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**NUTRITIONAL STUDIES ON *LOTUS*
CORNICULATUS CONTAINING CONDENSED
TANNINS TO INCREASE REPRODUCTIVE RATE
AND LAMB GROWTH UNDER COMMERCIAL
DRYLAND FARMING CONDITIONS**

A thesis presented in partial fulfilment of the requirements for
the degree of

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New Zealand


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DECLARATION

The studies presented in this thesis were completed by the author whilst a Postgraduate student in the Institute of Veterinary, Animal and Biomedical Science, Massey University, Palmerston North, New Zealand. I hereby affirm that the content of this thesis is original research conducted by the author. All views and conclusions are the sole responsibility of the author. All references to previous work are included in the References section of each chapter. Any assistance received during the preparation of this thesis has been acknowledged.


I certify that the content of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree. I certify that to the best of my knowledge any help received in preparing this thesis, and all sources of materials used, have been acknowledged in the thesis.




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ABSTRACT

Five rotational grazing experiments were carried out at Massey University's Riverside farm, in the Wairarapa, on the East Coast of the Southern North Island, New Zealand, to compare the effects of feeding *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) dominant pasture upon sheep year round productivity. These studies also investigated under grazing, seasonal and annual net herbage accumulation rate and seasonal dynamics of undisturbed (i.e. non-grazed) net herbage accumulation rate of *L. corniculatus* relative to that of grass-dominant pasture. Aspects of *in vivo* digestibility of dry matter (DMD), organic matter (OMD), digestible organic matter in the dry matter (DOMD) and metabolisable energy (ME) concentration of *L. corniculatus* at different stages of maturity over the spring, summer and autumn were investigated in three indoor digestion trials.

1. Two field experiments (Chapter 2) were conducted during spring to assess the effects of grazing mixed age undrenched ewes on *L. corniculatus* ($n = 50$) or pasture ($n = 50$) and their lambs (mainly twins) on live weight (LW), wool production, faecal nematode egg count (FEC) and dag score. In Experiment 1 (18 October 2000 to 21 January 2001) and Experiment 2 (3 October 2001 to 2 January 2002) both forages were fed *ad libitum*. Total condensed tannin (CT) concentration in the diet selected was 24 to 27 g CT/kg DM for *L. corniculatus* and 1.4 to 1.5 g CT/kg DM for pasture. The LW gain, weaning LW and wool production were consistently greater ($P < 0.001$) for lambs grazing *L. corniculatus*, in either

Experiment 1 (258 vs. 189 g/day; 36.1 vs. 30.1kg; 1.17 vs. 0.98 kg) and in Experiment 2 (247 vs. 162 g/day; 31.8 vs. 24.1kg; 1.17 vs. 0.81 kg), respectively. Ewe and lamb dag scores were strongly and positively correlated with dag weight ($P < 0.001$) and generally increased with time in sheep grazing pasture, whilst grazing on *L. corniculatus* consistently reduced dag score. FEC in ewes grazing pasture showed a post-parturient rise (PPR) following lambing, whilst ewes grazing *L. corniculatus* had a reduced PPR in FEC. Up to day 70, FEC in lambs grazing *L. corniculatus* was lower than that for lambs grazing pasture, but between day 70 and the end of both experiments (approximately day 90), FEC in lambs grazing *L. corniculatus* increased to similar values as for pasture-fed lambs. FEC was not correlated with dag score or dag weight in ewes or lambs grazing pasture, but these indices were weakly and positively correlated in ewes and lambs grazing *L. corniculatus*, suggesting that lowering FEC on *L. corniculatus* also reduced dag formation.

It was concluded that under dryland farming conditions, the use of *L. corniculatus* during the spring/early summer lactation period can increase lamb LW and wool production, whilst eliminating the need for pre-lambing anthelmintic drenching and probably reducing the amount of insecticide needed to control flystrike. These effects compared to pasture are probably due to higher digestibility, higher ME concentration, higher voluntary feed intake (VFI), and to the effect of CT in reducing rumen protein degradability and controlling internal parasites in sheep grazing *L. corniculatus*. The absence of endophyte in *L. corniculatus* may have also have contributed to these effects.

2. During 2001 and 2002 (Chapter 3), grazing trials from February to November were conducted for 279 days (Experiments 1) and 285 days (Experiment 2), to compare the effects of grazing shorn mixed age Romney ewes in light condition on *L. corniculatus* versus pasture during the mating period (9 weeks, Experiment 1) and 11 weeks (Experiment 2). In Experiment 2, the length of time (days) that ewes need to graze *L. corniculatus* before mating to maximise reproductive performance was also investigated. Common objectives in both Experiments were to measure forage feeding effects on ewe wool production and LW of their lambs at weaning.

In Experiment 1, groups of ewes ($n = 100$) were fed on either *L. corniculatus* or pasture at a herbage allowance of 1.8 kg green DM/ewe/day for the first three weeks of feeding and increased to *ad libitum* (2.3 kg green DM/ewe/day) during the mating period for two cycles. In Experiment 2, groups of 75 ewes grazed *L. corniculatus* for 42, 21, 10 and 0 days before a synchronised oestrus, with pasture being grazed for the balance of the 42 days. All *L. corniculatus* groups continued grazing *L. corniculatus* for a further 5 weeks. Feed allowance was initially 2.0 kg green DM/ewe/day, increased to 2.3 kg green DM/ewe/day during the mating period over the two cycles. At the end of *L. corniculatus* feeding in both experiments the groups were combined and grazed on pasture until weaning. Total CT concentration in the diet selected was 18 to 29 g CT/kg DM for *L. corniculatus*, with only trace amounts in pasture.

In Experiment 1 mating ewes on *L. corniculatus* compared to pasture increased number of lambs born and lambs weaned per ewe lambing by 16 and 32% units respectively ($P < 0.05$), due to more multiple and less single births ($P = 0.06$) and to reduced lamb mortality ($P < 0.05$) between birth and weaning. In Experiment 2, increasing the numbers of days of grazing *L. corniculatus* before ovulation (0, 10, 21, 42 days) linearly increased ovulation rate ($P < 0.05$), lambs born and lamb weaned by up to 16% units, but had no effect upon lamb mortality. Mating ewes on *L. corniculatus* increased wool production ($P < 0.01$) and fibre length ($P < 0.05$) in Experiment 1 but not in Experiment 2. Grazing *L. corniculatus* had no effect on lamb birth weight and only small positive effects on weaning LW.

It was concluded that, under commercial dryland farming conditions, the use of *L. corniculatus* during the mating season in late summer/autumn can be used to increase reproductive efficiency and wool production, with the largest responses in years with exceptionally dry autumn periods. These effects are probably due to the higher digestibility and ME concentration of *L. corniculatus* than pasture and to the CT in *L. corniculatus* reducing rumen protein degradability and leading to greater essential amino acid (EAA) absorption from the small intestine. Effects of forage CT upon the uterine microenvironment at the time of conception, implantation and early foetal growth, need to be investigated in future studies. It is also suggested that effects of mating on *L. corniculatus* upon lamb mortality between birth and weaning should be further investigated with ewe numbers/treatment increased from 100 to 350.

3. During the summer of 2002/2003, another grazing trial (Chapter 4: 95 days) compared the effects of grazing *L. corniculatus* and pasture on LW and the dynamics of nematode parasite infection in Suffolk x Romney weaned lambs fed *ad libitum*. Half of the lambs ($n = 30$) grazing either *L. corniculatus* or pasture received oral anthelmintic at the start and at monthly intervals (regular-drenched groups), whilst the remaining 30 lambs in each treatment only received oral anthelmintic when mean faecal nematode egg counts (FECs) exceed 1,000 eggs/g wet faeces (trigger-drenched groups), which occurred on day 58 only for both groups. Trigger and regular-drench lambs grazed separate areas. Total CT concentration in the diet selected was 40 to 31 g CT/kg DM for *L. corniculatus*, with only trace amounts in pasture.

Regular-drenched lambs grazing *L. corniculatus* had significantly higher LW gain (298 g/day) and carcass weight gain (133 g/day) than all the other groups, whilst trigger-drenched lambs grazing *L. corniculatus* had significantly greater LW gain (228 g/day) and carcass gain (99 g/day) than regular-drenched (200; 66 g/day) and trigger-drenched (187; 63 g/day) lambs grazing pasture. Carcass fatness was significantly lower for trigger-drenched lambs than for regular-drenched lambs, when fed either *L. corniculatus* or pasture. Dag score was consistently lower for regular-drenched lambs grazing *L. corniculatus* than pasture; trigger-drenched lambs showed similar effects up to day 48, with no differences between the two groups thereafter. Regular anthelmintic treatment maintained FECs at low values, while parasitised lambs on *L. corniculatus* tended to have higher FECs than pasture-fed lambs. Relative to trigger-drenched lambs that grazed pasture, grazing

trigger-drenched lambs on *L. corniculatus* had significantly reduced worm burdens of *Haemochus contortus*, *Teladorsagia spp.*, *Nematodirus spp.* and *Cooperia spp.* at slaughter, but greater burdens of *Trichostrongylus spp.*, *Chabertia ovina*, *Oesophagostomum spp.* and *Trichuris ovis* were present in *L. corniculatus*-fed lambs.

It was concluded that grazing *L. corniculatus* under dryland farming conditions compared to pasture can increase LW gain of weaned lambs, whilst reducing reliance on anthelmintic drenches to control parasites. These effects are probably due to increased protein supply from the action of CT enabling the lambs to have a higher LW gain when carrying a parasite burden, and to *L. corniculatus* better maintaining its high ME value under drought conditions. Using *L. corniculatus* to finish weaned lambs without anthelmintic drenches for a seven-week period is proposed.

