fasting increases the intake rate, reduces selectivity and alters the grazing preference of sheep (Villalba and Provenza 1999; Newman *et al.* 1994). Our method of feeding sheep *ad libitum* and then fasting for one hour prior to the preference test seemed to work well in this experiment as the sheep appeared to be neither hungry nor satisfied and so effectively displayed their preference when offered the forages. In general the sheep showed less interest in the forage at the senesced phase (personal observation), and this was supported by their lower intake rate and an increase in variability in the choice of forage selected (Table 5.5). This probably reflects the changes in the physical and chemical characteristics of the forages with maturity. A lower intake rate has been shown to be related to a number of physical and chemical forage characteristics, for example shear strength, grinding energy, fibre and nitrogen content (Henry *et al.* 1996).

One of the challenges in designing this experiment was to select appropriate forage combinations to obtain a measure of preference that could be compared to the field studies. By using an indirect comparison of the relative preference of the sheep between the legumes by offering them with annual ryegrass and wild radish we avoided using the large number of pots of forage that would have been required to make direct comparisons. Establishing plants for this indoor study was the most resource-consuming aspect of this indoor method and the use of indirect comparisons allowed us to compare between more legumes. Managing the pots after the plants were established was not difficult because they required only a relatively small area and the plants could be intensively managed. Reticulation ensured that a water deficit did not reduce plant growth so that adequate plant biomass was reliably available to use in the preference test. Testing for preference relative to 2 weeds doubled the capacity of this method to screen potential ‘companion’ pasture legumes against target weeds and shows how the method could be adapted to many other situations to determine the preferential motivation of grazing animals.

The food preferences of individual animals, even those that seem genetically similar, differs widely (Arnold 1964; Scott and Provenza 1999). The wethers in our study varied in their relative preference for wild radish, ryegrass and the six legumes at all phases of plant growth. For example, the relative preference of one sheep for ryegrass at the vegetative phase was 0.70 and 0.09 for radish, while for another the relative preference was 0.28 for ryegrass and 0.42 for radish. Despite this variation, it was clear that the six sheep, on average, preferred ryegrass to radish at this phase. Nevertheless, the wide variability in the preferences of individual sheep makes it difficult to generalise about their forage preferences. When using knowledge of the preferences of sheep to aid pasture management decisions such generalizations are necessary and useful, provided they accurately represent the flock.

In this study the relative preference was reported as the mean of six individual sheep and the small sample size may not accurately reflect the flock average, especially given the variability in preference that was found between the wethers. Ideally, the sheep used to assess relative preference should be a representative sample of the flock. In the two field studies this was assured because the method required a large plant biomass to be offered and therefore needed a relatively large number of animals for the preference test. Flocks of sheep ranging in size from 30 – 80 individuals were used in the field studies. As a result any differences in the relative preference of individual sheep should have had a negligible effect on the estimated relative preference of the whole flock. The consistency in preference we found between the two methods gives us confidence that the six animals selected for the indoor study were probably quite representative of the flock.

As mentioned earlier in this discussion the type and abundance of alternative forage is critical in determining the animal's preference for a particular forage. In addition, forage depletion by grazing has a considerable influence on preference as the animal eats. The forage biomass offered to the sheep decreased at a reasonably consistent rate as the sheep selectively removed their preferred forage and this could be accurately modelled using a quadratic function ($R^2 > 0.99$) at each of the three phases of plant growth. The rate of intake by the sheep also decreased each time the forage was re-offered and the preference of the sheep for the initially preferred forage decreased as the forage offered was depleted.

Due to the change in spatial, chemical and physical characteristics of the forage each time the pots were re-offered we considered that relative preference was only valid for intake measured before the preferred plant parts became so depleted that they restricted selection. We defined this as the period before the sheep's rate of intake decreased significantly. When offered the three pots of forage the sheep grazed for at least two minutes before their intake rate decreased at all phases of plant growth, so forage intake over this time was used to calculate relative preference (α). However, because we could not confidently predict this prior to the experiment the forage was offered to the sheep in six separate 1-minute periods. The information on forage availability and its relationship with rate of intake gained from this experiment could be used to calculate the grazing time required in future experiments so that the material does not need to be re-offered. However, an advantage of re-offering the forage in 1-minute periods was that the animal was compelled to re-consider its choice each time the forage was offered.

Some of the implications of the influence of plant morphology found in this study have already been discussed in this chapter (e.g. plant height and forage accessibility). Nevertheless, the differences in the spatial distribution of the different forages was probably exaggerated by the plants being grown in pots which elevated them above the ground surface and reduced inter-plant interactions compared to pasture plants in the field. The spatial arrangement of forage can exert a marked affect on selection by herbivores (Hodgson 1982). At the reproductive phase forage material of the wild radish, snail medic and biserrula plants extended further outside the perimeter of the pots than the other genotypes. Despite this, with the exception of forage density, the spatial characteristics of the forage that we measured were not related to the grazing preference of the sheep. In contrast, Hodgson and Jamieson (1981) found that sward height and not bulk density was important in determining grazing preference. However, they conceded that it was difficult to understand why there was no effect of bulk density and suggested that sward height may simply have had an overriding influence of sward height. Sward height was probably not a factor in our experiment because the plants were of adequate height so as not to restrict intake rate. The only exception was probably Dalkeith subterranean clover at the vegetative phase as discussed previously.

In addition to the weight lost due to grazing during the experiment, it was thought that the pots of plants would lose some weight as a result of evapo-transpiration. We found that the pots did lose weight and the amount of weight lost differed depending on the plant contained in the pot. However, we found that relative to the weight lost by defoliation by the sheep, the weight loss through evapo-transpiration very small and considered it not necessary to adjust for in calculating intake.

One of the concerns in offering the forage as a three way choice was that the centrally located pot would bias the animal's selection because of different accessibility compared to the pots positioned right or left of centre. We found that there was no bias in the forage selection of sheep between the three positions from where the forage was offered. As expected there was no bias toward either the left or right hand side, in agreement with Ganskopp (1995) who considered that turning preferences need not be considered when designing decision-based models of grazing behaviour in goats. We concluded that the position of the three rings did not affect the choice of forage so the dimensions of the feed trolley were probably adequate not to disrupt the preferential grazing exhibited by the sheep.

Chapter 6

Changes in pasture composition when a legume of low preference by sheep is introduced in a grazed pasture

Introduction

Friend and Kemp (2000) stated that "grazing management is the least understood and poorly used [management tool] for weed control in Australia". Sheep graze pastures selectively and pasture weeds are rarely eaten in preference to the desirable species (Friend and Kemp 2000). Under these conditions the weeds often persist in the pasture decreasing the feeding value of the pasture, especially at the end of the season, and the weed burden is carried over into the following pasture or crop. In contrast to this common pattern of pasture deterioration under grazing our aim was to use the ability of herbivores to manipulate plant diversity, within a pasture, to make use of grazing as a tool for weed management more effective by promoting the persistence of desirable pasture plants.

A strategy for weed control we considered is to incorporate into a pasture phase a highly productive legume which, when actively growing, is less preferred for grazing by sheep than a target crop weed. Sheep grazing this pasture should be compelled to eat more of the weed species and avoid grazing the legume, thereby effectively reducing the weed seed set and its subsequent germination in the crop phase. Annual ryegrass (*Lolium rigidum*) is a common weed in West Australian grain crops, which can severely impact yield (Medd *et al*. 1981). Although it is a weed in crops it has a high preference by sheep, particularly during its vegetative phase, and this makes it a good target for management by grazing in a pasture phase. We hypothesised that sowing into a pasture phase a productive legume that has a low short-term relative preference by grazing sheep when it is green would compel the sheep to graze annual ryegrass and improve the control of weed of crops in a grazed pasture.

This chapter comprises 4 parts, Part A, B, C and D, each describing an experiment to determine effects of grazing on the botanical composition of a pasture that was established.

Part A - Allandale 1

Introduction

The results of a preliminary experiment conducted in 1998 (General Introduction) indicated that sheep have a low preference for Prima gland clover (*Trifolium glanduliferum*) relative to Conquest annual ryegrass (*Lolium rigidum*) at the reproductive stage and a low preference for *Lathyrus* sp. relative to Conquest annual ryegrass at the vegetative stage. Based on these results these two genotypes were chosen as the 'companion' legumes in two experiments to test the effect on pasture composition of incorporating a less preferred legume into a grazed pasture phase. We hypothesised that the proportion of annual ryegrass in a grazed pasture sown to either gland clover or lathyrus will decrease compared to the proportion of annual ryegrass in an ungrazed pasture. We also hypothesised that the amount of seed set by annual ryegrass in a mixed lathyrus and annual ryegrass pasture grazed over two 1-week periods (early and late in the growing season) would be lower than the amount of annual ryegrass seed set in a pasture with grazing in only a single 1-week period late in the growing season.

Part A - Materials and Methods

A grazing experiment was established at Allandale Farm, Wundowie, 70 kilometres east of Perth (116.3 E, 31.8 S) on a gravely sand soil, pH ($CaCl_2$) 4.8 in June, 2000. Two pastures were established; i) a gland clover cv. Prima and annual ryegrass cv. Wimmera mixed pasture (27/06/00) and ii) a *Lathyrus cicera* cv. Chalus and annual ryegrass cv. Wimmera mixed pasture (27/06/00). These two pastures were assigned to two different grazing regimes to measure the effect of grazing on pasture composition.

Establishment and grazing of Prima gland clover and Wimmera annual ryegrass pasture
Seed of Wimmera annual ryegrass (25 kg/ha) and inoculated and lime pelleted seed of Prima gland clover (15 kg/ha) were sown as a mixture in a 1.2 ha plot. The plot was

divided by fencing into 4 x 30 m x 80 m plots to be grazed as four replicates and a 1 x 20 m x 120 m plot was excluded and left ungrazed. Two 2 m x 2 m exclosures were placed in each grazed plot prior to grazing to assess the biomass of ungrazed pasture and its composition.

Six Merino wether hoggets (approx. 45 kg) were allocated to each of the four plots in early September when food-on-offer was about 800 kg/ha. The sheep were removed from the plots in mid-October, which was earlier than was planned due to the lack of spring rain and the subsequent short growing season. Pasture samples were collected immediately prior to the sheep being put onto the pasture and every two weeks after that using a 0.5 x 0.5 m quadrat (5 quadrats/plot, 2 quadrats/exclosure), within which pasture composition was measured (General Materials and Methods).

Establishment and grazing of Chalus L. cicera *and Wimmera annual ryegrass pasture*

Seed of Wimmera annual ryegrass (25 kg/ha) and inoculated and lime pelleted seed of Chalus *L. cicera* (40 kg/ha) were sown as a mixture in a 1.32 ha plot. The plot was divided by fencing into 12 x 30 m x 30 m plots to be grazed and a 1 x 20 m x 120 m plot used as a sheep holding area. One 2 m x 2 m exclosure was assembled in each of the 12 grazed plots prior to grazing to assess the ungrazed pasture biomass. At 71 days after sowing, 6 groups of 5 Merino wether hoggets (approx. 45 kg) were allocated to 6 of these plots, for one week and then removed. At 114 days after sowing 12 groups of 8 Merino wether hoggets (approx. 50 kg) were allocated to all 12 plots, for one week and then removed. The pasture composition was measured before and after each of the two periods of grazing using a 0.5 m x 0.5 m quadrat taking samples from both grazed and ungrazed pastures (4 quadrats/plot, 2 quadrats/exclosure; General Materials and Methods). Estimates of annual ryegrass seed production (General Materials and Methods) across the plots were made in late October.

Site management

Weeds that had germinated before seeding were killed with an application of Roundup® herbicide (2 L/ha) prior to seeding. At seeding, double superphosphate + Cu, Zn, Mo (130 kg/ha) and KCl (40 kg/ha) was applied to the pasture.

Statistical analysis

Analysis of variance and LSD (Fisher's protected LSD) were used to determine the statistical significance of the results. These analyses were conducted using the SuperANOVA computer software package (Abacus Concepts Inc, CA, USA).

Part A - Results

Prima gland clover and Wimmera annual ryegrass pasture

The annual ryegrass content was the same for the grazed and ungrazed pastures at week 0 and week 2 ($P > 0.05$). The proportion of annual ryegrass in the mixed gland clover and annual ryegrass pasture was higher in the grazed than the ungrazed pasture at week 4 (0.81 *v* 0.62; $P < 0.01$). Over the 4-week period there was about a 10% increase in the annual ryegrass content of the pasture that was grazed ($P < 0.01$) while the overall proportion of annual ryegrass in the ungrazed pasture did not change ($P > 0.05$); Figure 6.1.

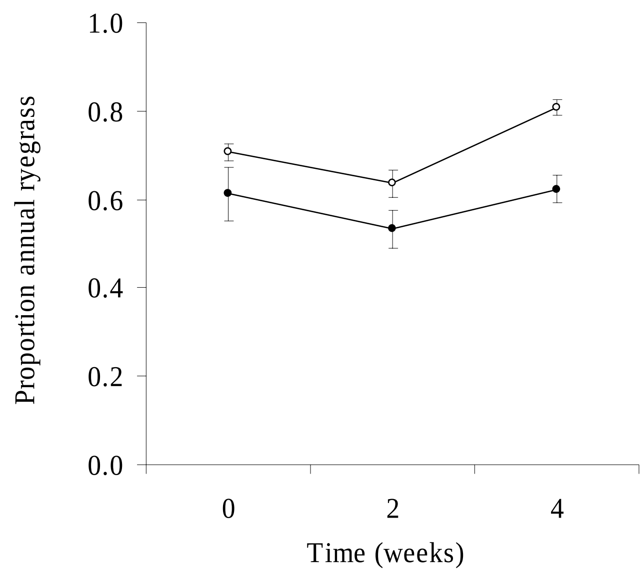

Figure 6.1. Proportion of annual ryegrass in a gland clover and annual ryegrass-sown pasture that was grazed (○) by Merino hoggets or kept ungrazed (●). Pasture composition

was determined prior to grazing, then after 2 and 4 weeks of grazing. Error bars are standard errors of the means.