4. A three-year study (Chapter 5; November 2000 to October 2003) was conducted to compare, under grazing conditions, seasonal and annual grazed net herbage accumulation rate and seasonal dynamics of undisturbed (i.e. non-grazed) net herbage accumulation rate of *L. corniculatus* relative to grass-dominant pasture. Prediction equations to estimate standing DM in *L. corniculatus* and pasture from the rising plate meter (RPM) and sward surface height were also generated.

L. corniculatus and pasture growing in a moderate fertility and low-pH soil (pH 5.35) accumulated similar total herbage masses (24.3 vs. 24.1 t DM/ha) over the 3-year period, with the DM production being greater for *L. corniculatus* than for

pasture during 2000–2001, producing more DM during summer/autumn drought conditions. The net herbage accumulation rate from undisturbed areas of *L. corniculatus* and pasture were similar in spring, summer and autumn. Seasonal variation in the calibration regressions fitted to estimate herbage mass of *L. corniculatus* non-destructively, suggested a combination of destructive and non-destructive methods are needed to assess herbage mass. It was concluded that *L. corniculatus* has the potential to increase the performance of a pasture-based sheep dryland farming system due to its ability to grow in acidic soils, its tolerance of drought conditions during summer/autumn and its seasonality of feed supply.

5. Three digestion experiments involving cryptorchid weaned lambs were conducted for 14 days over the spring, summer and autumn to determine changes in *in vivo* digestibility of DM, OM, digestible OM in the DM and ME concentration of *L. corniculatus* at different stages of maturity. *In vivo* digestibility samples were then used as standards to investigate if the enzymatic *in vitro* system of Roughan and Holland (1977) could predict OMD and DOMD of CT-containing *L. corniculatus*. Digestibility of *L. corniculatus* declined as it matured, but the rate of decline was much less than occurs for temperate grasses and for white clover. It was concluded that the *in vitro* enzymatic system of Roughan and Holland (1977) can be used to predict OMD and DOMD of *L. corniculatus*, provided a standard curve involving *in vivo* data generated with *L. corniculatus* is used. Using a standard curve with *in vivo* data from pasture led to bias which increased at lower OMD values. Reasons for the consistent differences between *L. corniculatus* and

pasture standard curves are discussed, including possible effects of residual bound CT in lowering *in vitro* digestibility.

From this series of experiments, this study is the first to report that relative to conventional perennial ryegrass/white clover, mating ewes on *L. corniculatus* under grazing conditions may reduce post-natal lamb mortality. It is also the first study to show that grazing sheep on *L. corniculatus* can maintain productivity during spring and summer with reduced dependence on anthelmintic drench input. It is concluded that whole farm modelling, mechanical harvesting and conservation strategies, selection of *L. corniculatus* germplasm for creeping-type plants more suited to grazing and the integration of new crops containing secondary compounds, such as chicory, should be considered to support major advances in sustainable dryland sheep farming systems.

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FOR THEIR ENDLESS LOVE, PATIENCE, ENCOURAGEMENT

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CHAPTER 1.
A LITERATURE REVIEW

1.1 *LOTUS CORNICULATUS* AS A FORAGE PLANT

1.1.1 Introduction

Legumes are an important component of feeding systems in temperate zones to fix nitrogen and to produce high quality forage as a feed for livestock (Hoffman *et al.*, 1993; Panciera and Sparrow, 1995). The genus *Lotus* comprises 80 to 200 annual and perennial species distributed throughout the world (Seaney and Henson, 1970).

The centre of origin of the old-world *Lotus* species is the Mediterranean. *Lotus corniculatus* L. (birdsfoot trefoil) is naturally distributed throughout Europe (Seaney and Henson, 1970), extending into Asia as far as the Himalayas and southward to include Eastern and Northern Africa (Jones and Turkington, 1986). *Lotus corniculatus* species have been introduced to over 200,000 ha in Eastern Canada, along the East and West coasts of the U.S.A (1 million ha) and in Mexico (Seaney and Henson, 1970; Turkington and Franko, 1980; Scott and Charlton, 1983; Beuselinck and Grant, 1995).

Furthermore, introduced *L. corniculatus* is widely distributed in China, India and South American countries (Brazil, Uruguay, Argentina and Chile) (Jones and Turkington, 1986) where approximately 250,000 ha are sown every year (Frame *et al.*, 1998). In tropical regions *L. corniculatus* is restricted to mountain altitudes in the range of 730 to 3100 m (Turkington and Franko, 1980; Jones and Turkington, 1986; Frame *et al.*, 1998).

Two perennial *Lotus* species are used for forage production in the world (Frame *et al.*, 1998). In New Zealand big trefoil (*Lotus pedunculatus* Cav., syn. *Lotus uliginosus* Schkuhr.) and birdsfoot trefoil (BFT) (*Lotus corniculatus* L.) were introduced in the early 1900's (Levy, 1918), as pioneer legumes to develop scrub-covered land (Frame *et al.*, 1998). However, today's BFT is recognised as a hay crop and as a perennial non-bloating pasture, which provides excellent feed for ruminants (Alison and Hoveland, 1989b; Beuselinck and Grant, 1995).

1.1.2 Agronomic characteristics

Lotus corniculatus is the most variable species in the *Lotus* genus (Turkington and Franko, 1980; Jones and Turkington, 1986). Birdsfoot trefoil is a cross-pollinated perennial legume. Plants live between 2 and 4 years (Beuselinck and Grant, 1995). In appearance BFT is mainly non-rhizomatous, predominantly winter-dormant especially in cold areas, and summer-active. Birdsfoot trefoil is tolerant of low soil fertility, drought conditions, soil acidity and impeded drainage (Heinrichs, 1970; Douglas and Foote, 1993; Pollock and Scott, 1993; Bologna *et al.*, 1996; Kemp *et al.*, 1999).

Birdsfoot trefoil has a taproot (up to 1 m in length), that becomes woody with age (Jones and Turkington, 1986), However, the root system is less deep than lucerne (*Medicago sativa*) (Beuselinck and Grant, 1995). The root system has several secondary lateral branches. It forms a thick fibrous root system in the topsoil (Turkington and Franko, 1980), which leads to its better persistence on undrained shallow soils than *Medicago* species (Foulds, 1978; Frame *et al.*, 1998).

There is a substantial difference in stem and leaf morphology within *L. corniculatus* (Seaney and Henson, 1970). The aerial system of BFT consists principally of numerous well-branched stems arising from a single crown (Alison and Hoveland, 1989a; Beuselinck and Grant, 1995; Bologna *et al.*, 1996). Stems emerge prostrate, erect or ascending, usually 10 to 50 cm (but up to 1m) (Jones and Turkington, 1986; Kallenbach *et al.*, 1996). After grazing or cutting, regrowth emerges from axillary buds located on the upper parts of the cut shoot (Nelson and Smith, 1968). Stems are slender, solid, round in cross section at the base, and square at the top, ranging from glabrous to pubescent (Turkington and Franko, 1980).

Leaf shape may vary from obovate, rounded or oblanceolate. Leaves are attached alternatively on opposite sides of the stem (Seaney and Henson, 1970). Leaves are pentafoliolate, 6 to 20 mm long and 1 to 9 mm wide, and entire to minutely serrate (Jones and Turkington, 1986). Each leaf has three leaflets attached to the terminal end of the petiole and two smaller green leaflets attached at the base (Turkington and Franko, 1980; Beuselinck and Grant, 1995), which are habitually hairless (Scott and Charlton, 1983). Similar to white clover (*Trifolium repens* L.) during darkness the leaflets close around the petiole and stem (Seaney and Henson, 1970; Beuselinck and Grant, 1995).

Birdsfoot trefoil is a long-day plant. It requires between 16 and 18 hours of day length for full flowering (Beuselinck and Grant, 1995). The inflorescence is a typical umbel with 4 to 8 florets attached on a rather erect peduncle (3 to 10 cm) (Seaney and Henson, 1970) arising from the axil of upper leaves (Turkington and Franko,

1980). Each floret consists of a calyx with 5 united sepals, 2 to 6 mm long, glabrous, toothed or pubescent and a characteristic legume corolla with 5 petals (Jones and Turkington, 1986), which are generally bright yellow to coppery or brick red at some stage (Frame *et al.*, 1998).

Seed pods are cylindrical, 15 to 30 mm long and 3 mm diameter (Turkington and Franko, 1980; Scott and Charlton, 1983) often impermeable to water (Seaney and Henson, 1970). As they ripen, they change colour from green to brown and are almost black as they mature (Frame *et al.*, 1998). An average of 5 to 6 pods are produced at right angles to the top on the peduncle (thus 'bird's-foot trefoil as the common name) (Seaney and Henson, 1970; Jones and Turkington, 1986). Each pod contains 15 to 20 seeds attached to the ventral suture. At maturity the pods split along both sutures and twist spirally, and violently discharge the seed (Seaney and Henson, 1970).

Seeds are irregularly rounded, somewhat flattened and very small, 1.3 to 1.5 mm x 1.0 mm. At maturity they vary in colour from olive to brownish to almost black and are frequently speckled. Their weight varies from 1.11 mg to 1.67 mg (Turkington and Franko, 1980; Jones and Turkington, 1986). Seed quality varies and depends upon maturity at harvesting and the crop-handling processes used (Frame *et al.*, 1998). Unless the seed coat is scarified or treated to allow absorption of moisture, the hard seed will not germinate (Seaney and Henson, 1970).

1.1.3 Uses

Lotus corniculatus is recognised as a high-quality pasture, hay crop and seed crop with an important role on soils that are imperfectly drained as less fertile and dry (McGraw and Marten, 1986). Birdsfoot trefoil is more tolerant than lucerne and clovers to acidic, saline and calcareous soils (Turkington and Franko, 1980; Schachtman and Kelman, 1991). In addition to forage production, its attractive flowers for honey production (Frame *et al.*, 1998) and perennial-life cycle make BFT popular for plantings on highway slopes and medians for beautification, soil improvement and erosion control (Beuselinck and Grant, 1995).

The seasonal production of BFT suggests that in farm management the legume is an alternative pasture for grazing throughout the summer period (Van Keuren *et al.*, 1969; Bologna *et al.*, 1996). However, if a pure stand of BFT is conserved as hay, it requires cutting at early flowering. *Lotus corniculatus* may be less tolerant of defoliation than clovers, but is more tolerant than lucerne (Scott and Charlton, 1983). Waghorn *et al.* (1998) suggested that BFT should be used for pastoral farming in Central Otago and the East Coast of South and North Islands in New Zealand.

1.1.4 Cultivars

In the early 1900's *Lotus major* (*L. pedunculatus* Cav.), *Lotus hispidus* (Body's clover or hairy birdsfoot trefoil), *Lotus angustissimus* (Slender birdsfoot trefoil) and *L. corniculatus* were species of trefoil of agricultural importance in New Zealand (Levy, 1918). Significant variability exists within BFT germplasm for further

selection of new cultivars (Beuselinck and Grant, 1995). More than 200 introductions have been screened from several overseas sources in dryland sites in the South and North Islands of New Zealand during the last decades (Scott and Charlton, 1983).

From this screening only eight erect cultivars were considered promising for dryland conditions by Scott and Charlton (1983) and Lowther *et al.* (1987); Cascade (USA), El Boyero (Uruguay), Franco (Italy), Ginestrino (Chile), Granger (USA), Maitland (Canada), San Gabriel (Brasil) and Tana (USA). However, the semi-erect cultivar Grasslands Goldie adapted for grazing is the most commonly available in New Zealand (Bologna *et al.*, 1996; Waghorn *et al.*, 1998).

1.1.5 Establishment and growth

The ability of BFT to establish is conditioned by seed weight and seed size (Beuselinck and McGraw, 1983; Beuselinck and Grant, 1995), and by sowing depth (Woodman *et al.*, 1990). In addition, Laskey and Wakefield (1978) reported lack of moisture at the time of seeding, environmental conditions and poor competition with weed, companion crops, and BFT itself to influence establishment.

Germination in BFT is conditioned by temperature (Hur and Nelson, 1985) and is rapid in the range between 10⁰ C and 20⁰ C (Pancieria and Sparrow, 1995). However, germination and seedling emergence in BFT are not the limiting process to achieve an adequate legume establishment (Bologna *et al.*, 1996). The failure is

due to the birdsfoot trefoil's poor seedling vigour (Seaney and Henson, 1970; Foulds, 1978; Beuselinck and Grant, 1995).

High seedling losses occur in May-June or August (Bologna *et al.*, 1996; Woodman *et al.*, 1997). Nodulation failures and slow initiation of symbiotic nitrogen fixation affect the early growth of BFT. This is to a large extent dependent on ineffective inoculation techniques, the strain of *Rhizobium* used and death of the *Rhizobium*, and soil temperature (Laskey and Wakefield, 1978; Chapman *et al.*, 1990).

Late winter or early spring is the ideal time to sow BFT in New Zealand to survive the onset of dry soil conditions (Woodman *et al.*, 1997). However, in dry environments a dilemma exists between sowing deeper to guarantee access to the moisture or sowing shallow to ensure emergence (Woodman *et al.*, 1990). In most situations BFT should be sown alone because it is non-competitive and slow during establishment compared with most pasture plants used in New Zealand (Douglas *et al.*, 1990). Pure stands of BFT provide high quality forage. However, weed invasion may induce earlier renovation than for a legume-grass system (Marten and Jordan, 1979).

Seeding rates between 3 and 5 kg/ha are suggested in oversowing. For cultivated soils, seeding rates up to 10 kg/ha of viable seed are recommended for establishment of a pure stand of BFT (Waghorn *et al.*, 1998). Pure stands of *L. corniculatus* reach the highest yield when it is sown in rows 15 cm apart (Turkington and Franko, 1980). Some reseeding takes place if established swards

are allowed for seed production in mid-summer each year (Scott and Charlton, 1983; Alison and Hoveland, 1989b).

1.1.5.1 Rhizobial requirements

In New Zealand, soils are not infected with the *Rhizobium sp.* for BFT in most of the country, except for those few areas where BFT or *Lotus tenuis* have become naturalised. As a consequence, birdsfoot trefoil requires inoculation with the commercial *Rhizobium* strain NZP2238 (Scott and Charlton, 1983) to avoid the limited root growth problem (Panciera and Sparrow, 1995).

On acid soils around pH 5.5 and less, management recommendations include inoculating at 5 times the manufacturer's stipulated rate and drilling with the incorporation of 10% of gum arabic for coated seed and oversowing within 24 hours of inoculation at not more than 12 mm depth (Chapman *et al.*, 1990). However, nodulation failure in acid soils may be reduced by both seed pelleting and broadcasting lime (Woodman *et al.*, 1997; Waghorn *et al.*, 1998). Furthermore, elemental sulphur (S) and phosphatic (P) fertiliser for seed coating also need to be considered in the moister areas (Pollock and Scott, 1993).

1.1.5.2 Nitrogen fixation

Legumes can use nitrogen (N) derived from soil or N₂-fixation (Gault and Peoples, 1993). Annual nitrogen fixation in pure stands of BFT is estimated at 90kg N/ha (Heichel *et al.*, 1985). The early growth and the N₂-fixing ability of the plant are largely dependent on the strain of *Lotus* rhizobia, root temperature (Kunelius and

Clark, 1970), soil pH (Foulds, 1978) and aluminium concentration (Edmeades *et al.*, 1991).

The capacity of nodules to sustain N₂-fixation may be affected by plant species and the competition between vegetative regrowth and nodules for reserve carbohydrates, and current photosynthate as well as the removal of photosynthetic tissue from the forage legume at harvest (Cralle and Heichel, 1981; Vance *et al.*, 1981).

Trefoil and lucerne adapt by different mechanisms to the stress of successive harvests. Since lucerne has a lower nodule mass than BFT (Beuselinck and Grant, 1995). There is substantial evidence that BFT exhibits a greater reduction in total nitrogenase activity (TNA) than lucerne after grazing or harvest and the recovery in TNA is more rapid in lucerne than in trefoil (Cralle and Heichel, 1981). This is due to lucerne's capacity to retain nodules with normal growth and function after harvest. In contrast, in BFT, as for soybeans, nodule function after grazing or harvest is dependent on renewed shoot growth, and the initiation of a new pink nodule population (Vance *et al.*, 1981). These differences may reflect a lower cost in energy for N₂-fixation in lucerne than in BFT (Cralle and Heichel, 1981; Vance *et al.*, 1981). As a consequence, BFT requires at least between 11 and 21 days of regrowth before showing normal TNA activity (Cralle and Heichel, 1981).

1.1.5.3 Soil pH effects

Birdsfoot trefoil is an alternative legume for different ecological niches (Schachtman and Kelman, 1991). It is found in the soil pH range from 4.5 to 8.0

(Jones and Turkington, 1986). BFT tends to grow better than lucerne on poor fertility acid soils. However, BFT shows a better vigour on soil at pH 6.5 than at low soil pH values (Turkington and Franko, 1980; Jones and Turkington, 1986).

1.1.5.4 Plant density

Pasture is a dynamic community of plants with new tissue being formed continually through growth and old tissue disappearing through the process of decay, senescence and death (Korte *et al.*, 1987). The farmer has direct control over the population density through the sowing rate. However, two antagonist objectives need to be considered. First, to sow a population that results in early canopy closure and second to delay senescence and decay at the end of the growing season (Hodgson, 1990). There must be a balance among rate of canopy closure, total biological yield and economic yield (McKenzie *et al.*, 1999).

For perennial legumes, such as BFT, it is critical that the proper population be achieved with the initial sowing (Chapman *et al.*, 1990; Miller and Stritzke, 1995). Forage yield per plant decreases asymptotically as plant population density (PPD) increases and forage yield/m² is high and relatively constant at 60 plants/m². However, BFT requires as a minimum a PPD of 30 plants/m² for high yields of dry matter (DM) (McGraw *et al.*, 1986).

Birdsfoot trefoil is largely dependent upon carbohydrate production from leaves rather than root reserves (Turkington and Franko, 1980). The interaction between height and interval of defoliation, and disease incidence has a preponderant effect on the population dynamics of BFT (Ayala, 2001). Results from Bologna *et al.*

(1996) showed that during a 17-month study a grazing interval of 2 weeks resulted in a survival of 35% of the established plants. In contrast, defoliation interval of no shorter than 4 weeks killed only 13% of the established plants in environments of the South Island in New Zealand. Ayala (2001) stated that a reduction of BFT population could be expected under close defoliation in the short term. However, more time is required to show a decline in stand density with less intensive defoliation (8-10) cm and increased grazing interval (Ayala, 2001) because plant density is also affected by natural reseeding (Taylor *et al.*, 1973).

1.1.5.5 Dry matter production

Several studies overseas and in New Zealand have evaluated dry matter production in BFT (Table 1.1).

Dry matter production is influenced by several factors such as cultivar, latitude, soil pH, management of the sward and environmental conditions. In New Zealand, the most productive season of DM production of *L. corniculatus* is the summer (Ayala, 2001), with approximately 50% of the annual yield produced over that period (Bologna *et al.*, 1996).

Table 1.1. Annual dry matter yields (t/ha) of *Lotus corniculatus* L.