In the grazed plots the biomass of annual ryegrass in the pasture increased by 40% from week 0 to week 4 of grazing (P < 0.01) while the overall biomass of gland clover did not change (P > 0.05); Figure 6.2. In the ungrazed control, the biomass of gland clover increased by 3.5 times (P < 0.01) and the biomass of annual ryegrass increased by 2.5 times from week 0 to week 4 of grazing (P < 0.01).

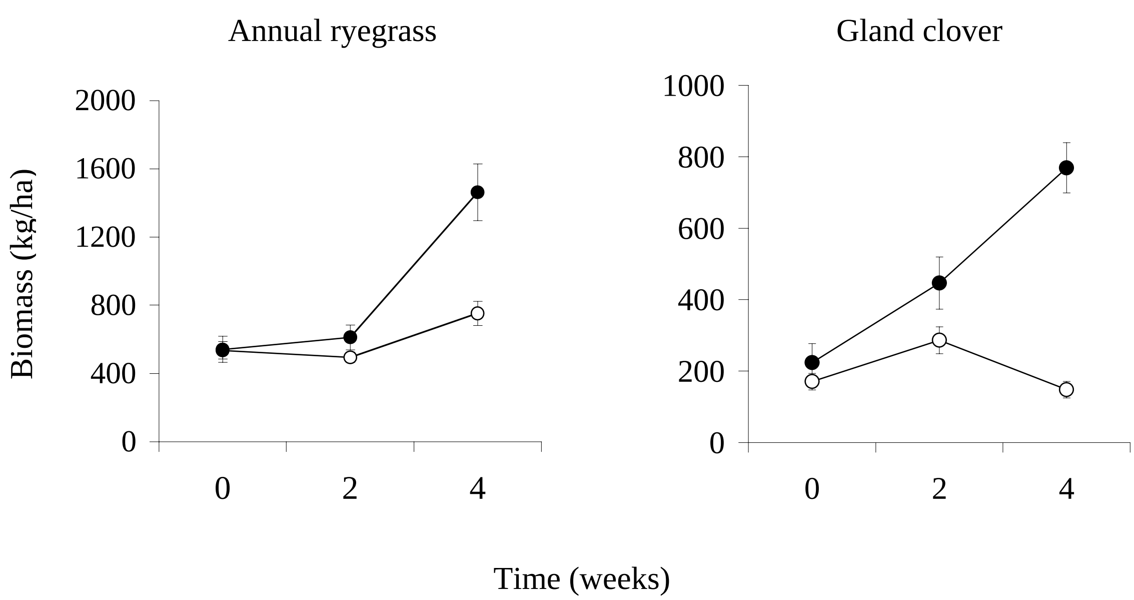

Figure 6.2. Change in the biomass (kg/ha) of gland clover and annual ryegrass in a mixed gland clover and annual ryegrass-sown pasture that was grazed (○) by Merino hoggets or kept ungrazed (●). Pasture composition was determined prior to grazing (0 weeks), then after 2 and 4 weeks of grazing. Error bars are standard errors of the means.

Chalus lathyrus and Wimmera annual ryegrass pasture

The proportion of annual ryegrass in the pasture did not change as a result of grazing in mid-September (Table 6.1). However, by mid-October the proportion of annual ryegrass in the pasture that had been grazed for one week in mid-September had increased by 40% compared to the pasture that was kept ungrazed (0.81 *v* 0.58; P < 0.05).

The total pasture biomass prior to grazing in mid-September was about 840 kg DM/ha and was reduced by about 7% after one week of grazing. The pasture biomass in mid-October was reduced by the early grazing compared to the ungrazed control (2440 *v* 3150 kg DM/ha; P = 0.09). Annual ryegrass seed set was not reduced by early grazing compared to the ungrazed control (30100 *v* 26600 seeds/m^2; P = 0.33). Grazing for 1 week in mid-October reduced annual ryegrass seed set by 66% in the previously ungrazed pasture and by 77% in the previously grazed pasture.

Table 6.1. Proportion of annual ryegrass in the pasture, the overall pasture biomass (kg DM/ha) and annual ryegrass seed set (seeds/m^2) in a mixed pasture sown to lathyrus and annual ryegrass to which 4 grazing treatments were applied; Grazed early or not, then grazed late or not

September (Early grazing)		October (Late grazing)			
Grazing treatment 1	Annual ryegrass content	Grazing treatment 2	Annual ryegrass content	Pasture biomass (kg DM/ha)	Estimated seed set (annual ryegrass seeds/m^2)
Ungrazed	0.72 ± 0.03	Ungrazed	0.58^{a} ± 0.04	3150^{c} ± 450	26600^{b} ± 3700
		Grazed (1 week)	0.74^{b} ± 0.04	1670^{ab} ± 160	9100^{a} ± 1800
Grazed (1 week)	0.71 ± 0.03	Ungrazed	0.81^{b} ± 0.03	2440^{bc} ± 310	30100^{b} ± 2300
		Grazed (1 week)	0.82^{b} ± 0.03	1360^{a} ± 110	7000^{a} ± 1100

Values within columns for each phase of growth with different superscripts are different (P < 0.05). Error terms are standard errors of the means.

Part A – Discussion

The hypothesis that the proportion of legume would increase in a grazed pasture sown with a legume that was previously shown to be less preferred by sheep was not supported in either experiment. Under continuous grazing the proportion of annual ryegrass in the mixture of gland clover and annual ryegrass pasture increased marginally indicating that the annual ryegrass was not being selectively removed. The proportion of annual ryegrass appeared to increase in the grazed pasture. However, this was probably not a result of selective grazing. It was more likely that the annual ryegrass was better established and able to recover from grazing more quickly due to its superior winter growth habit. Therefore we suggest that sheep exhibited either indifference or equal preference between gland clover and annual ryegrass and that the short-term relative preference was not a good predictor of the overall changes in the composition of grazed pastures in this experiment.

Grazing sheep on the lathyrus and annual ryegrass pasture for one week at the vegetative phase increased the proportion of annual ryegrass measured at the reproductive phase compared to the ungrazed control. However, total pasture biomass at the reproductive phase was not reduced by early grazing. In the ungrazed pasture the proportion of annual ryegrass decreased over time indicating that lathyrus may compete better with annual ryegrass if it is not grazed. In view of these results, our hypothesis that early grazing would reduce the seed set by annual ryegrass was rejected. Pasture composition did not change immediately due to early grazing, but when pasture composition was measured in October the proportion of annual ryegrass in the previously grazed pasture was higher than in the pasture that had not been grazed. Annual ryegrass may have a better tolerance to grazing compared to lathyrus and its recovery and growth following grazing may be more rapid as a result. Decumbency, or a prostrate growth habit, and a high number of stems are traits that are associated with a plant's tolerance to grazing (Rossiter 1966; Small 1996). The persistence of annual ryegrass in pastures has been related to their tolerance to grazing (Norton 1982). A lack of these characteristics in lathyrus compared to annual ryegrass may have contributed to its lower competitiveness under grazing.

Seed numbers were not reduced by early grazing. Although not significant ($P > 0.05$), annual ryegrass seed numbers may have been increased by the short period of early grazing. Young *et al.* (1996) found that grazing the annual ryegrass species *Lolium multiflorum* prior to when one-third of the primary tillers lose their apical meristem can increase seed production. These results indicate that annual ryegrass is tolerant of early grazing and in order to improve weed control by grazing at this time a pasture legume that is clearly avoided by sheep, is tolerant of grazing and is highly competitive appears to be needed. Continuous grazing may be more effective to reduce seed production by the target weed since the weed's reserves are more likely to be depleted, especially if the weed is grazed when relatively mature (Friend and Kemp 2000).

There are a number of possible explanations as to why the two legumes that were selected for their low short-term relative preference did not become dominant in the pasture. One of these is that the preliminary study may not have been robust enough to identify whether sheep really do have a low preference for gland clover and lathyrus. Other explanations have to do with the complex nature of the preferential grazing behaviour of herbivores and these are discussed further in the other discussion sections of this chapter.

Part B - Allandale 2

Introduction

Based on the results from screening 19 legumes for their relative preference by sheep (Chapter 4), we selected three legumes to evaluate the effect of sowing a less preferred legume on pasture composition. At the vegetative phase of growth, where the neutral relative preference (α) was 0.050, the short-term relative preference of sheep was low for Sava snail medic (0.009) and Casbah biserrula (0.015) compared to annual ryegrass (0.092); Chapter 4. Dalkeith subterranean clover with its moderate relative preference (0.060) was also selected, since its prostrate growth habit under grazing rather than its feeding characteristics were expected to facilitate selective grazing. Annual ryegrass often does not persist well in grazed, subterranean clover dominant, pastures and MacNish and Nicholas (1987) found that sowing some subterranean clover cultivars (particularly *T. subterraneum* cv. Dinninup) into the pasture markedly reduces the grass component of the pasture under grazing. Subterranean clover is uniquely adapted to grazing because of the position of its inflorescence relative to the leaf canopy and also its seed burial (Rossiter 1966). Pannell *et al.* (2003), in developing their Ryegrass Integrated Management (RIM) model, assumed the reduction in annual ryegrass plants in grazed subterranean clover pastures would be almost twice that in either a Cadiz serradella or volunteer pasture grazed at a standard grazing intensity over two years.

The three legumes that were chosen have exhibited good growth rates on gravely sand soils and were selected as the ‘companion’ legumes that would be likely to reduce the seed set by annual ryegrass in a grazed pasture phase. We hypothesised that the proportion of the annual ryegrass would decrease in a grazed pasture sown with annual ryegrass and a ‘companion’ legume compared to an ungrazed pasture and that annual ryegrass seed set in the grazed pasture sown with annual ryegrass and either biserrula, snail medic or subterranean clover would be lower than in a pure annual ryegrass pasture sown with the same density of annual ryegrass plants.

Part B - Materials and Methods

A grazing experiment was established at Allandale Farm, Wundowie (116.3 E, 31.8 S), 70 kilometres east of Perth on a gravelly sand soil in June, 2001. Four different pastures were established by sowing; i) a mix of Casbah biserrula (5 kg/ha) and Wimmera annual ryegrass (10 kg/ha), ii) a mix of Sava snail medic (20 kg/ha) and Wimmera annual ryegrass (10 kg/ha), iii) a mix of Dalkeith subterranean clover (10 kg/ha) and Wimmera annual ryegrass (10 kg/ha) and iv) Wimmera annual ryegrass alone (10 kg/ha).

The seed of the legumes was lime pelleted and inoculated with the appropriate inoculum. Weeds that had germinated before seeding were killed with an application of Roundup® herbicide (2 L/ha) prior to seeding. At seeding, 3:2 P:K fertiliser was applied at 130 kg/ha. The pastures were fenced into six replicate plots each 25 m x 100 m, each made up of four sub plots of 25 m x 25 m of the four pastures. In early August three of the 6 replicated plots were continuously stocked with 6 Merino hogget wethers weighing approximately 40 kg, while the other three plots were kept ungrazed. Within each plot, the hoggets had access to the four pastures at the same time. The hoggets were removed from the plots in mid October. The pasture composition in the plots was measured every 2 weeks for both the grazed and ungrazed treatments and the number of seeds of annual ryegrass was estimated in mid October (General Materials and Methods). Pasture composition in the plots was visually estimated in the following season (July 2002) using the dry weight rank method ('t Mannetje and Haydock 1963), with three replicates for each sub plot.

Statistical analysis

Analysis of variance and LSD (Fisher's protected LSD) were used to determine the statistical significance of the results. These analyses were conducted using the GenStat® (Version - 6.1.0.200) statistical package.

Part B - Results

The proportion of annual ryegrass in the plots with a mix of subterranean clover and annual ryegrass that were grazed tended to decrease from 0.69 to 0.49 (P = 0.14) over the 70 days of grazing. There was a similar, but not significant trend for the proportion of annual ryegrass to decline in the grazed plots containing the mixture of biserrula and annual ryegrass (Figure 6.3). The proportion of annual ryegrass in the grazed plots of snail medic and annual ryegrass did not change (P = 0.58). The proportion of subterranean clover in the grazed plots sown with a mix of subterranean clover and annual ryegrass increased over the 70 days from 0.15 to 0.32 (P = 0.11), but in the plots sown to snail medic the proportion of snail medic decreased from 0.16 to 0.04 (P = 0.06). There was no overall change in the proportion of biserrula in the plots where this legume was sown with annual ryegrass (P = 0.84). The proportion of annual ryegrass and legume in the ungrazed plots did not change throughout the 70 days the other plots were grazed (Figure 6.4). Other pasture species, such as broadleaf weeds, comprised the remaining pasture component that was neither annual ryegrass nor legume.

The amount of annual ryegrass seed set in mid October was up to 270 times greater in the ungrazed pastures compared to the grazed pastures. There were no significant differences in the amount of annual ryegrass seed set due to the legume that the annual ryegrass was sown with, for either the grazed or ungrazed plots (P > 0.05); Table 6.2. In the grazed plots the germination of annual ryegrass in the following season was markedly reduced compared to the ungrazed plots. In the grazed plots broadleaf weeds were dominant and annual ryegrass made up only about 10% of pasture biomass, in contrast annual ryegrass was dominant in the ungrazed plots, which contained only about 15% broadleaf weeds (Table 6.2).