Forage	Year	Country and district	Total t/DM/ha	Reference
<i>L. corniculatus</i> L	90-91	U.S.A, Alaska	3.0	Pancieria and Sparrow (1995).
Cv. Dawn	69-72	U.S.A, Kentucky	2.6	Taylor <i>et al.</i> (1973).
Cv. Viking	70-72	U.S.A, Kentucky	2.6	Taylor <i>et al.</i> (1973).
<i>L. corniculatus</i> L	95-96	U.S.A, Iowa	7.2	Sleught <i>et al.</i> , (2000).
<i>L.corniculatus.</i> L	85-86	New Zealand, Wairarapa	6.3	Douglas <i>et al.</i> (1990).
Cv. Granger	93	New Zealand, Wairarapa	10.1	Douglas and Foote (1993).
Cv. G. Goldie	94-96	New Zealand, Canterbury	13.1	Bologna <i>et al.</i> (1996).
Cvs. San Gabriel, Ganador, Boyero and Quimey	95	Chile	6.7-8.9	Acuna (1995) (cited in Ayala, 2001).
<i>L.corniculatus.</i> L	96	Uruguay	9.0	Carambula <i>et al.</i> (1996) (cited in Ayala, 2001).
Cvs. INIA Draco, San Gabriel	98-00	Uruguay	5.5	Ayala (2001).
Cvs. G. Goldie, Steadfast	98-00	Uruguay	2.1	Ayala (2001).

The lowest DM production is in the spring and late autumn-winter (Douglas and Foote, 1993). According to Gervais (1988) in Quebec (Canada) the DM yield increased significantly with advancing maturity up to the midbloom stage in the spring. In contrast, subarctic environmental conditions depress the growth response during the whole year (Pancieria and Sparrow, 1995). Typical annual yields for dryland areas in New Zealand range from 6.3 to 13.1 t/ha (Table 1.1) (Douglas *et al.*, 1990; Douglas and Foote, 1993).

1.1.5.6 Persistence and pest resistance

The persistence of BFT under grazing conditions is based on the survival of the plants originally established, the development of a soil-seed bank and seasonal seedling recruitment (Bologna *et al.*, 1996). Birdsfoot trefoil has also been found to be strongly influenced by the effect of cutting height and cutting frequencies on root growth (Nelson and Smith, 1968; Cralle and Heichel, 1981; Vance *et al.*, 1981; Alison and Hoveland, 1989a; Panciera and Sparrow, 1995).

Birdsfoot trefoil requires 20 to 30 day grazing intervals and 6 to 10 cm defoliation heights during spring and moderate cutting heights (≥ 6 cm), and rest periods, during summer (Ayala, 2001). However, to avoid effects on the quality and persistence of *L. corniculatus* defoliation in late autumn, cutting height of less than 4 cm and harvests, or grazing periods, in winter should be avoided (Ayala, 2001).

In New Zealand BFT is comparatively free from diseases and pest damage (Waghorn *et al.*, 1998). However, internationally fungal species, pests and viruses affecting crown, roots, and foliage, have been described widely by Seaney and Henson, (1970), Turkington and Franco, (1980), Jones and Turkington, (1886), Beuselinck and Grant (1995) and Frame *et al.* (1998).

The reduction in competitive ability or persistence of BFT results in the invasion of mainly perennial ryegrass (*Lolium perenne* L.) and white clover in fertile swards (Douglas and Foote, 1993), and weed invasion elsewhere (Beuselinck and Grant, 1995).

From this perspective, Douglas and Foote (1993) stated that the most appropriate use of pure stands of BFT is as a short-term (less than 3 years) forage with a short duration mob-stocking and uninterrupted regrowth. In addition, rotational grazing prevents excessive selection of *Lotus* in mixtures with grass. However, by allowing reseeding in the two first years, greater longevity would be achieved (Waghorn *et al.*, 1998). In addition, Templeton *et al.* (1967) stated that swards of BFT for seed production persisted for more than 12 years.

1.1.6 Management

Each pasture species is adapted to particular combinations of temperature (altitude), fertility and soil moisture (Seaney and Henson, 1970). Even though BFT is considered to be a long-lived perennial legume, its management has shown to have a considerable influence on its persistence (Van Keuren and Davis, 1968; Van Keuren *et al.*, 1969).

Considerable research (Van Keuren and Davis, 1968; Scott and Charlton, 1983; Chapman *et al.*, 1990) has shown that BFT should be managed similarly to lucerne, with mob stocking and rotational grazing. However, a low stocking rate (SR) must be avoided to reduce selectivity by grazes and the rejection of stems (Waghorn *et al.*, 1998). But, at the same time, the efficient utilisation of BFT swards would include a grazing management that increases the intake of leaves rather than stems due to leaves' low content of lignin (John and Lancashire, 1981).

Rotational grazing promotes reseeding and greater photosynthetic area after grazing, which reduces the demands on the carbohydrate reserves in the roots

during regrowth (Beuselinck and Grant, 1995). Intervals between defoliation for the original plants to survive under grazing should not be shorter than 4 weeks (Bologna *et al.*, 1996). This is consistent with the view that defoliation must be not lower than 8 cm (Scott and Charlton, 1983) and also consistent with maximum seedling recruitment (Bologna *et al.*, 1996).

1.1.7 Chemical composition

1.1.7.1 Nutrient concentration

The nutritive value of BFT is high as pasture, silage or hay (Table 1.2) (Seaney and Henson, 1970) and is comparable with lucerne and other legumes (Frame *et al.*, 1998).

Table 1.2. Mean values of chemical composition (g/kg dry matter (DM)) and *in vitro* organic matter digestibility (% OMD) in the diet select by sheep grazing *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie).

	Season		
	Spring/summer	Summer	Summer/autumn
<i>Total N</i>	35.5	35.5	38.3
Water soluble CHO	95	ND	84
Pectin	40	ND	39
Lignin	65	66.2	88
NDF	286	251	346
ADF	218	197	271
Total CT	44.5	37.0	20.8
<i>In vitro</i> OMD	73.3	73.5	79.6

❖ Results from Douglas *et al.* (1995); Wang *et al.* (1996a,b); Min *et al.* (1998); Barry *et al.* (1999); Douglas *et al.* (1999); Min *et al.* (1999); Min *et al.* (2001).
ND; not determined.

Birdsfoot trefoil maintains its quality during maturity better than most forage crops (Alison and Hoveland, 1989b). Nitrogen concentration remains high until very late in the growing season (Li, 1989). The content of soluble carbohydrates and structural fibre depends on the proportions of leaf and stems, and stage of maturity (Waghorn, *et al.*, 1998).

Birdsfoot trefoil contains condensed tannins (CT) that bind strongly with protein after chewing, reducing protein degradation in the rumen and increasing essential amino acid (EAA) absorption in the small intestine (Wang *et al.*, 1996b; Barry and McNabb, 1999). CT concentration may decrease more rapidly throughout the growing season in species with high concentrations than in those with low concentrations. However, in general CT concentration decreases throughout the summer months (Roberts *et al.*, 1993).

1.2 EFFECT OF CONDENSED TANNINS IN *LOTUS CORNICULATUS* UPON NUTRIENT UTILISATION

1.2.1 Chemical properties

Tannins and lignins are synthesised in the plant by the same shikimic acid biochemical pathway (Fig.1.1) (Swain, 1979). Two groups of tannins have been classified according to their structural types, the hydrolysable tannins (HT) and the condensed tannins (CT) or proanthocyanidins (PA) (McLeod, 1974).

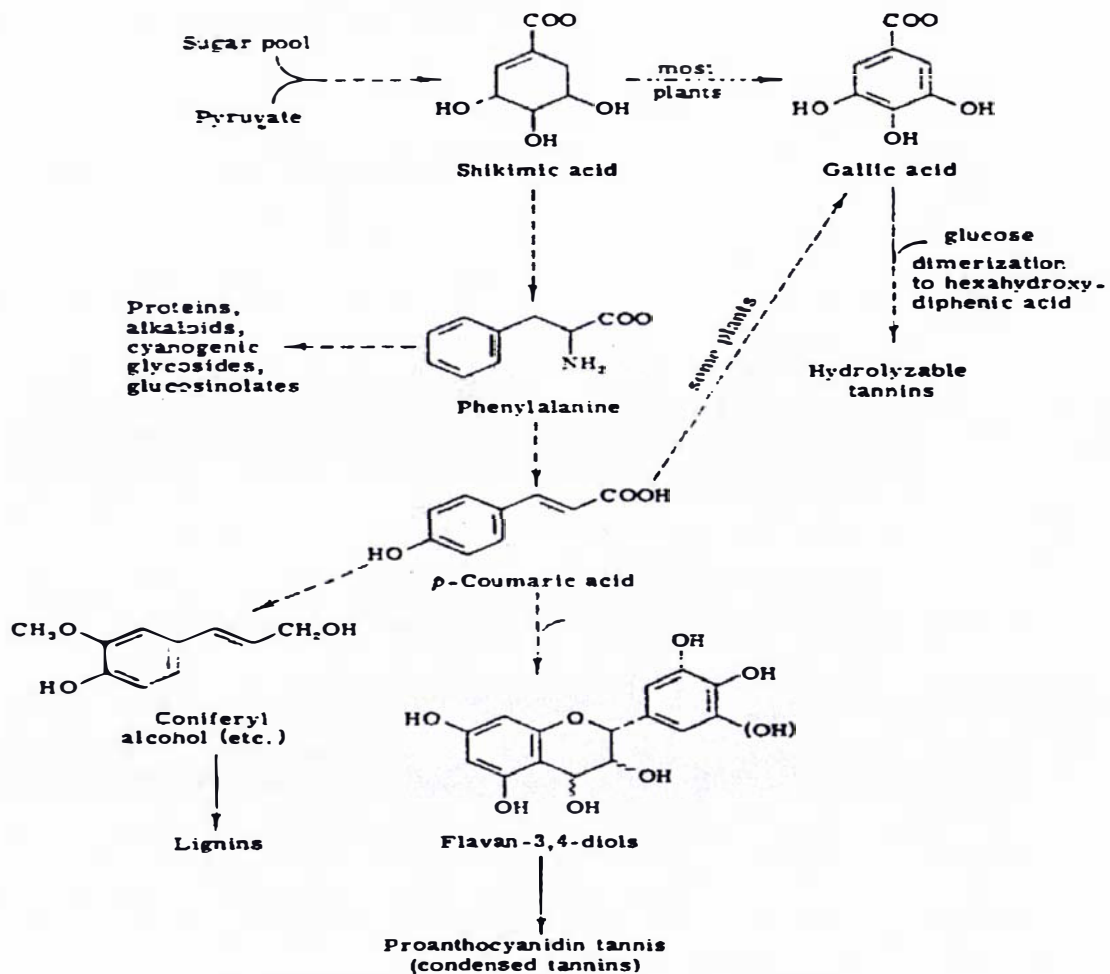


Figure 1.1. Biosynthetic origins of hydrolyzable and condensed tannins, and lignin, in plants (Swain, 1979).

Condensed tannins structurally can be regarded as being formed by the condensation of flavan-3-ol-units (catechin, epicatechin, galocatechins and epigallocatechin) to form dimers and higher oligomers, which mainly contain 4-8 linkages (Swain, 1979). Foo *et al.* (1996) reported an average molecular mass of the CT from *L. corniculatus* in the range 1800-2100 and a predominance of epicatechin units (67%) in the procyanidin-type polymer. In contrast, the average molecular mass of the CT from *L. pedunculatus* was 2200, with the polymer being of the prodelphinidin type, with epigallocatechin (64%) as the major extender unit

(Foo *et al.*, 1997). Hence, *L. pedunculatus* and *L. corniculatus* CT differ considerably in their chemical structure, and, hence, are likely to differ in their physiological properties and in their effect on protein digestion in ruminants fed fresh forages (Foo *et al.*, 1997).

The reactivity of CT with proteins is based in two mechanisms, hydrogen (H) bonding, which is reversible, and oxidative coupling, which is not reversible (McLeod, 1974; Swain, 1979). Most of the positive effects of CT in ruminant nutrition are associated with its great affinity for leaf protein (Jones and Mangan, 1977).

The stability of the CT-protein complex is pH dependant (Jones and Mangan 1977). Condensed tannins in the near neutral pH range form CT-protein complexes, which are stable and insoluble at rumen pH (6.5-7.0). However, the protein-CT complex is unstable and releases protein in the abomasum (pH 2.5-3.0), which is then available for digestion in the small intestine (pH 8.0-9.0) (Mangan, 1988).

The tannin-protein complex is reversible by polyethylene glycol (PEG) in the range between 200 and 6,000 molecular weight (MW), at a pH range of 2.0-8.5 (Jones, 1965). Polyethylene glycol forms insoluble complexes with tannins through hydrogen bonding between phenolic hydroxyl groups in the CT (or other tannin moiety) and ether oxygen atoms in the polyethylene glycol chain (Jones, 1965). Jones and Mangan (1977) stated that the protein is released from the protein-tannin complex due to an exchange reaction with PEG, which in turn reacts with

CT to form an insoluble complex. However, protein release from the protein-tannin complex is negatively correlated with the amount of tannin in the complex and the age of the complex prior to addition of PEG.

1.2.2 Condensed tannins, location and concentration

Condensed tannins are present in the vacuoles of some plants, including temperate pasture legumes (Swain, 1979; Foo *et al.*, 1982; Barry, 1989).

Table 1.3. Influence of season and low and high soil fertility on condensed tannin concentrations (% of DM) in cultivars of *Lotus corniculatus* L. and *Lotus pedunculatus* (Grasslands Maku).

	Season			
	Summer		Autumn	
	Low	High	Low	High
<i>Lotus corniculatus</i>				
El Boyero	3.04	3.06	1.96	1.41
Empire	0.56	0.39	0.34	0.13
Granger	3.89	2.87	2.52	2.09
Maitland	2.71	2.62	2.19	1.16
Winnar	0.57	0.35	0.30	0.14
<i>Lotus pedunculatus</i>				
Maku	8.61	8.42	6.80	5.80

❖ Adapted from Lowther *et al.* (1987).

The data in Table 1.3 indicates that, except for cv. El Boyero, growing BFT under conditions of low, compared with high, soil fertility, resulted in a moderate increase in CT concentration. A reduction of CT concentration among cultivars and species was found in the autumn period compared to summer (Roberts *et al.*, 1993). Consistent differences in CT concentration between different cultivars of *L. corniculatus* are apparent.

1.2.3 Condensed tannins and voluntary feed intake

Voluntary feed intake (VFI) is determined by the relationship between the animal, feeding environment and properties of the feed (Mertens, 1994).

Table 1.4. Effect of CT concentration on the voluntary feed intake in sheep fed *Lotus sp.* Effects were deduced from comparing sheep fed each species with and without PEG supplementation.

Species	CT concentration g/kg/DM	Effect on VFI	Reference
<i>L. pedunculatus</i>	63-106	- 27 %	Barry and Duncan (1984).
<i>L. pedunculatus</i>	55	-12%	Waghorn <i>et al.</i> (1994).
<i>L. corniculatus</i>	32-57	Slightly increased	Douglas <i>et al.</i> (1995).
<i>L. corniculatus</i>	50	Modified by feed allowance	Douglas <i>et al.</i> (1999).
<i>L. corniculatus</i>	44.5	NC	Wang <i>et al.</i> (1996a).
<i>L. corniculatus</i>	34	NC	Wang <i>et al.</i> (1996b).
<i>L. corniculatus</i>	28	NC	Min <i>et al.</i> (1998).
<i>L. corniculatus</i>	24	NC	Barry <i>et al.</i> (1999).
<i>L. corniculatus</i>	24	NC	Luque <i>et al.</i> (2000).
<i>L. corniculatus</i>	22	NC	Waghorn <i>et al.</i> (1987).
<i>L. corniculatus</i>	17.6	Increased	Min <i>et al.</i> (2001).
<i>L. corniculatus</i>	17	NC	Min <i>et al.</i> (1999).

❖ NC; no change.

Studies conducted in New Zealand with *Lotus sp.* (Table 1.4) suggest that levels of CT less than 40 g/kg DM in *L. corniculatus* do not depress VFI of sheep. In contrast, higher levels of CT in *L. pedunculatus* (60-100 g/kg DM) reduce VFI. This detrimental effect of CT on VFI is closely associated with the high intake of free CT, which is related to the total CT content (Barry and Forss, 1983).

1.2.4 Condensed tannins and rumen digestion of protein

The concentration and type of CT in the diet both influence the positive effects of CT on rumen protein digestion (McLeod, 1974; Reid *et al.*, 1974).

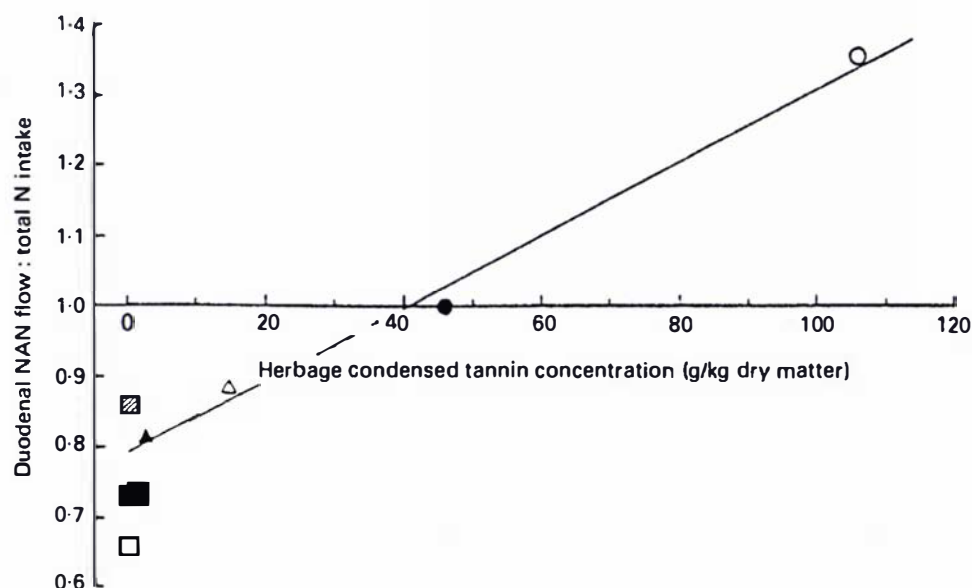


Figure 1.2. Duodenal non-ammonia nitrogen (NAN) flow per unit total nitrogen intake as a function of herbage condensed tannin concentration in sheep fed on *Lotus* species. (○) High- and (●) low-tannin *Lotus pedunculatus*. (△), high- and (▲) low-tannin *Lotus corniculatus*. Results are compared with the non-tannin containing herbage; (▨), short rotation ryegrass, (□) perennial ryegrass, and (■) white clover. All results are for an N intake of 28 g/d and refer to fresh forages. Adapted from Barry and McNabb (1999).

In fresh *Lotus* species, duodenal non-ammonia N (NAN) flow per unit total N intake increases linearly with increasing dietary reactive CT in the range 0 -110 g/kg DM (Fig. 1.2) (Barry and McNabb, 1999). Duodenal NAN flow is equivalent to total N intake at a CT concentration of approximately 40 g/kg DM (Barry and McNabb, 1999).

Consequently, CT reduce both the loss of N as ammonia (NH₃) absorbed from the rumen, estimated as 20-35% of N intake, which occurs in ruminant feeding systems with fresh forages (20-25% CP) (Waghorn and Barry, 1987) and an extra energy cost of 12 kcal/g of excess NH₃ detoxified in the liver (Van Soest, 1994).

1.2.5 Condensed tannins and amino acid absorption from the small intestine

Feeding experiments with sheep (Waghorn *et al.*, 1987, 1994) have shown that CT in *L. corniculatus* and *L. pedunculatus* exert differing effects upon the digestion and absorption of amino acids (Table 1.5).