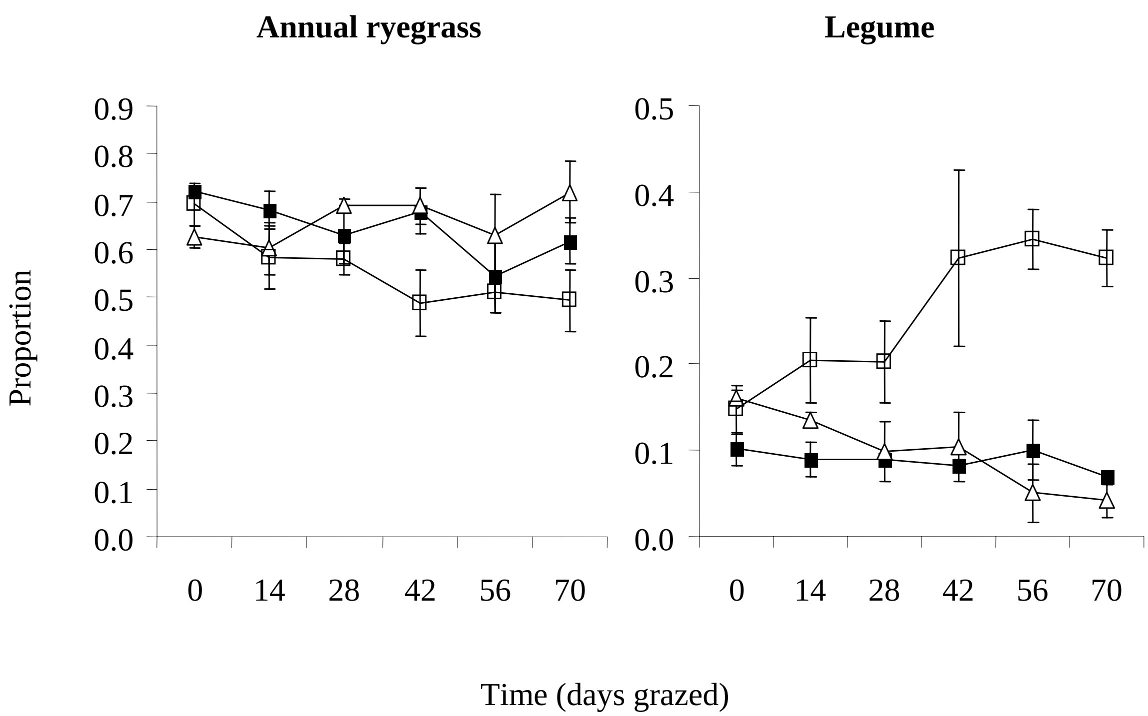

Figure 6.3. Proportion of annual ryegrass or legume in a mixed pasture sown with annual ryegrass and either biserrula (■), snail medic (△) or subterranean clover (□) that was set-stocked with Merino hoggets. Error bars are standard errors of the means.

Annual ryegrass **Legume**

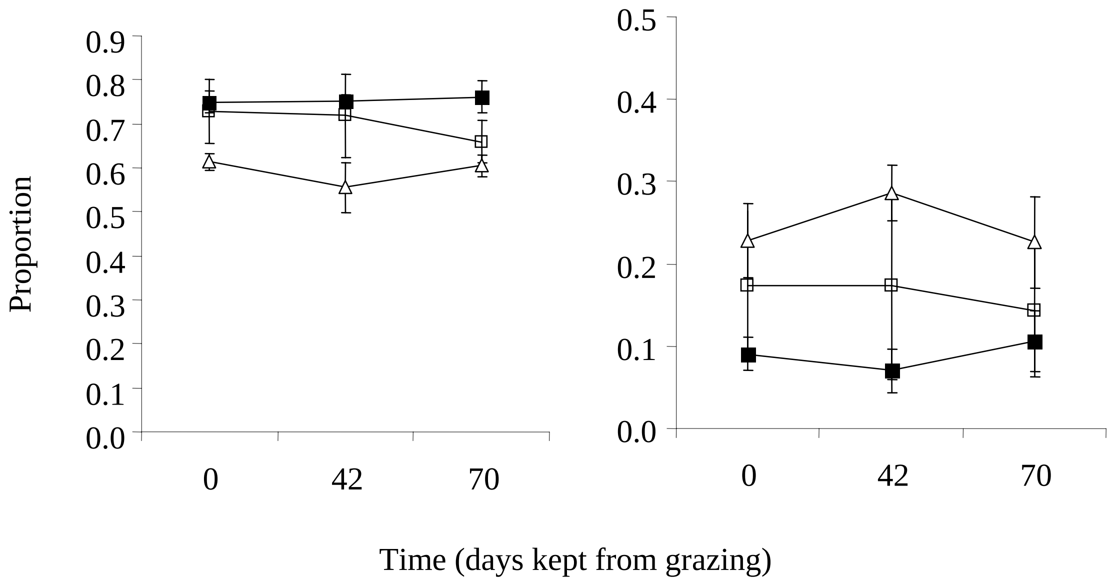

Figure 6.4. Proportion of annual ryegrass or legume in a mixed pasture sown with annual ryegrass and either biserrula (■), snail medic (△) or subterranean clover (□) that was not grazed. Error bars are standard errors of the means.

Table 6.2. Annual ryegrass seed set (seeds/m^2) in a mixed pasture sown with annual ryegrass and either biserrula, snail medic or subterranean clover and the proportion of legume, broadleaf and annual ryegrass plants regenerating in the pasture in the subsequent season

Grazing treatment	Pasture Treatment	Seed set 2001 (seeds/m^2)	Germinating plants 2002 (estimated proportion (%) of dry weight)		
		Annual ryegrass	Legume	Broadleaf	Annual ryegrass
Grazed	Biserrula	15^a ± 5	20.4 ± 0.7	70.2^a ± 0.0	9.4^a ± 0.7
	Annual ryegrass	30^a ± 6	17.6 ± 2.5	70.2^a ± 0.0	12.2^a ± 2.5
	Snail medic	73^a ± 18	18.4 ± 1.8	70.2^a ± 0.0	11.5^a ± 1.8
	Sub. clover	107^a ± 46	17.6 ± 1.8	70.2^a ± 0.0	12.2^a ± 1.8
Ungrazed	Biserrula	4071^b ± 517	12.8 ± 2.4	17.0^b ± 2.4	70.2^b ± 0.0
	Annual ryegrass	5267^b ± 153	14.9 ± 3.1	14.9^b ± 3.1	70.2^b ± 0.0
	Snail medic	3661^b ± 469	17.0 ± 1.9	12.8^b ± 1.9	70.2^b ± 0.0
	Sub. clover	4847^b ± 581	14.2 ± 2.5	15.6^b ± 2.5	70.2^b ± 0.0

Values within columns with different superscripts are different ($P < 0.05$). Error terms are standard errors of the means.

The final pasture biomass for the four pastures was reduced by about 70% due to grazing (Figure 6.5). There were no significant differences in the pasture biomass between the plots of the four pastures. The biomass of annual ryegrass in the grazed plots at day 70 was highest in the plots of snail medic mixed with annual ryegrass and lowest in the plots of subterranean clover mixed with annual ryegrass. However, the biomass of annual ryegrass in the pasture was highly variable and the differences were not significant (1991 *v* 1105 kg/ha; P = 0.29); Figure 6.6. In the mixture of snail medic and annual ryegrass the annual ryegrass plants appeared to be grazed less than in the other plots (personal observation). In the ungrazed plots the biomass of annual ryegrass was highest in the pure annual ryegrass pasture, but this also was not significant. The reduction in annual ryegrass biomass due to competition from the legumes was around 20% but this difference, too, could not be validated statistically due to the high variability of the components and biomass.

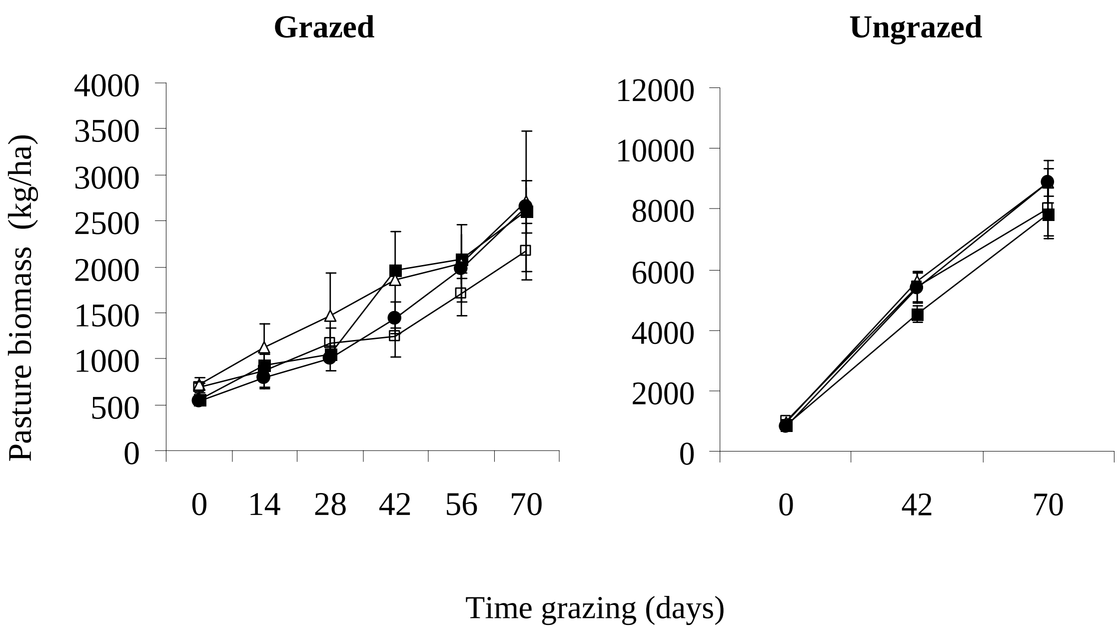

Figure 6.5. Total pasture biomass (kg/ha) in mixed pastures sown with annual ryegrass alone (●) or annual ryegrass with either biserrula (■), snail medic (△) or subterranean clover (☐) and either grazed or kept ungrazed. Error bars are standard errors of the means.

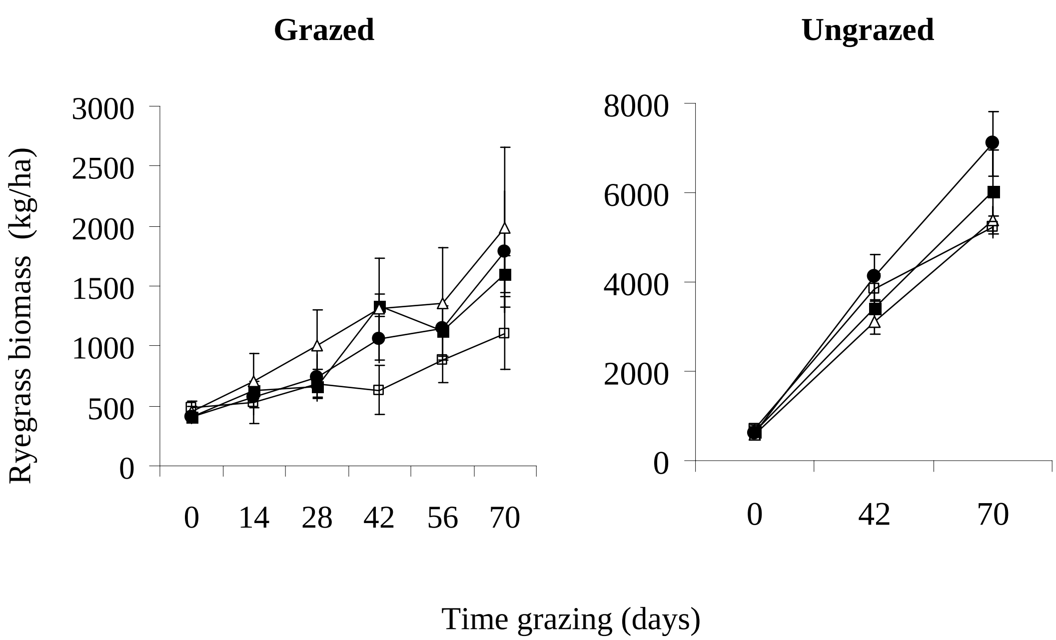

Figure 6.6. Annual ryegrass biomass (kg/ha) in mixed pastures sown with annual ryegrass alone (●) or annual ryegrass with either biserrula (■), snail medic (△) or subterranean clover (☐) and either grazed or kept ungrazed. Error bars are standard errors of the means.

Part B - Discussion

The proportion of annual ryegrass in the grazed plots sown with Casbah biserrula or Dalkeith subterranean clover decreased, supporting our hypothesis. However, the proportion of annual ryegrass in the snail medic plots that were grazed did not change. The amount of annual ryegrass seed set did not vary between the four pasture mixtures for both the grazed and ungrazed treatments so the second hypothesis was not supported.

In this study it was noted that sheep appeared to avoid grazing annual ryegrass plants that were close to the snail medic. Similarly, the trend in pasture biomass suggested that the plots with a mixture of snail medic and annual ryegrass were grazed less than the other pasture plots. This indicates that sheep may seek to substitute their grazing site rather than select the preferred components from a site with a high proportion of an undesirable species. It has been reported that plants can be protected from herbivory through the presence of a neighbouring plant that is avoided by the grazing animal (Rousset and Lepart 2002).

Grazing, irrespective of whether the herbivore exhibits selectively, is widely adopted as a tool for weed control. The effectiveness of grazing at a set stocking rate to reduce annual ryegrass seed production was highlighted in this study. Annual ryegrass seed numbers were reduced by about 99% by grazing the plots while the pasture biomass was reduced by only about 70%. The large difference in the proportion of annual ryegrass in the pasture in the following year between the previously grazed and ungrazed pastures (~10% *v* ~70% annual ryegrass) clearly demonstrated the marked impact of grazing. Capeweed was dominant in the following year for the grazed pasture while annual ryegrass was dominant in the ungrazed pasture (Table 6.2). An increase in the proportion of grass in pastures that are left ungrazed is commonly observed (Rossiter 1966), and Broom and Arnold (1986) showed that selective grazing by sheep can disadvantage annual ryegrass in mixed pastures. Selective grazing of annual ryegrass seed heads in pastures by sheep and cattle was reported by Matthews *et al.* (1996) and may be a result of their high relative preference by livestock. Additionally, allelopathic activity has been identified in annual ryegrass residues (San Emeterio *et al.* 2004) and may have contributed to the dominance of annual ryegrass in the ungrazed pasture, particularly given the large amount of dry annual ryegrass residue standing in the pasture at the end of the previous season. It is generally accepted that the preference of sheep for grasses decreases relative to legumes as the pasture matures (Arnold 1962). However, the nutritional value of stem decreases more rapidly than leaf (Norton 1982) so the leaf and seed of the plant may retain a higher relative preference. Alternatively, the height of the seed heads and therefore their relative elevation in the sward may have improved their accessibility for grazing, and thereby increased their selection by sheep (Louault *et al.* 1997).