Table 1.5. The effect of condensed tannins in sheep fed fresh *Lotus corniculatus* (*L. c*; 22 g/kg DM) and *Lotus pedunculatus* (*L. p*; 55 g/kg DM) upon the digestion of amino acids. Effects of CT were assessed through intraruminal infusion of polyethylene glycol (PEG, MW 3500) into half the animals fed each forage.

	Essential*				Non-essential*			
	<i>L. c</i>		<i>L. p</i>		<i>L. c</i>		<i>L. p</i>	
	CT-acting	PEG	CT-acting	PEG	CT-acting	PEG	CT-acting	PEG
Amino acids intake (g/d)	98.9	98.9	91.6	103.5	97.9	97.9	87.6	98.9
Abomasal flow (g/d)	84.6	55.5	106.8	93.7	68.5	60.0	84.3	77.7
Proportion of intake	0.86	0.56	1.17	0.91	0.70	0.61	0.96	0.79
Apparent loss in the rumen (g/d)	14.3	43.4	-15.2	9.8	29.4	37.9	3.3	21.2
Proportion of intake loss in the rumen	0.14	0.44	-0.17	0.09	0.30	0.39	0.04	0.21
Apparent absorption from small intestine	58.8	36.2	71.0	73.5	37.4	41.3	50.8	57.2
Proportion abomasal flow	0.70	0.65	0.66	0.78	0.55	0.69	0.60	0.74
Proportion of intake	0.59	0.37	0.78	0.71	0.38	0.42	0.58	0.58

* Essential: Histidine, Isoleucine, Leucine, Lysine, Phenylalanine, Threonine, Tyrosine (phenylalanine as a only source), Valine. Arginine values are excluded from this comparison.

• Non-essential: Alanine, Asparagine, Glutamate, Glycine, Proline, Serine.

Adapted from Barry and Blaney (1987), Waghorn *et al.* (1987) and Waghorn *et al.* (1994).

Condensed tannins in *L. corniculatus* increased both the flux of EAA (52%) and non-essential amino acids (NEAA) (14%) through the abomasum. A similar trend was observed in *Lotus pedunculatus*, but the magnitude of the response was lower than in BFT, 14% for essential amino acids (EAA) and 8% for NEAA. Rumen fermentation in CT-acting sheep fed BFT resulted in a smaller loss of EAA (14% of intake) than in NEEA (30% of intake). However, when PEG was given, a net loss of both EAA and NEAA (44% and 39% of intake respectively) was shown from the rumen.

In contrast, in CT-acting sheep fed *L. pedunculatus* there was a net gain of EAA (17% of intake) and a small loss of NEAA (4% of intake) across the rumen, but when PEG was given there was a lower net loss of both EAA and NEAA than in BFT. Additionally, the apparent absorption (proportional abomasal flow) in BFT of EAA was not different for the CT acting and PEG sheep, but it was affected for NEAA. However, CT depressed the apparent absorption of both EAA and NEAA in sheep fed *L. pedunculatus*.

The effects of tannin treatment on increasing the apparent absorption (proportion of intake) of EAA in the small intestine was higher for sheep fed BFT (59%) than in *L. pedunculatus* (10%). In contrast, CT reduced the apparent absorption of NEAA by 10% of intake for sheep fed BFT, but was without effect for sheep fed *L. pedunculatus*.

1.2.6 Condensed tannins and fibre digestion

The effects of CT upon fibre digestion have been examined with sheep fed *Lotus* species by comparisons of duodenal flows and ruminal and post-ruminal digestion. Apparent digestibility and rumen digestion of plant fibre (cellulose, hemicellulose and lignin) are inversely related to the CT content (Table 1.6) (Barry and Manley, 1984; Barry *et al.*, 1986a).

Table 1.6. Digestion of structural carbohydrates (cellulose, hemicellulose) and readily fermentable carbohydrates (soluble CHO + pectin) in sheep fed *Lotus pedunculatus* differing in total condensed tannin content and free condensed tannins due to applications of high (1), low (2) and zero (3) rates of polyethylene glycol (PEG; MW 3350). Together with effect of high (4) and low (5) soil fertility levels on condensed tannin concentration. Apparent digestibility, rumen digestion and post-ruminal digestion are expressed as proportions of feed intake.

	Barry <i>et al.</i> (1986b)			Barry and Manley (1984)	
	14	45	95	46	106
Total CT (g/kg DM)	14	45	95	46	106
Free CT (g/kg DM)	2⁽¹⁾	5⁽²⁾	15⁽³⁾	3⁽⁴⁾	14⁽⁵⁾
Structural carbohydrates					
Cellulose					
Apparent digestibility	0.74	0.75	0.75	0.78	0.63
Rumen digestion	0.73	0.72	0.69	0.69	0.53
Post-ruminal digestion	0.01	0.03	0.06	0.09	0.10
Hemicellulose					
Apparent digestibility	0.72	0.66	0.72	0.73	0.56
Rumen digestion	0.61	0.52	0.49	0.44	0.21
Post-ruminal digestion	0.11	0.14	0.23	0.28	0.35
Readily fermentable carbohydrates					
Apparent digestibility	0.98	0.98	0.97	0.95	0.93
Rumen digestion	0.83	0.77	0.89	0.80	0.78
Post-ruminal digestion	0.15	0.21	0.08	0.15	0.16

Values of CT up to 22 g/kg DM in BFT appear to have no effect upon rumen fibre digestion (Barry, 1989). In contrast, CT concentration in the range 46 to 106 g/kg DM in *L. pedunculatus* markedly depressed rumen hemicellulose digestion and slightly depressed rumen cellulose digestion. Effects on apparent digestibility of these were lower, due to increases in post-ruminal digestion in sheep fed the higher CT *L. pedunculatus* (Barry *et al.*, 1986b).

Benoit and Starkey (1968) and Barry and Duncan (1984) stated that the rumen decomposition of compounds of large molecular weight, such as cellulose and hemicellulose can be reduced due to the action of free (i.e. unbound) tannin, which inactivates both microbial and digestive enzymes in the initial hydrolysis of the carbohydrates.

Rumen digestion of readily fermentable carbohydrates (RFC), defined as water-soluble carbohydrates (WSC) and pectin, is only very slightly depressed by CT up to a concentration of 106 g/kg DM, even though RFC are almost completely digested in the whole digestive tract (Barry and Manley, 1984; Barry *et al.*, 1986b).

1.2.7 Condensed tannins and hormonal response

Endocrine concentrations in sheep fed *L. pedunculatus* have shown that diets with the lowest value of CT have the highest plasma concentration of both 3,5,3' -triiodothyronine (T_3) and free T_3 (Barry *et al.*, 1986a) (Table 1.7).

Table 1.7. Condensed tannin effect on plasma hormone concentration in sheep fed *Lotus pedunculatus* differing in total condensed tannin content (TCT) due to applications of high (1), low (2) and zero (3) rates of polyethylene glycol (PEG; MW 3350).

	TCT*	TCT*	TCT*
	14 g/kg DM	45 g/kg/ DM	95 g/kg/ DM
Growth hormone (GH) ($\mu\text{g/l}$)	2.3	2.8	4.8
Total 3,5,3' -tri-iodothyronine (T_3) (nmol/ l)	1.57	1.29	1.16
Free T_3 (relative units)	1.52	1.43	1.12
Insulin-like growth factor (IGFI) ($\mu\text{g/l}$)	45.5	38.7	47.5
Insulin-like growth factor (IGFII) ($\mu\text{g/l}$)	1056.3	1286.7	987.0
Insulin (mU/ l)	22.4	22.4	20.0
Glucagon (ng/ l)	239.1	94.5	152.4

*Diets contained 2, 5 and 15 g/kg DM of free CT.

Adapted from Barry *et al.* (1986a).

In contrast, Barry *et al.* (1986a) found that a positive and linearly relationship existed between plasma growth hormone concentration ($\mu\text{g/l}$) and CT concentration (g/kg DM) in sheep fed *L. pedunculatus*, but there was no other effect on plasma concentration of the other hormones measured. Effects of daily ovine GH hormone may be related to a reaction of free CT in inactivating gut-wall proteins by H bonding Barry *et al.* (1986a). Previous research with sheep (Davis *et al.*, 1970a, 1970b and Muir *et al.*, 1983) has shown daily GH hormone injections to be related to increased levels of plasma glucose, insulin and N gain.

According to Muir *et al.* (1983) GH has an immediate, short-term insulin-like effect on adipose tissue producing adipose tissue refractoriness to the insulin-like growth factor, which is followed by an extended, lipolytic response when GH is chronically increased.

1.3 LOTUS CORNICULATUS AND FEEDING VALUE

Feeding value (FV) is defined as the animal production response to the herbage consumed by the ruminant under unrestricted grazing conditions (Ulyatt, 1973). FV is typically measured as liveweight gain per day in growing animals and as the milk yield per day in lactating animals. The components of FV are voluntary feed intake (VFI) and nutritive value/unit DM eaten; the latter can further be subdivided into the digestive process and the utilisation of absorbed nutrients. In a comparison of non-CT-containing herbages, Ulyatt (1973) concluded that the FV of legumes was greater than that of grasses and that approximately half of the differences in FV between herbages were due to differences in VFI.

1.3.1 Wool growth and body growth

Wool growth is dependent upon the absorption of EAA from the small intestine and the availability of sulphur-containing amino acids (SAA) (Reis, 1979). However, marginal protein deficiency exists when ruminants are fed on fresh temperate forages due to high degradation rates of forage proteins in the rumen. Consequently, there is a large absorption of NH_3 from the rumen, leaving absorption of SAA from the small intestine that is below animal requirements for these amino acids (AA) (Barry, 1982).