Competition between the legumes and annual ryegrass appears to have reduced the biomass of annual ryegrass by about 20%, but the differences were not significant and greater replication would be required to validate this. In this strategy it is expected that inter-plant competition will also have a significant effect on weed growth and seed set independent from grazing. An additional complexity is that legumes may potentially assist grass growth, for example by nitrogen transfer. In annual pastures most of the contribution of nitrogen to the soil by plants comes from the breakdown of above-ground plant material and to a lesser extent from the decomposition of roots and nodules of senesced plants at the end of the growing season. However, there appears to be some direct transfer of symbiotically fixed nitrogen from legumes to grasses during the growing season from plants that die early and also from legume root exudates (Ta and Faris 1987).

We conducted this study to investigate whether annual legumes with a low, short-term relative preference by sheep have a competitive advantage over annual ryegrass in a grazed pasture. The results support the hypothesis but show that the relationship between short-term relative preference and diet selection by sheep in pastures is far from simple. We can conclude that there are many factors apart from the short-term relative preference of sheep among pasture species that contribute to the effect of grazing on pasture composition.

Part C – Merredin

Introduction

In this section we report on a collaborative project with the West Australian Department of Agriculture, which was supervised by Dr Clinton Revell. The project was initiated to determine whether the pasture composition and seed set by volunteer annual ryegrass (an established population of annual ryegrass that had been previously introduced to the paddock) differed as a result of sowing legumes of high and low relative preference by sheep. The design and establishment of this experiment was undertaken by Dr Revell to develop further our work on the short-term relative preference of sheep among legumes as a screening method for potential 'companion' legume candidates. My contribution was to provide background on the appropriate legumes for the grazing study and to measure and analyse the seed set by annual ryegrass in the grazed pastures.

The short-term relative preference of sheep for snail medic cvs. Kelson and Sava and vegetative biserrula cv. Casbah have been found to be low compared to other annual legumes and annual ryegrass (Chapter 4). Therefore these two plants were selected as 'companion' legumes to reduce the seed set by annual ryegrass in a grazed pasture. The short-term relative preference of sheep for burr medic cv. Santiago is moderate to high compared to other annual legumes and annual ryegrass and was suited to the soil type of the experimental site so this plant was selected to be used as a positive control.

We hypothesised that sowing biserrula or snail medic into a grazed pasture that contains volunteer annual ryegrass will reduce the seed set by annual ryegrass more than in a pasture sown with burr medic.

Part C – Materials and Methods

The experiment was established at the Department of Agriculture's Merredin Research Station, Western Australia (31.5 S, 118.2 E) on a red sandy loam soil with a pH ($CaCl_2$) of

5.0. The site had a significant population of volunteer annual ryegrass. Three pasture treatments were sown in May 2001 in a randomised complete block design with three replications. The pasture treatments sown were Casbah biserrula (*Biserrula pelecinus*) at 15 kg/ha, Sava snail medic (*Medicago scutellata*) at 26 kg/ha and Santiago burr medic (*Medicago polymorpha*) at 23 kg/ha.

The plots were top-dressed with about 50 kg/ha of double superphosphate at seeding and 50 kg/ha of KCl in early August. The pastures were sown in 9, 0.5 ha (45 m x 112 m) plots, without the application of a knockdown herbicide for weed control. Six Merino wether sheep (approx. 50 kg liveweight) were allocated to graze each plot (12 DSE/ha) in the last week of August when the food-on-offer was about 900 kg/ha. An additional 5 m x 45 m of each plot was excluded from grazing to assess pasture production when ungrazed. About 20 - 25% of the pasture consisted of naturally regenerating grasses, dominated by annual ryegrass. The sheep were maintained on the plots until the last week of October, for a total of eight weeks.

Annual ryegrass seed production was assessed in October based on tiller numbers and length of inflorescence in 12 grazed and 2 ungrazed $0.25m^2$ quadrats per plot and assuming four seeds per centimetre of inflorescence.

Statistical analysis

Analysis of variance and LSD (Fisher's protected LSD) were used to determine the statistical significance of the results. These analyses were conducted using the SuperANOVA computer software package (Abacus Concepts Inc., CA, USA).

Part C – Results

Sowing the annual legume Casbah biserrula into a grazed pasture reduced the seed set by annual ryegrass to 6% of that of the Santiago burr medic positive control (311 *v* 5396 seeds/m^2; $P < 0.05$). The seed set by annual ryegrass in the plots sown with Sava snail medic did not differ significantly from the control. Both the number of tillers/m^2 and the length of the seed heads were reduced in the biserrula plots that were grazed compared to the grazed burr medic plots (Table 6.3).

In the ungrazed plots, there was no effect of the legume cultivar sown on the number of annual ryegrass tillers, estimated seed set or the length of seed heads (Table 6.3).

Table 6.3. Effect of grazing and pasture cultivar on annual ryegrass tillers and estimated seed set

Pasture cultivar	Casbah biserrula	Sava snail medic	Santiago burr medic
Ungrazed			
Annual ryegrass tillers/m^2	85 [a]	93 [a]	145 [a]
Estimated seed set (no/m^2)	4240 [a]	5352 [a]	7504 [a]
Length of seed head (cm)	13 [a]	15 [a]	13 [a]
Grazed			
Annual ryegrass tillers/m^2	16[a]	171[b]	139[b]
Estimated seed set (no/m^2)	311[a]	6522[b]	5396[b]
Length of seed head (cm)	5[a]	9[b]	10[b]

Values within rows with different superscripts are different ($P < 0.05$).

Part C - Discussion

The hypothesis that sowing snail medic and biserrula would reduce annual ryegrass seed set in a grazed pasture more effectively than sowing burr medic was only partially supported. Sheep consistently avoided snail medic plants in our short term selection studies, but in the field we found no evidence to suggest that the sheep avoided eating snail medic and targeted annual ryegrass. This was not the case with biserrula. This study gave strong evidence to support anecdotal observations that other pasture species are targeted when sheep graze a biserrula dominant pasture (Loi, Revell and Kolb; pers. comm.).

The mechanisms behind the apparent avoidance of biserrula and the targeting of alternative plants in the biserrula pasture are still unclear. Although biserrula plants appeared to be less readily selected by sheep than burr medic (personal observation), biserrula was still consumed and quite evenly grazed. Therefore the degree of aversion was not strong enough to constitute full feed refusal by the sheep. Perhaps sheep were selecting a preferred dietary mix consisting of more annual ryegrass to support rumen fermentation and function better from the forage available. Nevertheless, it seems preferable in managing grazed pastures to reduce weeds if the sheep can also derive some nutritional benefit by eating some of the avoided species when alternative forage is scarce.

There are several possible explanations for the sheep apparently not seeking substitute forage on the snail medic pasture as was predicted from our short term preference studies. The sheep may have been physically unable to exclusively select annual ryegrass from a snail medic pasture. The growth habit of snail medic is such that it becomes entwined with annual ryegrass plants and may be difficult for sheep to distinguish and select out. Grazing of the snail medic plots appeared to be patchy compared to the burr medic and biserrula plots (personal observation). Sheep may have selected patches containing proportionally less snail medic rather than trying to distinguish between individual plants that were growing adjacent to each other. Milne *et al.* (1979) characterise this distinction by introducing the concept of “site selection” and “bite selection”. By his definition patchy grazing would be a consequence of a high degree of site selection but little bite selection. Similarly, Louault *et al.* (1997) suggest that increased defoliation of annual ryegrass compared to white clover was a result of the low vertical availability of white clover rather than the sheep’s preference between the two types of forage.

Alternatively, the sheep may have become accustomed to the unpalatable characteristics of the snail medic plants and ultimately incorporated the plant into their diet. Rook *et al.* (1997) contend that palatability or short-term preference is unlikely to persist in itself. Often low preference is linked to undesirable chemical attributes of the plant that the animal identifies after it familiarises itself with the plant and has a negative postingestive experience. With novel foods, the herbivore could identify a plant as objectionable prior to sampling and therefore deny itself the opportunity to assess the plants feeding value. However, by association and familiarity the herbivore increases its acceptance of the forage. This mechanism is described by McClymont (1967) as habituation, defined as a "Reduction in selection against a material as a result of repeated or continued exposure to it when the selection has had no beneficial effect on function". This appears to be describing a tolerance of the animal to an undesirable taste, smell or texture that, as determined by experience, did not have a positive effect (or a severely adverse effect) on the animal. The preferences of sheep for typically unpalatable plants is often found to increase when the sheep have increased exposure to the plant, especially when they are young (Willms 1977; Matthews 1987; Walker *et al.* 1992; Distel *et al.* 1996).

High levels of rumen-degradable protein in biserrula may have contributed to the apparent aversion response in the sheep. Prins and Beekman (1989) found that buffalos eating plants with high levels of rumen-degradable protein can experience malaise and subsequently reduce their intake of that food presumably because they accumulate high levels of rumen ammonia, causing toxicoses. Sheep have been found to reverse their preference for clover to grass when infused with ammonia (Cosgrove *et al.* 1999), indicating that sheep may have the capacity to regulate their intake of rumen degradable protein to avoid excessive levels of rumen ammonia. Goto *et al.* (1983) found that sheep prefer cocksfoot with higher levels of water soluble carbohydrates and lower nitrogen, suggesting that excess plant nitrogen can cause sheep to avoid selecting the forage. Unfortunately we did not measure the level of rumen-degradable protein in the biserrula forage, but our plant analysis revealed the crude protein content in biserrula was in excess on 30% at the late vegetative phase of plant growth and could easily be as high as 35% prior to this. However, other annual legumes are also likely to have similarly high levels of rumen-degradable protein and have not been observed to create an aversion, so the possibility that the high soluble protein in biserrula creates an aversive response is not without question, especially given that the aversion exists for biserrula at flowering where the concentration of protein is lower than at the vegetative phase.

It is also possible, that some other aversive compound, for example a plant secondary compound, is present in biserrula. There are a wide variety of plants and plant compounds that have been found to induce food aversions in herbivores, many of these have been identified (Provenza 1996a). These compounds act via postingestive feedback mechanisms to reduce the selection of specific forages, by associating the forage with a negative digestive experience.

The amount of seed set by annual ryegrass in the grazed pastures sown with either snail medic or biserrula, differed markedly. As in part B, we concluded that factors other than short-term relative preference among annual legumes contribute to the success of legumes in promoting favourable changes in pasture composition where a potential ‘companion’ legume is introduced into a pasture phase.

Part D – Wickepin

Introduction

As reported in Part C, Casbah biserrula (*Biserrula pelecinus*) was found to be a particularly good candidate to make annual ryegrass less competitive in a grazed pasture. The aim of this study was to explore this further and determine whether Casbah biserrula is more effective than Dalkeith subterranean clover (*T. subterraneum*) in reducing the seed set by annual ryegrass in a grazed pasture. Further, we aimed to determine whether grazing isolated pastures sown with either biserrula or subterranean clover (referred to as Specific grazing) or allowing sheep to graze across both biserrula and subterranean clover plots (Open grazing) would impact on the selective grazing of annual ryegrass. We hypothesised that the seed set by annual ryegrass would be lower in a grazed pasture with Casbah biserrula than in a grazed pasture with Dalkeith subterranean clover and that confining sheep to a plot where a single legume was sown would be result in a greater reduction in annual ryegrass seed set.

Part D - Materials and Methods

The experiment was conducted from May to November 2002, on a property near Wickepin in Western Australia (32° 46' S, 117° 30' E), on a gravelly sand soil pH ($CaCl_2$) 5.7 at the surface. The site had previously been cropped with lupins in 1999 and with wheat in 2000 and 2001. The stubble residue from the 2001 wheat crop was burned prior to seeding. Four pasture treatments (Table 6.4) were sown with CSBP Super3Potash applied at 120 kg/ha and BigPhosTE applied at 75 kg/ha fertiliser during seeding.

Table 6.4. Sowing rates for the 4 pasture treatments

Pasture treatment	Sowing rates
Biserrula (low ryegrass)	Biserrula[1], 20 kg/ha; annual ryegrass[2], 5.4 kg/ha
Biserrula (high ryegrass)	Biserrula, 20 kg/ha; annual ryegrass, 10 kg/ha
Sub. clover (low ryegrass)	Sub. clover[3] 40 kg/ha; annual ryegrass, 5.4 kg/ha
Sub. clover (high ryegrass)	Sub. clover 40 kg/ha; annual ryegrass, 10 kg/ha

[1]*Biserrula pelecinus* cv. Casbah
[2]*Lolium rigidum* cv. Wimmera
[3]*Trifolium subterraneum* cv. Dalkeith

The pastures were fenced to allow three grazing treatments within each pasture treatment. The grazing treatments were:

(i) Specific grazing, where weaner Merino wethers were allocated, in groups of 15, to an area of 100 m x 100 m (15 sheep/ha) on each of the four pasture treatments.

(ii) Open grazing, where 30 weaner Merino wethers were allocated to an area of 400 m x 50 m (15 sheep/ha) with access to all of the four pasture treatments.

(iii) No grazing, where sheep were excluded from the pasture for the duration of the experiment.

The weaners were weighed, condition scored (on a scale 0-5) and allocated (after being sorted into groups of equal mean weights) to the pastures on September 10 (Day 0) when it was estimated that the food-on-offer (FOO) was 1000 kg/ha. The weaners were maintained on the plots until November 18. The body weight and the condition score of the weaners was measured between Days 0 to 44 (Period 1) and between Days 45 to 70 (Period 2). Analysis of variance was conducted using the SuperANOVA computer software package (Abacus Concepts Inc, CA, USA).

Measurements were made of FOO and pasture composition on Day 0 by sampling 6 x 0.10 m^2 quadrats (General Materials and Methods) in each of the three grazing treatments for each of the four pasture types. Estimates were made of annual ryegrass seed set on October 15 based on the number of tillers and the length of the inflorescence for 6 x 0.10 m^2 quadrats in each grazing treatment (General Materials and Methods). The data were analysed by comparison of means with t-tests.

Part D - Results

Prior to grazing the legume biomass was higher in the subterranean clover (high and low annual ryegrass) pastures than in the biserrula (high and low annual ryegrass) pastures (1380 and 1530 *v* 440 and 450 kg DM/ha; $P < 0.01$); Table 6.5. Subterranean clover was observed to senesce and dry-off 2-3 weeks earlier than biserrula (personal observation).

Table 6.5. Biomass (kg DM/ha) of legume, annual ryegrass and other pasture components in the 4 sown pastures measured 10 September, 2002

Pasture component	Biserrula		Subterranean clover	
	low ryegrass	high ryegrass	low ryegrass	high ryegrass
Legume	450	440	1530	1380
Annual ryegrass	350	670	560	610
Other	120	310	140	210
Total	920	1420	2230	2200

The estimated annual ryegrass seed set was greater in the high ryegrass Biserrula pasture than in the high ryegrass Subterranean clover pasture for both the Specific grazing (5500 *v* 2700 seeds/m^2; $P = 0.047$) and Open grazing treatments (10000 *v* 4100 seeds/m^2; $P = 0.019$); Table 6.6. Of the Specific grazing treatments the high ryegrass Biserrula pasture had the highest estimated annual ryegrass seed set (5500 seeds/m^2), while the seed set in the low ryegrass Subterranean clover pasture was the lowest at 1100 seeds/m^2.

Table 6.6. Estimated seed set/m^2 of annual ryegrass in pasture sown with biserrula (20 kg/ha) and subterranean clover (40 kg/ha) and with annual ryegrass at high (10 kg/ha) and low (5.4 kg/ha) rates and assigned to 3 grazing treatments

Grazing treatment	Biserrula		Subterranean clover	
	low ryegrass	high ryegrass	low ryegrass	high ryegrass
Specific grazing	2800	5500	1100	2700
Open grazing	5300	10000	4800	4100
No grazing	32700	36600	21500	33000

The Specific grazing treatment was about twice as effective as the Open grazing treatment in reducing annual ryegrass seed set, and for both grazing treatments there was a marked reduction in annual ryegrass seed set compared to the ungrazed control (Table 6.6).

For the Specific grazing treatments, the weaners grazing the low ryegrass Biserrula pasture had a higher growth rate than those grazing the low ryegrass Subterranean clover pasture (195 *v* 165 g/h.d; P = 0.013); Table 6.7. Similarly, the weaners grazing the high ryegrass Biserrula pasture grew faster than the weaners grazing the high ryegrass Subterranean clover pasture (202 *v* 150 g/sheep.day; P = 0.013); Table 6.7.

Table 6.7. Growth rate (g/h.d) of weaner Merino wethers grazing 5 different pastures, measured over 2 periods; September 10 to October 24 (Period 1) and October 25 to November 18 (Period 2), 2002

Grazing period	Biserrula (n = 15)		Subterranean clover (n = 15)		Open grazing (n = 30)	P-value
	low ryegrass	high ryegrass	low ryegrass	high ryegrass		
1. (Day 0 to 44)	230^{c}	218^{bc}	192^{b}	146^{a}	222^{bc}	0.000
2. (day 45 to 70)	135^{ab}	175^{c}	117^{a}	155^{bc}	165^{bc}	0.027
1 & 2. (day 0 to 70)	195^{c}	202^{c}	165^{b}	150^{b}	201^{c}	0.000

Values within rows with different superscripts are different (P < 0.05).

In period 2, the weaners grazing the high ryegrass Biserrula pasture had the fastest growth rate (175 g/h.d), while the weaners grazing the low ryegrass Subterranean clover pasture had the lowest growth rate (117 g/h.d); Table 6.7. The condition score of the weaners at each weighing did not differ between any of the pasture treatments (Table 6.8).

Table 6.8. Change in condition score of weaner Merino wethers grazing 5 pastures, measured over 2 periods; September 10 to October 24 (Period 1) and October 25 to November 18 (Period 2), 2002

Grazing period	Biserrula (n=15)		Subterranean clover (n=15)		Open grazing (n=30)	P-value
	low ryegrass	high ryegrass	low ryegrass	high ryegrass		
1. (day 0-44)	.57	.41	.51	.34	.46	0.669
2. (day 45-70)	-.14	.15	-.10	.16	.04	0.180
1 & 2. (day 0 -70)	.43	.55	.41	.50	.50	0.911

Values within rows with different superscripts are different (P < 0.05).

Part D – Discussion

Our hypothesis that the seed set by annual ryegrass would be lower in a grazed biserrula pasture than in a grazed subterranean clover pasture was not supported in this experiment. The higher annual ryegrass seed set in the biserrula pasture was probably a consequence of differences in the pattern of feed availability between the two pasture types that was generated in response to the dry seasonal conditions. Spring rainfall in 2002 was unusually low, with only 29 mm of rain recorded for Wickepin from September 10 to November 18. The grazing management strategy we applied probably needed an extended period of grazing while the plants were growing for the selective grazing behaviour of sheep to translate into large changes in pasture composition, i.e. a reduction in the annual ryegrass and the legume to become the dominant pasture species. Under these dry conditions pastures were heavily grazed and the forage selected by the sheep was most likely modified by the lack of feed on offer.

Although the initial biomass of the subterranean clover pasture was almost double that of biserrula (perhaps due to the higher seeding rate), we observed that much of the subterranean clover senesced within the first month after the pastures were grazed. Thus with limited feed available, the sheep appeared to graze the annual ryegrass more intensely. On the other hand biserrula, which is known to be deep rooted and resilient in dry conditions, was able to survive and grow when any rain fell during this period. This would have increased the amount of biserrula available during the later stages of the experiment. Compared to the subterranean clover pastures, there would have been less grazing pressure on the annual ryegrass during seed set as the sheep could supplement their diet with biserrula. Evidence that the extra biserrula was utilised is supported by the higher growth rates of the sheep grazing biserrula pastures throughout this experiment.

Although seasonal conditions clearly have a major impact, it is possible that Dalkeith subterranean clover can be a useful 'companion' legume to reduce the annual ryegrass component of a pasture. Subterranean clovers have a prostrate growth habit that reduces their accessibility to sheep and this generally makes them tolerant of grazing. MacNish and Nicholas (1987) found that some subterranean clover cultivars (particularly Dinninup) became dominant when sown into volunteer barley grass and broadleaf weed pastures when grazed by sheep. However, other subterranean clover varieties had less effect on pasture composition and a high proportion of grasses and broadleaf weeds was maintained. In their Ryegrass Integrated Management (RIM) model Pannell *et al.* (2003) report that in two years a subterranean clover pasture stocked at a standard grazing intensity can reduce the number of annual ryegrass plants setting seed by 70% compared to 40% in a serradella pasture.

The slightly lower annual ryegrass seed set in ungrazed subterranean clover pastures may indicate that competition from subterranean clover reduced the vigour of the annual ryegrass plants independent of grazing.

This experiment has highlighted the considerable impact that seasonal conditions have on weed management during a grazed pasture phase. Ideal conditions for manipulating pasture composition by selective grazing include a long growing season and maintaining the FOO so that forage availability is neither limiting nor in excess. The potential for a grazed biserrula pasture to reduce the seed set by annual ryegrass during a pasture phase was not demonstrated under the conditions of this experiment.

Discussion

The hypothesis that sowing into a pasture phase a productive legume that has a low short-term relative preference by grazing sheep when it is green would compel the sheep to selectively graze the undesirable weed species and improve its control was only partially supported. We found that where a legume is avoided in a pasture and alternative forages are targeted, weed seed set can be spectacularly reduced compared to pastures where the introduced legume is not avoided. However, we also found that selecting for low short-term relative preference is not a reliable method to identify legumes that will be avoided, or advantaged, in annual pastures. Measuring the short-term relative preference of sheep for annual legumes may be a useful guide to the screening of annual legumes for their potential to be used as a ‘companion legume’. However, this method is unlikely to clearly identify those legumes that are or are not the best to use to assist grazing for weed control. In order to implement grazing management strategies to improve weed control other complex plant-animal interactions should also be considered in addition to short-term relative preference.

In ‘cafeteria’ type field experiments where short-term relative preference is determined, a wide range of legumes are available while mixed pastures typically only have a few dominant species. Similarly, selective grazing may be facilitated better for sheep in the ‘cafeteria’ study compared to the mixed gland clover and annual ryegrass pasture. The greater spatial heterogeneity of pasture components in a mixed pasture compared to those monoculture swards may decrease the sheep’s selectivity. However, perhaps more importantly, the short-term relative preference of sheep is likely to differ from their preference after a period of familiarisation with the forage.

The olfactory stimulus that sheep receive from a novel plant, or its initial preference for the plant, may contribute only marginally to whether or not a sheep selects that plant from a mixed pasture because of this grazing experience (Rook *et al.* 1997). Factors such as plant growth habit (Kydd 1966; Rossiter 1966), postingestive feedback from chemo-, osmo- and mechano-receptors during digestion (Provenza 1996a) and the relative abundance and distribution of the pasture plants (Heady and Torell 1959; Hodgson 1982) can significantly modify short-term preference of forage when sheep graze a mixed pasture, so the change in pasture composition due to grazing may not be as predicted.

The relative availability and overall pasture status, which contribute to the animal factors that affect grazing behaviour, also have a large influence on selective grazing behaviour. In Part C we reported that biserrula had considerable potential to improve weed control in a grazed pasture phase. However, in Part D we reported that the seed set by annual ryegrass was not reduced after biserrula was introduced into the pasture and that the low rainfall in spring may have contributed substantially to this result.

The results of chapter 6 suggest that there are a number of criteria for a 'companion' legume species that is less preferred by sheep to be successful in driving the desired changes in pasture composition. First the species must be less preferred by the grazing animal over an extended period of time, i.e. the animal must not increase its preference as it becomes familiar with the forage. Second, the less preferred legumes must be able to be easily differentiated by the grazing animal so it is able to select alternatives and so 'patch' grazing does not occur. Third, the 'companion' legume must be tolerant of grazing, and have good vigour, providing strong competition against the target weeds.

The implementation of improved grazing management is an avenue to manage weeds as the need for alternatives arises due to the current unsustainable use of many groups of chemical herbicides. Carter (1990) contends that grazing animals have a vital role in weed control and that this is often more effective, more sustainable and far cheaper than herbicides. It is likely that farmers will increase their use of sheep as an option for weed control because of the reliability, economic viability and effectiveness of a grazed pasture phase for weed control.

Chapter 7

Aversive behaviour in sheep grazing a biserrula-dominant pasture

Introduction

The food preference of sheep depends on the animal's prior experience as well as its innate behaviour. That sheep show partial and changing preference is not widely recognised, although evidence for this aspect of grazing is mounting (Parsons *et al*. 1994). Sheep learn from their mother, other sheep and their own grazing experience and their preferences among forage options changes accordingly. The preference of sheep for novel forage may differ from its preference when the sheep has had some experience in grazing the forage and the individual experience of a herbivore can invoke powerful shifts in diet selection (Arnold and Maller 1977; Provenza 1995). Therefore preference among forages should be considered in relation to the previous dietary history of the animal.

The proportion of annual ryegrass plants was unusually low in a newly sown pasture of *Biserrula pelicinus* cv Casbah when grazed by sheep in spring (Revell and Thomas 2004). Annual ryegrass seed production in the established Casbah pasture was only about 6% that in similar pastures sown to Santiago burr medic and Sava snail medic (Revell and Thomas 2004). It has been suggested that the reduction in annual ryegrass seed set is the result of the unpalatability of Casbah to sheep, particularly during flowering. However, the results of our field study (Chapter 4) and the indoor study (Chapter 5) where we measured short-term preference of sheep for a number of legumes revealed that biserrula in the reproductive phase is not objectionable to grazing sheep. Furthermore, snail medic, which was shown to be less preferred in these studies, did not produce the same reduction in annual ryegrass seed set under grazing when it was sown as a companion pasture. The results from another study showed that plant characteristics causing sheep to avoid certain forages in a short-term preference test may contribute little to actual selection between a pasture legume and annual ryegrass by sheep in the field (Rook *et al*. 1997).

The short-term preference of sheep for a novel food is controlled by the olfactory stimulus (palatability) of the forage during investigation and prehension. At this time the acceptance or rejection of a particular food is based on either a positive or negative sensory experience. However, as an animal becomes familiar with a food its response to the food is modified by postingestive feedback signals from chemical compounds that are released during rumination and digestion (effects of nutrients and toxins on chemo-, osmo- and mechano-receptors) (Provenza 1995). An experienced sheep will therefore use its senses primarily to distinguish between forage options, rather than determining the acceptability of the forage. Therefore, we could expect that the forage selected by an animal that is familiar with a range of plants genotypes will differ from that of an inexperienced animal.

Parsons *et al*. (1994) found that sheep with a history of eating white clover initially selected more grass than clover when given a choice. However, after a period of grazing this selection reversed and they ate more of the diet to which they had previously been exposed. Similarly Provenza (1996b) notes that in some unpublished work "lambs fed nutritionally balanced apple- or maple-flavoured food one day prefer the alternate flavour the next day, and the decrease in preference is even more pronounced when the food is either low (90% NRC [National Research Council daily energy requirement determined for sheep]) or high (110% NRC) in energy". Cropper *et al*. (1986) found that sheep adjusted their selection to maintain an 'optimal' protein intake. The results from these studies support the view that there are postingestive feedback signals associated with the protein in digesta. These are examples of sheep modifying their food selection in response to different eating experiences and this may explain the selective targeting of weeds in biserrula-dominant pastures (Revell and Thomas 2004). In view of this we hypothesised that sheep will develop an aversion to biserrula when they graze a pasture in which it is a major constituent.

Materials and Methods

Experimental design

The experiment was conducted with pasture swards at three phases of growth; these were defined as vegetative (before flowering), reproductive (during flowering and seeding) and senesced (dead plant material). At each phase of plant growth five groups of 10 Merino wether hoggets each grazed either pasture sown with i) 100% biserrula, ii) 100% crimson clover, iii) 100% annual ryegrass, iv) 50% biserrula and 50% annual ryegrass or v) 50% crimson clover and 50% annual ryegrass.

After the hoggets had grazed the plots for 4 weeks they were then allocated to plots containing an equal number of rows of biserrula, crimson clover and annual ryegrass (test swards). Paired quadrats (30 x 100 cm) were taken before and after grazing to measure intake in order to calculate relative preference (General Materials and Methods). The wethers were taken out of the test swards when approximately 50% of the plant biomass originally on offer was removed. At the vegetative phase the hoggets were given access to the test swards for 1.5 days. At the reproductive phase the hoggets were given access to the test swards for 3.5 days. At the senesced phase the hoggets were given access to the test swards for 3 days.