CT in *L. pedunculatus* (McNabb *et al.*, 1993) and in *L. corniculatus* (Wang *et al.*, 1994) reduced the degradation of SAA to inorganic sulphide in the rumen, increased the rate of transulphuration of methionine to cystine in the body as well as cystine flux to body synthetic reactions.

Table 1.8. Wool production and liveweight gain (LWG) of sheep grazing *Lotus sp.*, lucerne (*Medicago sativa*), sulla (*Hedysarum coronarium*) and pasture (*Lolium perenne/Trifolium repens*) with or without polyethylene glycol (PEG) supplementation.

Reference	Plant fed									
	<i>Lotus corniculatus</i>		<i>Lotus pedunculatus</i>		Lucerne		Sulla		Pasture	
	CT-acting	PEG	CT-acting	PEG	CT-acting	PEG	CT-acting	PEG	CT-acting	PEG
Wool growth										
Terrill <i>et al.</i> 1992							117 ¹	105	115	97
Terrill <i>et al.</i> 1992							80 ¹	83	74	77
Robertson <i>et al.</i> 1995	0.71 ¹	ND	0.57	ND	0.71	ND	0.80	ND	0.69	ND
Douglas <i>et al.</i> 1995	133 ¹	ND			123	ND				
Douglas <i>et al.</i> 1999	146 ¹	ND					135	ND		
Barry, 1985			8.5 ²	9.5						
Barry, 1985			7.8 ²	8.9					7.1	8.7
Wang <i>et al.</i> 1996b	12.1 ²	10.9			10.8	10.2				
Clean fleece weight (kg)										
Douglas <i>et al.</i> , 1995	2.78	ND			2.25	ND				
Min <i>et al.</i> 1998	2.53	2.28								
Douglas <i>et al.</i> 1999	1.63	1.62					1.45	1.53		
Min <i>et al.</i> 1999	1.35	1.31							1.09	1.14
Luque <i>et al.</i> 2000	1.69	1.73							1.54	ND
Min <i>et al.</i> 2001	1.71	1.61							1.41	ND
LWG (g/day)										
Barry, 1985			125 ³	166						
Terrill <i>et al.</i> 1992							233 ⁴	278	175	136
Terrill <i>et al.</i> 1992							194 ⁴	222	169	209
Wang <i>et al.</i> 1996b	203 ⁴	188			185	178				
Douglas <i>et al.</i> 1995	275 ⁴	ND			263	ND				
Douglas <i>et al.</i> 1995	228 ⁴	ND			183	ND				
Douglas <i>et al.</i> 1999	215 ⁴	207					227	236		
Robertson <i>et al.</i> 1995	208 ⁴	ND	232	ND	243	ND	226	ND	166	ND

- ❖ 1 = mg/100 cm² per day; 2 = g/day; 3 = drenched ewes; 4 = drenched lambs.
- ❖ ND; not determined.

A review of many year's data (Table 1.8) showed that wool growth rate and clean fleece weight of sheep grazing pure stands of BFT was equal to or higher than on

other temperate legumes or perennial/white clover pasture. PEG supplementation indicated that CT in BFT increased wool production by up to 11%. This was achieved with no increase in VFI and could be explained by a possible increase in EAA absorption (especially SAA) caused by the action of CT.

Additionally, the data in Table 1.8 shows that, relative to perennial ryegrass, *L. pedunculatus*, lucerne and sulla, liveweight gain of sheep was higher when grazing BFT, with the differences averaging + 35%, 28%, 5% and 4% respectively. However, there was no evidence to support CT making a positive contribution to this effect, as judged by response to PEG supplementation.

1.3.2 Milk yield and composition

The yield and efficiency of milk and milk protein production in cows fed on fresh forage diets of high protein content and digestibility are limited by the amount of AA absorbed from the small intestine relative to energy (Rogers *et al.*, 1980). Penning *et al.* (1988) found that when ewes were fed on fresh ryegrass in weeks two to seven of lactation, daily milk yield, milk protein concentration and lamb growth rates were increased as a result of feeding supplementary protein of low rumen degradability, with no effect on live weight or body condition of the supplemented ewes. This confirmed that availability of EAA also limited milk production in lactating ewes fed high quality fresh forage.

A study conducted by Wang *et al.* (1996a) showed that CT in BFT reduced the potential rumen degradability of the protein of lactating ewes rearing two lambs and increased milk yield and the secretion rates of protein and lactose without

affecting VFI. In this experiment, control ewes (CT-acting) and PEG-supplemented (CT-not acting) ewes had no differences in milk yield at peak lactation. However, as lactation progressed the PEG group produced 21% less milk than CT-acting ewes (Fig. 1.3).

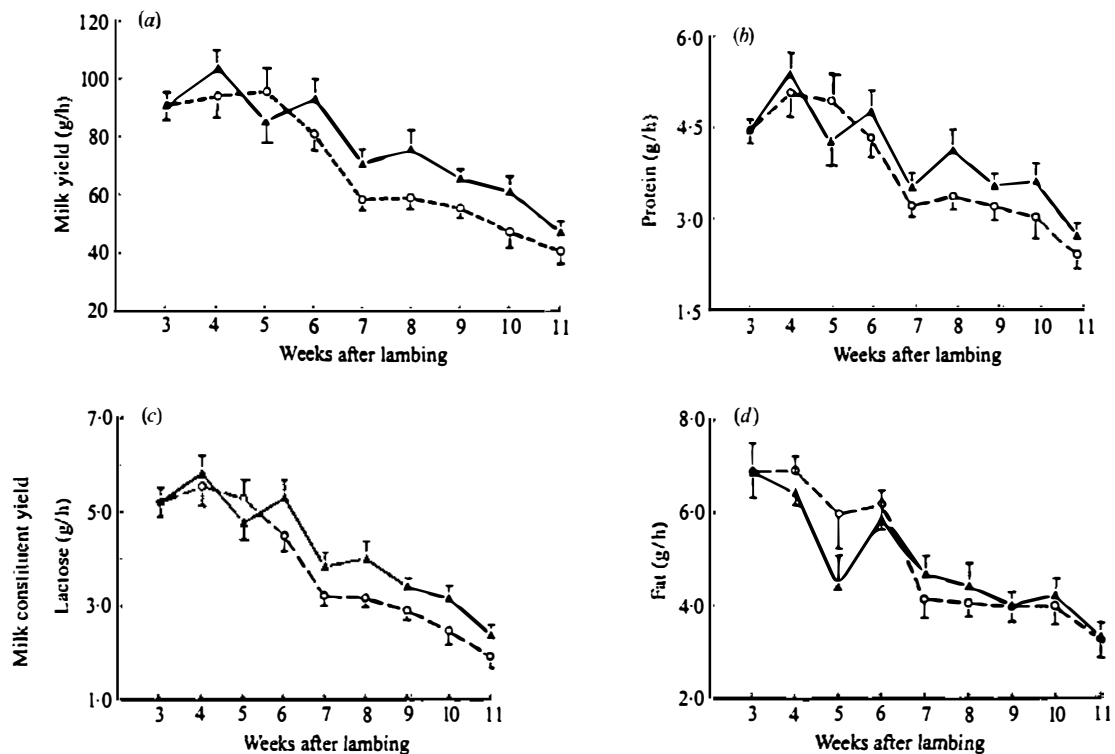


Figure 1.3. Milk production (g/h) (a) and yields (g/h) of (b) protein, (c) lactose, and (d) fat in the milk of twin lactating ewes grazing *Lotus corniculatus*. Control ewes (▲); ewes given twice-daily oral supplementation of polyethylene glycol (PEG; MW 3500) (○). Means are for 14 ewes per treatment. Vertical bars represent S.E.S. (Wang *et al.*, 1996a).

Milk protein, lactose and fat yields (g/h) decreased through the lactation period. However, CT-acting ewes produced more milk protein (14%) and lactose (12%), and had a lower fat concentration in their milk than the comparable PEG supplemented ewes from mid to late lactation. However, there were no differences

in total fat secretion (g/h) between CT-acting and PEG groups in the whole grazing trial.

1.3.3 Reproductive performance

Reproductive performance is dependant on the food availability and quality of the food ingested (Smith, 1991). Ovulation rate (OR) can be increased when grazing sheep increase intakes of both energy and protein (Smith, 1985). Experimental dietary protein manipulation conducted by Cruickshank *et al.* (1988) showed that multiple ovulations were increased by 18% when ewes were supplemented with abomasal infusions of soy protein isolate and lactalbumin from days 8 to 17 of 4 successive oestrous cycles.

Table 1.9. Effect of grazing ewes on *Lotus corniculatus* L. or perennial ryegrass/white clover pasture (*Lolium perenne*/*Trifolium repens*), with or without supplementation with polyethylene glycol (PEG; MW 3500), on ovulation rate (corpora lutea/ewe mated), lambing (lambs born/ewe mated) and liveweight gain (LWG). Mean liveweight (LW) at the start of Experiments 1, 2 and 3 were respectively 54.2, 59.8 and 53.2 kg.

	Lotus corniculatus		Pasture		Reference
	CT-acting	PEG	CT-acting	PEG	
Ovulation rate at third cycle					
Experiment 1	1.78	1.56	1.33	1.35	Min <i>et al.</i> (1999)
Experiment 2	1.77	1.87	1.65	ND	Luque <i>et al.</i> (2000)
Experiment 3	1.79	1.58	1.48	ND	Min <i>et al.</i> (2001)
Lambing					
Experiment 1	1.70	1.42	1.36	1.36	
Experiment 3	1.69	1.39	1.22	ND	
LWG (g/day) during mating					
Experiment 1	40.3	33.8	18.6	4.5	
Experiment 2	-25.0	-20.0	-12.0	ND	
Experiment 3	22.3	16.3	43.2	ND	

ND: not determined.