After grazing the test swards, on each occasion, the 5 groups of wethers were combined and offered a pasture containing 1 hectare monoculture swards of annual ryegrass, crimson clover and biserrula for 5 days, so that all groups were offered the same grazing experience over this time. The wethers were then put into new test swards containing an equal number of rows of biserrula, crimson clover and annual ryegrass, and the relative preference of the sheep in each of the five groups was determined a second time.

Forage intake was estimated as the forage available before grazing minus the forage available after grazing. Relative preference was calculated from forage availability and estimated food intake using an adaptation of the Chesson-Manly selection index (see General Materials and Methods).

Experimental site

This experiment was established in June, 2000 on a gravelly sand soil, at Allandale Farm, Wundowie 70 kilometres east of Perth, Western Australia (116.3 E, 31.8 S). This area has a Mediterranean type climate with typically 80% of the total rain during the months of May to September.

Pasture establishment

As outlined in the experimental design, 5 pastures, each approximately 1 hectare, were established comprising combinations of the two legumes *Biserrula pelecinus* cv Casbah (biserrula) and *Trifolium incarnatum* cv Caprera (crimson clover) and *Lolium rigidum* cv Wimmera (annual ryegrass).

To provide plant material to assess the preferences of sheep, 30 blocks each consisting of 18 monoculture rows (20 m x 1.2 m) with equal numbers of rows of biserrula, crimson clover and annual ryegrass in random order were established. The 30 blocks were divided into 6 groups of 5 blocks and fenced so that fresh plant material could be offered to each of the 5 groups of wethers 2 times for grazing at 3 phases of plant growth and replicated 6 times.

The legume seed was inoculated and lime pelleted immediately prior to sowing. The pastures were sown using a conventional combine seeder and the seed was combined with 120 kg/ha of 3:2 P:K fertiliser. The monoculture rows were sown using a cone-seeder, and 160 kg/ha of 3:2 P:K fertiliser was applied at sowing. Weeds that had germinated prior to seeding were killed with an application of Roundup® herbicide at 2 L/ha.

An unacceptable density of wild radish plants germinated in the plots, and several chemical applications were applied to reduce their prevalence. The Group B herbicide, Broadstrike® was applied to the pastures sown with either annual ryegrass or crimson clover. Since this herbicide was not suitable for the control of wild radish in biserrula the Group C herbicide Bromocide® (bromoxynil) was applied to the 2 pastures containing biserrula. In addition the plot sown with 50:50 biserrula and annual ryegrass was grazed with 20 sheep for 3 days after it had been sprayed with Bromocide® to improve the effectiveness of the spray to kill the wild radish plants. In the preference testing plots Roundup® herbicide was applied to the wild radish plants using a weed wiper.

A significant number of red-legged earth mites were observed 4 weeks after the plots were sown so Lemat® insecticide was applied at 150 ml/ha. The biserrula plots became quite heavily infested with aphids at flowering so these were again sprayed with 150 ml/ha of Lemat® insecticide.

Seasonal conditions

A total of 556 mm of rain was recorded at Allandale Farm in 2002 (Table 7.1).

Table 7.1. Monthly rain (mm) recorded for Allandale Farm, 2002

Month	Rain (mm)
January	12.6
February	0.0
March	0.0
April	29.2
May	51.2
June	144.4
July	117.8
August	92.5
September	48.6
October	57.9
November	1.4
December	0.0

Animals

The 225 Merino wether hoggets used in this experiment were all from the commercial flock at the University of Western Australia's Allandale farm. The hoggets weighed between 40 and 50 kg liveweight and were 14, 15 and 17 months of age during the vegetative, reproductive and senesced phases of plant growth. At each phase the hoggets were fasted for 16 hours, weighed and condition scored and then sorted into groups of equal mean weight and assigned to their pasture treatment. At the conclusion of the preference testing the hoggets were fasted for 16 hours, weighed, and condition scored. Cool water was available to the sheep *ad libitum* at all times during this experiment.

Method to determine preference

The indoor method we developed for assessing relative preference (Chapter 5) would have been ideal for this experiment. However, the lack of facilities did not allow us to pursue this option. It was necessary to conduct this experiment at Allandale farm, so that large areas of pasture could be grown for the sheep to graze, but there were no individual sheep holding pens available at Allandale to use for the indoor method. Given the strict quarantine protocols it would not have been possible to transport the sheep to indoor facilities without considerable disturbance to the animals and it is not known what impact this might have had on the eating behaviour of the sheep.

Statistical analysis

Analysis of variance and LSD (Fisher's protected LSD) were used to determine the statistical significance of the results. These analyses were conducted using the GenStat® (Version - 6.1.0.200) statistical package.

Results

Relative preference (α)

There were clear differences in the relative preference (α) of Merino hoggets with different prior grazing experiences at each of the three phases of plant growth (Table 7.2). However, at all phases of plant growth, there were no significant differences between the groups after they were combined and grazed together with access to one hectare monoculture pastures of annual ryegrass, crimson clover and biserrula.

At the vegetative phase the group of hoggets that that previously grazed 100% biserrula pasture had the lowest preference for biserrula and this was significantly lower than the groups that grazed 50:50 biserrula/annual ryegrass, 100% crimson clover or 50:50 crimson clover and annual ryegrass (α; 0.02 *v* 0.32 , 0.41 and 0.19; Table 7.2). This pattern was repeated at the reproductive phase where the group of hoggets that had previously grazed 100% biserrula pasture had the lowest preference for biserrula and this was significantly lower than the groups that grazed 50:50 biserrula/annual ryegrass or 100% crimson clover (α; 0.09 *v* 0.60 and 0.41). At the senesced phase sheep that previously grazed 100% biserrula did not have a lower preference for biserrula than the other groups.

The groups of sheep that had prior grazing experience with the 50:50 legume/annual ryegrass tended to have a higher preference for the legume they were familiar with than those grazing the monoculture pastures sown to the same legume. For example at the reproductive phase the group grazing 50:50 biserrula/annual ryegrass had a higher preference for biserrula than the group grazing 100% biserrula (α; 0.60 *v* 0.09 ; $P < 0.05$). At the senesced phase the group grazing 50:50 crimson clover/annual ryegrass had a higher preference for crimson clover than the group grazing 100% crimson clover (α; 0.60 *v* 0.09 ; $P < 0.05$). This was a common trend across all of the three phases of plant growth.

Most of the groups of hoggets exhibited selectivity ($\alpha \neq 1/$(number of forage types available) (Chesson 1983); $P < 0.05$) in their choice between annual ryegrass, biserrula and crimson clover at the vegetative, reproductive and senesced phases of plant growth.

Table 7.2. Relative preference (α) of five groups of Merino hoggets for biserrula, crimson clover and annual ryegrass after each grazed different pastures (first grazing) and then grazed together on all three pastures (second grazing)

Growth Phase	Grazing time	Pasture experience	Biserrula	Crimson clover	Annual ryegrass
Vegetative	First grazing	Biserrula	0.02^{a} ± 0.02	0.67^{bc} ± 0.04	0.31^{b} ± 0.04
		Biserrula/ryegrass	0.32^{cd} ± 0.07	0.46^{a} ± 0.06	0.22^{ab} ± 0.07
		Crimson clover	0.41^{d} ± 0.06	0.49^{ab} ± 0.08	0.10^{a} ± 0.05
		Crimson clover/ryegrass	0.19^{bc} ± 0.05	0.52^{ab} ± 0.07	0.29^{b} ± 0.05
		Annual ryegrass	0.10^{ab} ± 0.05	0.81^{c} ± 0.07	0.09^{a} ± 0.06
		P-value	< 0.001	0.004	0.02
	Second grazing	Biserrula	0.03 ± 0.02	0.87 ± 0.07	0.10 ± 0.05
		Biserrula/ryegrass	0.04 ± 0.04	0.89 ± 0.05	0.07 ± 0.02
		Crimson clover	0.02 ± 0.02	0.81 ± 0.06	0.17 ± 0.05
		Crimson clover/ryegrass	0.12 ± 0.08	0.66 ± 0.10	0.22 ± 0.10
		Annual ryegrass	0.09 ± 0.04	0.83 ± 0.08	0.08 ± 0.06
		P-value	0.49	0.22	0.36
Reproductive	First grazing	Biserrula	0.09^{a} ± 0.06	0.49 ± 0.13	0.42 ± 0.11
		Biserrula/ryegrass	0.60^{c} ± 0.09	0.28 ± 0.07	0.12 ± 0.06
		Crimson clover	0.41^{bc} ± 0.05	0.44 ± 0.11	0.15 ± 0.07
		Crimson clover/ryegrass	0.20^{ab} ± 0.05	0.58 ± 0.07	0.21 ± 0.07
		Annual ryegrass	0.28^{ab} ± 0.09	0.38 ± 0.14	0.34 ± 0.08
		P-value	< 0.001	0.38	0.06
	Second grazing	Biserrula	0.39 ± 0.06	0.49 ± 0.05	0.12 ± 0.05
		Biserrula/ryegrass	0.40 ± 0.03	0.46 ± 0.05	0.14 ± 0.06
		Crimson clover	0.29 ± 0.08	0.51 ± 0.07	0.20 ± 0.05
		Crimson clover/ryegrass	0.38 ± 0.02	0.44 ± 0.03	0.18 ± 0.03
		Annual ryegrass	0.33 ± 0.07	0.33 ± 0.04	0.34 ± 0.08
		P-value	0.66	0.10	0.08
Senesced	First grazing	Biserrula	0.28^{b} ± 0.11	0.50^{b} ± 0.11	0.23 ± 0.09
		Biserrula/ryegrass	0.43^{b} ± 0.09	0.16^{a} ± 0.06	0.42 ± 0.10
		Crimson clover	0.21^{ab} ± 0.08	0.21^{a} ± 0.08	0.58 ± 0.10
		Crimson clover/ryegrass	0.03^{a} ± 0.03	0.64^{b} ± 0.08	0.34 ± 0.08
		Annual ryegrass	0.26^{ab} ± 0.06	0.41^{ab} ± 0.10	0.33 ± 0.05
		P-value	0.03	0.01	0.08
	Second grazing	Biserrula	0.31 ± 0.08	0.52 ± 0.06	0.17 ± 0.05
		Biserrula/ryegrass	0.37 ± 0.11	0.30 ± 0.07	0.33 ± 0.09
		Crimson clover	0.21 ± 0.08	0.44 ± 0.03	0.35 ± 0.08
		Crimson clover/ryegrass	0.28 ± 0.10	0.39 ± 0.11	0.33 ± 0.14
		Annual ryegrass	0.38 ± 0.07	0.28 ± 0.02	0.34 ± 0.06
		P-value	0.84	0.12	0.66

Error terms are standard errors of the means (n = 6). Values within columns for each growth phase and each grazing period with different superscripts are different ($P < 0.05$).

Forage availability

The biomass (g/m^2) of annual ryegrass and biserrula offered to the groups of sheep did not differ between treatments, while crimson clover biomass did differ among the treatment groups (Table 7.3). The biomass varied between the pasture species and their relative abundance changed between phases of plant growth due to the differing growth habits of the plants and their response to the environment (Table 7.3). The same area of pasture was offered at each of the three phases of plant growth and consequently the biomass offered to the sheep increased with maturity, but this increase was least between the reproductive and senesced phases of growth.

Intake of forages

Differences in intake (g DM/m^2) between the three pasture species and five prior grazing experiences closely followed the pattern reported for relative preference. There were clear differences in the intake of the three forages offered and their magnitude differed between the groups of hoggets according to their prior grazing history (Table 7.4).

At the vegetative phase an average of 38 g DM/m^2 was removed by 50 sheep in 1.5 days (1.1 kg/sheep.day), at the reproductive phase an average of 93 g DM/m^2 was removed by 50 sheep in 3.5 days (1.2 kg/sheep.day) and at the senesced phase an average of 77 g DM/m^2 was removed by 50 sheep in 3 days (1.2 kg/sheep.day). Therefore daily dry matter intake remained consistent across the three phases of plant growth.

Biomass of pasture for prior grazing experience

The pastures were managed to try to achieve monoculture stands of each of the pastures types sown. In the crimson clover and annual ryegrass pastures the options for chemical manipulation allowed us to achieve monocultures that were visually assessed as better than 95% pure. However, biserrula is generally sensitive to herbicides and this limited the options for weed management. As a result volunteer silver grass was not controlled and may have comprised up to 10% of the total plant biomass in the biserrula pastures.

The two pastures containing alternating legume/annual ryegrass plots were sown in equal areas and the pasture biomass in both the legume and annual ryegrass pasture did not differ significantly at the vegetative and reproductive phases of plant growth. However,

Table 7.3. Biomass (g DM/m^2) of biserrula, crimson clover and annual ryegrass offered to five groups of Merino hoggets after each had grazed different pastures (first grazing) and then grazed together on all three pastures (second grazing)

Growth Phase	Grazing Time	Pasture experience	Biserrula	Crimson clover	Annual ryegrass
Vegetative	First grazing	Biserrula	145 ± 15	187 ± 15	199 ± 9
		Biserrula/ryegrass	159 ± 11	189 ± 12	196 ± 20
		Crimson clover	143 ± 16	189 ± 6	184 ± 12
		Crimson clover/ryegrass	134 ± 8	197 ± 10	194 ± 13
		Ryegrass	145 ± 11	186 ± 6	180 ± 12
		P-value	0.74	0.94	0.84
	Second grazing	Biserrula	135 ± 14	219^b ± 13	166 ± 15
		Biserrula/ryegrass	119 ± 4	192^a ± 9	168 ± 15
		Crimson clover	103 ± 7	182^a ± 6	177 ± 7
		Crimson clover/ryegrass	132 ± 10	188^a ± 8	201 ± 17
		Annual ryegrass	134 ± 13	167^a ± 8	193 ± 30
		P-value	0.17	0.01	0.59
Reproductive	First grazing	Biserrula	265 ± 27	334^b ± 22	384 ± 14
		Biserrula/ryegrass	307 ± 37	269^a ± 23	416 ± 51
		Crimson clover	304 ± 10	298^{ab} ± 16	372 ± 41
		Crimson clover/ryegrass	291 ± 18	252^a ± 21	360 ± 27
		Annual ryegrass	325 ± 26	243^a ± 13	341 ± 37
		P-value	0.55	0.02	0.66
	Second grazing	Biserrula	291 ± 37	482 ± 24	410 ± 29
		Biserrula/ryegrass	266 ± 23	446 ± 39	291 ± 38
		Crimson clover	331 ± 28	483 ± 39	359 ± 30
		Crimson clover/ryegrass	281 ± 50	441 ± 18	327 ± 40
		Annual ryegrass	297 ± 31	403 ± 11	421 ± 34
		P-value	0.76	0.28	0.07
Senesced	First grazing	Biserrula	207 ± 17	406^{bc} ± 26	315 ± 32
		Biserrula/ryegrass	200 ± 13	299^a ± 14	295 ± 27
		Crimson clover	212 ± 23	359^{ab} ± 33	404 ± 65
		Crimson clover/ryegrass	270 ± 42	369^b ± 15	294 ± 21
		Annual ryegrass	271 ± 43	439^c ± 18	395 ± 26
		P-value	0.28	0.00	0.11
	Second grazing	Biserrula	219 ± 28	409 ± 38	255^a ± 6
		Biserrula/ryegrass	194 ± 32	311 ± 21	346^{ab} ± 34
		Crimson clover	214 ± 46	351 ± 7	383^b ± 39
		Crimson clover/ryegrass	305 ± 53	332 ± 47	391^b ± 20
		Annual ryegrass	287 ± 29	353 ± 34	358^b ± 43
		P-value	0.16	0.30	0.04

Error terms are standard errors of the means (n = 6). Values within columns for each growth phase and each grazing period with different superscripts are different (P < 0.05).

Table 7.4. Intake (g DM/m^2) of biserrula, crimson clover and annual ryegrass from test plots by five groups of Merino wether hoggets after each grazed different pastures (first grazing) and then grazed together on all three pastures (second grazing)

Growth Phase	Grazing Time	Pasture experience	Biserrula	Crimson clover	Annual ryegrass
Vegetative	First grazing	Biserrula	2.3^a ± 2.3	49.2 ± 23.3	53.2 ± 15.3
		Biserrula/ryegrass	43.1^c ± 10.2	43.3 ± 16.6	53.2 ± 17.9
		Crimson clover	42.4^c ± 9.5	35.6 ± 11.6	43.6 ± 13.1
		Crimson clover/ryegrass	31.2^{bc} ± 8.5	61.9 ± 24.2	62.8 ± 18.7
		Annual ryegrass	9.7^{ab} ± 4.6	29.4 ± 11.3	57.0 ± 19.4
		P-value	0.002	0.76	0.95
	Second grazing	Biserrula	4.8 ± 3.0	71.2 ± 31.5	66.2 ± 20.7
		Biserrula/ryegrass	3.7 ± 3.5	45.9 ± 20.0	49.6 ± 14.8
		Crimson clover	2.9 ± 2.6	46.0 ± 19.9	61.2 ± 19.3
		Crimson clover/ryegrass	7.9 ± 5.5	35.7 ± 15.9	29.8 ± 9.6
		Annual ryegrass	9.1 ± 5.1	41.2 ± 18.6	48.9 ± 16.0
		P-value	0.79	0.82	0.58
Reproductive	First grazing	Biserrula	11.5^a ± 9.1	48.6 ± 19.7	26.2 ± 10.8
		Biserrula/ryegrass	97.8^{bc} ± 10.1	31.7 ± 15.7	49.2 ± 14.7
		Crimson clover	116.2^c ± 24.2	73.8 ± 23.4	75.7 ± 24.8
		Crimson clover/ryegrass	52.0^{ab} ± 17.6	70.8 ± 23.5	56.8 ± 18.3
		Annual ryegrass	66.3^{bc} ± 25.7	41.2 ± 16.3	27.8 ± 7.8
		P-value	0.005	0.50	0.20
	Second grazing	Biserrula	112.9 ± 22.5	135.2 ± 44.5	147.3 ± 46.6
		Biserrula/ryegrass	115.2 ± 22.2	143.6 ± 53.0	121.3 ± 34.9
		Crimson clover	112.4 ± 34.5	132.5 ± 46.4	155.3 ± 47.0
		Crimson clover/ryegrass	131.4 ± 21.7	152.1 ± 49.8	150.7 ± 48.7
		Annual ryegrass	123.0 ± 36.8	89.6 ± 23.1	113.1 ± 41.0
		P-value	0.99	0.88	0.94
Senesced	First grazing	Biserrula	32.8^{ab} ± 12.8	56.5 ± 25.8	75.6 ± 27.8
		Biserrula/ryegrass	71.5^b ± 17.2	56.4 ± 20.1	22.6 ± 10.3
		Crimson clover	47.7^{ab} ± 20.4	32.9 ± 20.6	97.2 ± 42.1
		Crimson clover/ryegrass	4.9^a ± 3.8	96.4 ± 42.9	125.4 ± 43.6
		Annual ryegrass	69.3^b ± 18.0	102.0 ± 53.9	140.8 ± 41.2
		P-value	0.032	0.61	0.18
	Second grazing	Biserrula	81.3 ± 29.4	93.1 ± 30.9	172.2 ± 53.6
		Biserrula/ryegrass	70.2 ± 27.1	59.1 ± 15.1	49.2 ± 18.9
		Crimson clover	47.6 ± 21.5	78.1 ± 31.1	70.6 ± 19.8
		Crimson clover/ryegrass	115.7 ± 43.8	114.6 ± 36.5	64.1 ± 24.5
		Annual ryegrass	118.3 ± 42.1	54.7 ± 15.7	89.3 ± 27.9
		P-value	0.64	0.52	0.08

Error terms are standard errors of the means (n = 6). Values within columns for each growth phase and each grazing period with different superscripts are different ($P < 0.05$).

due to the grazing that was applied to the biserrula and annual ryegrass pasture to control weeds the biserrula biomass (kg DM/ha) in this pasture was lower than that in the biserrula only pasture (Table 7.5).

Table 7.5. Forage biomass (kg DM/ha) in pastures offered to sheep for prior experience

Growth Phase	**Pasture Experience**	**Biomass (kg DM/ha)**
Vegetative	Biserrula	$1693^{bc} \pm 173$
	Biserrula/ryegrass	$1090^{ab} \pm 163$
	Biserrula/**ryegrass**	$723^{a} \pm 178$
	Crimson clover	$1893^{c} \pm 87$
	Crimson clover/ryegrass	$1797^{bc} \pm 203$
	Crimson clover/**ryegrass**	$1743^{bc} \pm 223$
	Annual ryegrass	$1943^{c} \pm 228$
	P-value	0.006
Reproductive	Biserrula	$4327^{bc} \pm 505$
	Biserrula/ryegrass	$2577^{a} \pm 52$
	Biserrula/**ryegrass**	$2497^{a} \pm 413$
	Crimson clover	$4951^{c} \pm 195$
	Crimson clover/ryegrass	$3427^{ab} \pm 70$
	Crimson clover/**ryegrass**	$3520^{ab} \pm 481$
	Annual ryegrass	$3993^{b} \pm 262$
	P-value	< 0.001

Error terms are standard errors of the means (n = 6) for single or (n = 3) for dual species pastures. Bold text indicates species measured in pastures with dual species. Values within columns for each growth phase with different superscripts are different ($P < 0.05$).

Sheep growth rates and condition scores

The five groups of Merino hoggets offered different pastures for 4 weeks differed markedly in their growth rates at each of the phases of plant growth. At the vegetative phase hoggets grazing biserrula only, crimson clover only or crimson clover and annual ryegrass grew the fastest while hoggets grazing biserrula and annual ryegrass pasture had a lower growth rate. Hoggets grazing annual ryegrass grew at only 41 g/day and the growth rates of the sheep were consistently the lowest on annual ryegrass pasture at each of the three phases of plant growth (Table 7.6).

At the reproductive phase the pasture type did not significantly affect growth rates with the exception that hoggets grazing 100% annual ryegrass grew more slowly. At the senesced phase the hoggets grazing annual ryegrass only pasture and those grazing crimson clover and annual ryegrass lost weight. Sheep grazing the two ‘legume only’ pastures had the highest growth rate.

At the vegetative phase of plant growth the condition of the hoggets grazing pastures containing either crimson clover and annual ryegrass or crimson clover only gained almost one condition score over the four week period which was significantly higher than that for hoggets grazing the other three pasture types where the sheep gained only 0.25 to 0.5 of a condition score (Table 7.6).

Table 7.6. Liveweight, growth rate and change in condition score of Merino hoggets grazing different pastures

Growth Phase	**Pasture experience**	**Initial Weight**	**Final Weight**	**Growth rate (g/d)**	**Change in condition score**
Vegetative	Biserrula	35.6 ± 1.1	39.2 ± 1.2	101^{bc} ± 14	0.51^{ab} ± 0.11
	Biserrula/ryegrass	35.7 ± 1.0	38.3 ± 1.0	74^{ab} ± 19	0.48^{a} ± 0.11
	Crimson clover	35.6 ± 1.0	40.2 ± 1.0	130^{c} ± 17	0.95^{c} ± 0.17
	Crimson clover/ryegrass	35.4 ± 1.0	39.6 ± 0.9	123^{c} ± 18	0.88^{bc} ± 0.12
	Annual ryegrass	35.6 ± 1.0	37.1 ± 1.1	41^{a} ± 9	0.25^{a} ± 0.15
	P-value	0.99	0.26	0.001	0.003
Reproductive	Biserrula	39.2 ± 1.5	41.5 ± 1.4	81^{ab} ± 8	0.03^{ab} ± 0.06
	Biserrula/ryegrass	38.5 ± 1.2	42.0 ± 1.3	121^{b} ± 22	0.28^{bc} ± 0.09
	Crimson clover	39.2 ± 1.2	41.8 ± 1.2	90^{b} ± 13	0.44^{c} ± 0.11
	Crimson clover/ryegrass	38.7 ± 1.5	41.9 ± 1.4	109^{b} ± 22	0.35^{c} ± 0.13
	Annual ryegrass	38.8 ± 1.3	40.0 ± 1.2	42^{a} ± 10	-0.08^{a} ± 0.18
	P-value	0.99	0.80	0.01	0.001
Senesced	Biserrula	42.5 ± 1.4	43.3 ± 1.5	38^{c} ± 7	-0.08^{ab} ± 0.06
	Biserrula/ryegrass	42.9 ± 1.8	43.0 ± 2.0	5^{bc} ± 17	0.10^{bc} ± 0.07
	Crimson clover	42.5 ± 1.5	43.0 ± 1.3	24^{c} ± 25	0.33^{c} ± 0.10
	Crimson clover/ryegrass	42.4 ± 1.8	41.8 ± 1.9	-29^{b} ± 15	-0.18^{a} ± 0.14
	Annual ryegrass	42.8 ± 1.4	41.1 ± 1.3	-81^{a} ± 20	-0.10^{ab} ± 0.09
	P-value	0.99	0.84	< 0.001	0.004

Error terms are standard errors of the means (n = 10). Values within columns for each phase of growth with different superscripts are different ($P < 0.05$).

Discussion

Preference

This study supported the hypothesis that sheep grazing a biserrula pasture can develop an aversion to biserrula forage. However, it appears necessary to have a high proportion of biserrula in the pasture to create this aversive response. The relative preference of hoggets for biserrula was lowest in the group that had previously grazed a biserrula dominant pasture at both the vegetative and reproductive phases of plant growth. In contrast, the relative preference of hoggets for biserrula was markedly higher for sheep that had previously grazed a biserrula and annual ryegrass pasture at both the vegetative and reproductive phases. Therefore it appears that the hogget's familiarity with biserrula did not cause their low relative preference for this forage, rather the aversion was more likely a response to the high proportion of this plant in their diet.

Although the sheep that grazed a 100% biserrula pasture clearly avoided biserrula when subsequently given a choice, this was not the case for the group that grazed only crimson clover. The preference of this group for crimson clover was consistently as high as that of the other groups, except perhaps at the senesced phase. The sheep grazing pasture containing only annual ryegrass appeared to have a lower preference for annual ryegrass at the vegetative phase, but this was not observed at the reproductive or senesced phases.

Sheep grazing the 100% annual ryegrass pasture had the lowest growth rates at all phases of plant growth reflecting the feeding value of the annual ryegrass compared to the two legumes. This is interesting because when sheep have been observed to develop an aversion to forage it is generally associated with the low nutritional value of the forage or toxicoses resulting from the ingestion of the forage (Provenza 1995). Animals can develop aversions to foods that do not contain adequate nutrients and, as a result, depress growth (Gietzen 1993). Therefore, it is unclear why in this study the hoggets grazing 100% annual ryegrass appeared to develop this aversion to annual ryegrass at the vegetative phase of plant growth, but not at the reproductive or senesced phases. Given that growth was depressed on the 100% annual ryegrass pasture at each growth phase compared to the two legume pastures our expectation was that the preference of sheep for annual ryegrass would decrease across all phases of growth. However, at the reproductive phase the 100% annual ryegrass group appeared to be less selective compared to the other groups and displayed an equal preference for each of the three forages. The disappearance of the aversion to annual ryegrass after the vegetative stage may be associated with the formation and translocation of water soluble carbohydrates in the plants.

The length of time that the aversion to biserrula persists was not tested in this experiment. Nonetheless, we found that the preference between the groups after they had been grazed together for one week had reverted to the same levels suggesting the aversive response to biserrula could have been lost after one week. This might have been due to the social influence of the other sheep that grazed with them. In ruminant groups, dietary preferences of individuals are affected by the presence and behaviour of their conspecifics (Nicol 1995). Ralphs and Provenza (1999) reported that aversions to poisonous plants induced by lithium chloride could be maintained indefinitely provided the averted animals were kept separate from animals without an aversion. However, when the averted sheep were grazed with their non-averted cohorts they quickly lost the induced aversion. At the vegetative phase of this experiment the relative preference of the biserrula only group did not appear to improve (α; 0.02 *v* 0.03). In contrast, the biserrula only group, which had developed an aversion to biserrula, may have influenced the grazing preferences of the non-averted groups. However, at the reproductive phase this pattern was not observed and the preference of the 100% biserrula group appeared to increase after all of the groups were combined (α; 0.09 *v* 0.39), consistent with Ralphs and Provenza (1999) study. This difference may be linked to the strength of the aversion. In some instances sheep develop weak ‘transient’ food aversions that can be rapidly ameliorated when alternative foods become available, in other cases strong and persistent aversions develop even to forage of high nutritive value such as barley (Phy and Provenza 1998). The strength of an aversion is thought to be related to the severity of the negative postingestive experience, for which there are many interacting causes. It seems feasible that under some conditions the averted group would influence the grazing behaviour of the non-averted group and under other conditions the reverse might occur, depending perhaps on the type and intensity of the aversion.

In this study the aversion was created by the plant, presumably through naturally occurring compounds within the biserrula plant itself. In the case where an aversion response is induced artificially and there is no aversive agent inherent in the plant aversive behaviour may be more easily overcome. The presence or not of alternative forage may also play an important role. Sheep offered a single diet, linked to an induced aversion, began sampling this food and readily recognised that the toxin had been removed (Wang and Provenza 1997). Kimball *et al*. (2002) found that a LiCl induced aversion to citric acid flavoured food was prolonged considerably in sheep when alternatives to this paired food were available and the sheep had less incentive to sample other food. It would appear that there is some scope to use artificially induced aversions to manipulate pasture composition. To do this we would have to consider the animal's natural grazing ability. The accessibility of a particular plant species and the precision of the grazing animal in selecting the preferred forage are likely to contribute to inadvertent sampling and the potential for remediation of an aversion response.

What is the mechanism behind the aversion response of sheep to biserrula? It seems likely that there is a chemical compound in the plant that can create a postingestive food aversion. Because sheep only developed an aversion when forced to include a high proportion of biserrula in their diet the likely chemical agent may be either diluted sufficiently or neutralised when there is other forage in the sheep's diet, as seems to be the case when annual ryegrass is concurrently available in the pasture.

Sheep appear to target weeds in biserrula-dominant pastures and this could be a useful tool for improving the efficacy of weed control in grazed pastures. Sheep grazing Dinninup subterranean clover pastures have been found to target alternate forages, particular grasses (MacNish and Nicholas 1987), and perhaps a similar mechanism may exist for biserrula. The rate of intake (Wang 1997) and the preference (Colebrook *et al*. 1990) of sheep for Dinninup can be high, so postingestive feedback mechanisms probably modify the preference of sheep for this cultivar. Supporting this view, Francis (1973) reported that a subterranean clover strain (mutant 792) was less preferred by sheep due to its lack of β-glucosidase. The absence of β-glucosidase was suggested to contribute to the sheep ingesting higher levels of unhydrolysed glycosides and their subsequent low preference.

This mechanism may explain why sheep avoid subterranean clover cultivars such as Dinninup and Yarloop that have high levels of isoflavones. Perhaps it was in view of such contrasting observations that led Weston (1996) to remark that "the significance of low palatability as an intake constraint remains a vexed question".

Growth Rate

The growth rate of the sheep grazing the 100% biserrula pasture was not significantly reduced. At all phases of plant growth sheep grazing the 100% biserrula only pasture gained weight at the same rate as or faster than sheep grazing the other pastures. Therefore, although the hoggets developed an aversion to biserrula, they must have maintained their intake at a level that provided them with adequate nutrition. Chemical compounds such as tannins, saponin, nitrate and oxalate are associated with food aversions in sheep and are known to depress their voluntary food intake severely if their concentrations in the forage are high (Burritt and Provenza 2000). However, in this study the aversive plant characteristics were probably not strong enough to markedly reduce food intake. A low relative preference for a species where an animal has a choice does not necessarily result in a reduction in intake of the species when the animal has no choice (Arnold *et al*. 1966). This is also supported by the experiment described in Chapter 6 where annual ryegrass was obviously targeted, and consumed in preference to biserrula in the biserrula-sown pasture, but it was equally obvious that sheep had been grazing a significant quantity of biserrula. Due to the high proportion of biserrula in this pasture, it would have been unlikely that the sheep could have selected enough substitute forage to meet their nutritional requirements and achieve satiety if they had completely avoided eating biserrula. The positive changes in liveweight and condition score indicated that the sheep grazing biserrula gained weight at a rate comparable to that on the other pastures. However, the condition score of sheep grazing biserrula improved at a lower rate than sheep on the other pastures.

On balance, biserrula seems a likely candidate as a companion species to encourage sheep to graze weed species selectively but some questions remain to be resolved. For example, at the vegetative phase, the sheep on the 100% biserrula pasture were observed to be scouring more than the other groups despite their growth being unaffected, so there may be other implications for animal health associated with grazing biserrula dominant pastures. In addition, there has been some evidence that biserrula pasture may be mildly toxic to sheep and has been linked to photosensitivity (Revell, pers. comm.). Although such cases have been isolated, this highlights a need for further research to manage grazing in biserrula pastures.

Chapter 8

General Discussion

This thesis began with the general hypothesis that sowing a legume of low preference to sheep would improve the efficacy for control of target weeds of crops in a grazed pasture phase. In broad terms, the experimental evidence supports this proposition. Establishing and grazing a biserrula (*Biserrula pelecinus* cv. Casbah) pasture resulted in at least one instance of a spectacular decrease in the proportion of other species in the pasture and this was shown to be a direct result of the low preference of sheep for biserrula relative to the weeds.

The difficulty in testing the general hypothesis was the complex nature of creating a selective differential between pasture species and it is necessary to understand this better before reliable functional tests can be developed. The selective grazing behaviour of sheep was difficult to predict because of the number and changing nature of factors contributing to diet selection. In particular, the results from this study showed that the preference of sheep for weeds relative to the legume was influenced by a series of factors that included the physical and chemical composition of the legume and the weed, the potential intake rate, plant maturity, relative abundance of the species, differences among individual animals, environment, prior grazing experience and conditioned food aversion. Additional factors that may have contributed to grazing preference are cited in the literature review. Despite this complexity, consistent trends in grazing behaviour emerged and made it possible to understand this whole complex better and develop workable grazing management strategies.

Selective grazing to improve weed control

One of the key objectives of the general hypothesis was to identify potential candidates for the weed control strategy. However, identifying a mechanism that contributed to the selective targeting of weeds in a legume-sown pasture was of greater practical importance. It became clear that a conditioned food aversion through postingestive learning in sheep was more important than short-term preference/avoidance stimulated by the plants inherent palatability, in attempts to exploit selective grazing by sheep to remove weeds. This implies that we need a new approach to develop a strategy where companion legumes are used to improve weed control. Because postingestive signals are related to the concentration of specific secondary compounds, a 'cafeteria-type' study for preferences is probably of little value for identifying plants with inherent aversive characteristics because of the wide variety of forage from which the animals can select. The key issue here is that sheep generally select across a range of forages offered, particularly as preferred species become depleted. By increasing diet diversity their likelihood of eat enough of any particular forage type to elicit an aversion response to it is reduced. Even if they did, they are unlikely to associate this response with a particular pasture species given the diverse range of forages consumed (Duncan and Young 2002). A further problem with using cafeteria studies to identify potential candidate species to aid weed control in a grazed pasture phase is that the sheep's history of learned responses is not usually known. In addition, the relative abundance of different species may influence preference because the preference of sheep for forage is relative to the alternatives that are available, and the proportion of these on offer.

Selectivity

In this series of experiments, sheep were selective in their short-term relative preferences among annual legumes, and their ability to be selective is critical to the success of a weed management strategy. Selective grazing by sheep is widely reported, despite them being adapted to low quality diets and considered a grass/roughage feeder among ruminants (Hofmann 1989). In the short term 'cafeteria' experiment, where $\alpha = 0.05$ for neutral preference, the relative preferences ranged from 0.00 to 0.12 at the vegetative, 0.01 to 0.11 at the reproductive and 0.01 to 0.11 at the senesced phase of plant growth. Six legumes, vetch, chickpea, the snail medics, lotus and biserrula were avoided by sheep ($\alpha < 0.02$) at the vegetative phase compared to the weed of crops, annual ryegrass, which was preferred ($\alpha = 0.09$). That sheep exhibited selectivity in the short term across a wide range of novel forages is evidence that they can exhibit preferences before they have the opportunity to experience postingestive consequences. The sheep must have relied largely on their sensory capacity (taste, smell, touch and sight) in the selection of their diet, except for the small number of forage types for which they may have had some limited grazing experience previously. However, our study demonstrated that legumes of low short-term preference are not necessarily superior companion legumes for a weed control strategy.

Physical and chemical composition and potential intake rate

Sheep clearly demonstrated an ability to select among legumes and annual ryegrass for forage of higher nutritive value. Interestingly, this was observed at the reproductive and senesced phases of plant growth, but not at the vegetative phase. This may be due to the higher average nutritive value of the forages at the vegetative phase. At the reproductive and senesced phases sheep preferred forages of higher nitrogen content and digestibility, and lower fibre. In addition, it appears that senesced biserrula loses its aversive characteristics, as sheep grazing a pure biserrula sward did not have a low preference for biserrula at this time. At the vegetative phase compression energy was negatively related to relative preference, supporting the view that sheep may have been selecting for forage of higher potential intake rate at the vegetative phase.

Environment

In this study sheep offered novel forages were found to display consistent patterns of short-term preference in both the field and indoor experiments. For example, sheep readily ate crimson clover whether in the field or from pots in an indoor study, even though they had never eaten it before. Similarly, but in a negative sense, snail medic and vegetative chickpeas were consistently avoided both in the field and indoors without the sheep having seen these species previously. Thus, an indoor method may be useful for assessing preferences in the development of a weed management strategy.

Measuring relative preference using plants grown *in situ* presents unique challenges compared to determining preference among pre-harvested forages. The quantity of living plant material offered is not easily controlled because the growth rate of plants varies widely between species and individual plants as well as between seasons. Because of this, comparison of intake as a measure of preference can be inaccurate. We addressed this problem in this study by using the Chesson-Manly selection index, a function that adjusts intake according to the relative abundance of the plants to give a valid measure of relative preference.

Plant maturity

There may be greater opportunity to compel sheep to graze weeds selectively at the vegetative and reproductive phases due to changes in chemical composition as the pasture matures. With maturity, the nutritive value of the weeds (non-legume grasses and broadleaf plants) is likely to decline more rapidly than that of legumes and the sheep are likely to select more of the legume, particularly at senescence. In both the field and indoor preference tests, we found that the short-term relative preference of sheep for annual ryegrass (and wild radish in the indoor study) generally decreased relative to legumes as the plants matured.

Differences among individual animals

In some cases there were clear differences in the preferences of individual sheep in the animal house experiment to test preferences among pasture species offered growing in pots. For example, at the vegetative phase the relative preference of one sheep for ryegrass was 0.70 and 0.09 for radish, while for another the relative preference was 0.28 for ryegrass and 0.42 for radish. This result is in agreement with other literature that the food preferences of individual animals, even those that seem genetically similar, may differ widely (Arnold 1964) (Scott and Provenza 1999). However, there were general consistencies in the preferences of the individual sheep; for instance higher preference for legumes relative to weeds (annual ryegrass and wild radish) with maturity. Such generalizations are necessary to be able to use knowledge of the preferences of sheep to predict selective grazing outcomes.

Prior grazing experience and conditioned food aversions

Of the factors influencing preferential grazing, prior grazing experience and conditioned food aversion stood out as being the most clearly responsible for the increased effectiveness of grazing to control weeds of crops. The evidence supporting this view was that sheep grazing a biserrula dominant pasture develop a low preference for biserrula and consequently selected alternatives. The prior grazing experience of sheep, through postingestive feedback mechanisms, contributes to determine grazing preferences. The low preference of sheep for biserrula after grazing a biserrula dominant pasture was consistent with a conditioned aversion response (Table 7.2).

Biserrula and selective grazing behaviour

Where biserrula was dominant in a pasture, sheep were consistently found to target alternative pasture species selectively (Table 6.3, implied in Table 7.2, personal observations). This resulted in a marked change in pasture composition in pastures sown to biserrula. The mechanism or mechanisms that cause sheep to seek alternate feeds in a biserrula dominant pasture are still unclear. We can conclude that the initial preference of biserrula forage is not overly important as this was generally found to be moderate. The protein and digestibility of biserrula are comparable or better than most pasture species and the growth rates of sheep on pure biserrula pastures have been found to be as expected. It seems likely that some postingestive feedback mechanism (Provenza 1995) is involved in causing a food aversion and thereby reducing the relative preference of sheep for biserrula in biserrula dominant pastures. The discovery in Chapter 7 that sheep that have been grazing a biserrula-dominant pasture have a low relative preference for biserrula supports this view. However, no likely aversive compound has been identified. Possibilities include high rumen ammonia and plant secondary compounds, which are known to be associated with postingestive food aversions (Provenza 1996). Both the high protein of biserrula forage and the high incidence of plant secondary compounds in legumes (Baker and Dynes 1999) support these possibilities.

There is no evidence in either of the experiments described here or elsewhere that production from sheep is reduced on biserrula-dominant pastures. In fact, where biserrula has been compared with other pastures, the growth rate of sheep has been relatively high. It appears that the sheep maintain their voluntary food intake on biserrula pastures and selectively target other plants in addition to including a significant proportion of biserrula in their diet. The food aversion of sheep apparently does not result in the sheep totally avoiding biserrula. Rather, they select alternatives in combination with biserrula to achieve their preferred diet. We also found that sheep offered a mixed ryegrass and biserrula pasture preferred to incorporate a significant proportion of biserrula in their diet.

The information developed in this thesis suggests that a grazed pasture phase in cropping systems can be a useful inclusion in an integrated strategy to manage herbicide resistance. The selective grazing behaviour of domestic ruminants should be considered as a tool to manage pasture composition. Understanding the interactions between a diverse range of volunteer and commercial pasture plants and the domestic ruminants that feed on them in pastures is a key component in developing the strategy.

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