Recent experiments (Table 1.9) compared the reproductive efficiency of ewes mated whilst grazing BFT or perennial ryegrass/white clover. Results from these studies have shown that relative to pasture, in Experiments 1 and 3 grazing BFT (18 g CT/kg DM) increased both OR and lambing by 27% and 20% respectively without affecting VFI, with approx 50% of the response being due to action of CT. However, the increase in OR (10%) during the second experiment could not be explained by the action of CT in BFT (24 g CT/kg DM), as deduced from responses to PEG supplementation. The greatest responses in OR and lambing percentage from grazing BFT during mating, with the greatest contribution from CT, were in lighter ewes that gained live weight during mating (Experiments 1 and 3) and lowest responses were obtained in heavier ewes that lost small amounts of live weight during mating (Experiment 2).

Collectively, the data show that grazing on BFT increased wool growth, milk secretion and ovulation rate, with a component due to action of CT that does not involve any change in VFI. It would therefore seem that the CT in BFT may have increased FV through increasing nutritive value/DM eaten without affecting VFI, due to improving the efficiency of both the digestive process (for protein) and the efficiency with which absorbed AA are utilised, or other mechanisms not yet understood.

1.4 INTERNAL PARASITES AND DAG FORMATION IN SHEEP

1.4.1 Gastro-intestinal nematodes

After more than 180 years since the introduction of sheep in New Zealand from Australia and England (Vlassof and Mckenna, 1994), clinical diseases and stock

losses associated with detrimental effects of gastrointestinal nematode parasites (roundworms), such as *Trichostrongylus axei*, *Haemonchus contortus*, *Ostertagia spp.*, in the abomasum and *Cooperia spp.*, *Nematodirus spp.*, *Trichostrongylus spp.*, in the small intestine, continue to be a limiting factor for sheep pastoral systems (Vlassof and Mckenna, 1994; Vlassof, *et al.*, 2001).

Nematode life cycles are closely synchronized with the breeding cycle of their hosts (Vlassoff, 1982) and depend on the combination of their ecological requirements for survival and development outside the host (Familton and McAnulty, 1995), such as the prevailing conditions of moisture and temperature gradients, and the physical conditions of the pasture (Georgi, 1985). However, each component has many features that influence the type, epidemiology and severity of the infection (Brunsdon, 1982).

The typical life cycle of nematodes infecting sheep takes six weeks or more and comprises the egg and four larval stages (Charleston, 1982; Vlassoff, 1982). Given adequate conditions, morulate eggs pass out in the faeces, the L₁ stage larva develops in the egg, emerges, grows on pasture and moults to the L₂ stage and to the non-feeding infective L₃ stage, which is both more resistant to adverse conditions (Vlassof, *et al.*, 2001) and eaten by the suitable host (Charleston, 1982; Georgi, 1985). In contrast, the development of the L₃ stage in *Nematodirus* species occurs in the egg (Charleston, 1982).

The pathological processes, which comprise losses of plasma and epithelial cells with increased mucus production (Sykes and Coop, 2001) take place in the

abomasum, in the gastric glands of the fundic region and in the mucosal crypts of the small intestine, when two further moults are completed (Sykes and Poppi, 1982). The females once mated lay eggs and then the life cycle is completed (Charleston, 1982).

1.4.2 Larval and infection dynamics

The seasonal pattern of larval availability on pasture increases exponentially and is the result of the interaction between faecal egg output of lambs, weather and further contamination of the pasture (Brunsdon, 1981). A diagrammatic representation of this seasonality over a 10-year period is illustrated in Figure 1.4.

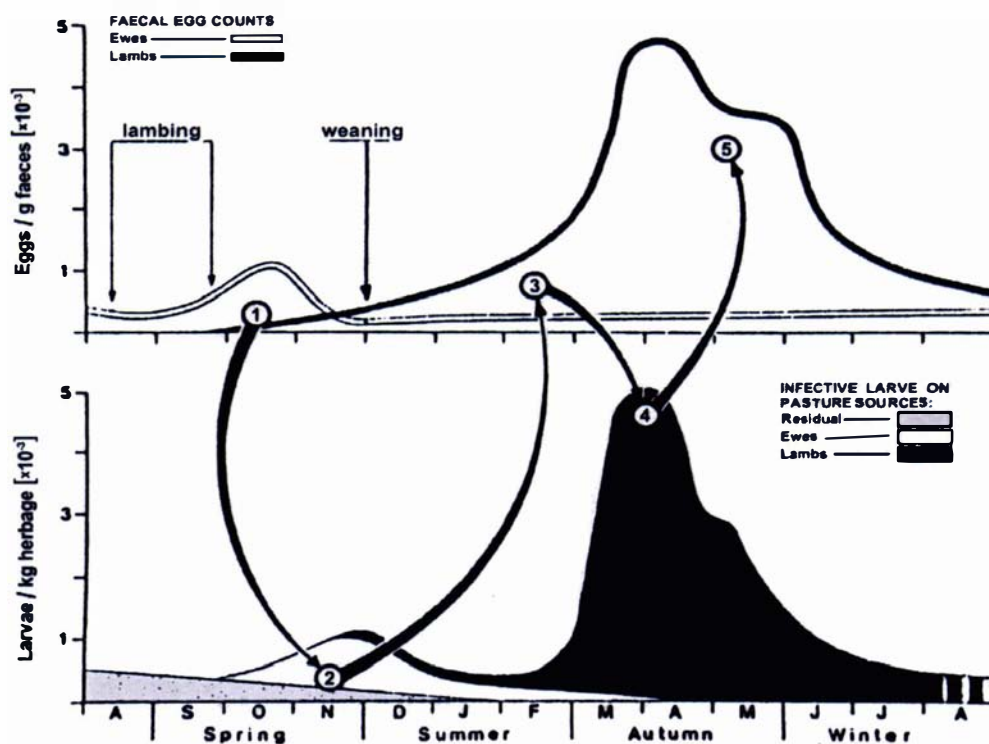


Figure 1.4. Representation of seasonal contamination by nematode egg output of un-drenched ewes and lambs, and the pattern of larval availability on the pasture (Brunsdon, 1981).

In spring, as a consequence of the stress imposed by sub-optimal feed intake over the winter, pregnancy and lambing status, the immune ability is disrupted resulting in a large increase in the output of nematode eggs in the faeces of the breeding ewe (post-partum rise, PPR) (Vlassof and McKenna, 1994; Vlassof, *et al.*, 2001). This spring rise in faecal egg count (FEC) ensures that infective larvae stages will be available in large numbers on the pasture to develop infection in a grazing flock (Georgi, 1985).

Such pastures become re-contaminated in spring when infective larvae develop to the first generation of roundworms in the host, which accumulate in growing animals in summer (Vlassoff, 1982). Nematode reproductive activity in lambs during late summer and early autumn is followed by a peak of infective larvae on pasture during autumn, which produces the second generation of nematode infection in lambs and more pathogenic effects in growing lambs than in mature animals during autumn and winter (Georgi, 1985). Nematodes over-winter both as infective-stage larvae on pasture and inhibited larvae in the lactating ewe are the source of infection for ewes and lambs in the following spring (Vlassoff, 1982).

Consequently, the pattern of L₃ stage on sheep pastures in New Zealand reaches the highest level in autumn and is related with the pattern of the succession of species in the host (Vlassoff, 1982; Vlassoff *et al.* 2001) but does not necessarily match changes in both (Beckett, 1993). However, danger periods are present during the whole year when times of nutritional stress suppress immunity in any class of stock (Ross, 1982).

Field studies reviewed by Vlassoff (1982) and Vlassof and McKenna (1994) showed that, even though larvae of the genera *Cooperia*, *Ostertagia* and *Trichostrongylus* are common for all areas, species of *Haemonchus* are more prevalent in the North than in the South Island, due to west-east moisture and north-south temperature gradients (Familton and McAnulty, 1995). Conversely, *Nematodirus* genera distribution is more prevalent in the South Island (Vlassoff 1982; Vlassof and McKenna, 1994).

Studies conducted by Beckett (1993) found that over a period of 15 months differential pasture larval counts on the East Coast of the North Island showed a specific seasonal peak distribution in which *Trichostrongylus* in July, *Nematodirus* in February, *Ostertagia* in March and *Haemonchus* genera in April-May were predominant. Additionally, from these studies, the mean percentage composition of larval species in pooled faecal cultures noted that the proportion of *Ostertagia* spp. declined in the autumn and *Trichostrongylus* spp. rose from summer to July. *Chavertia*, *Cooperia* and *Oesophagostomum* spp. were present in low proportions and tended to rise over the winter months. *Haemonchus* spp. larvae peaked in May.

Further, Vlassof and McKenna (1994) found that worm numbers in young sheep are dominated in late spring by *Nematodirus* spp. whilst *Strongyloides*, *Ostertagia* spp., *H. contortus* and small intestine *Trichostrongylus* spp. are prevalent during late summer/autumn, and *Cooperia* spp. and *T. axei* are abundant in autumn and winter.

1.4.3 Methods for measuring parasites

The use of parasitology tests, such as faecal egg count, larval identification from larval cultures and gastrointestinal worm counting, for the detection of nematode parasites of veterinary importance in naturally infected sheep have been used widely (Ministry of Agriculture, Fisheries and Food, 1986).

Eggs counted by floating them on a variety of solutions can be very useful in circumstances where animals are not available for *post mortem* examination, under the assumption that there is a clear relationship between total worm burdens and egg per gram of wet faeces (epg) (McKenna, 1981). Infective larvae cultured from faeces are identified by morphological and morphometric keys (Ministry of Agriculture, Fisheries and Food, 1986). *Post mortem* worm counts are expensive and, even though it provides an accurate assessment of the genera and number of worms present in each animal, several counts would be required to assess the parasite status on a sheep flock (McKenna, 1987).

Studies conducted by Larsen *et al.* (1994) showed that FEC of nematodes in ewes provide a measure of *post mortem* worm counts. In contrast, results from McKenna (1981) showed that, although for sheep beyond 12 months of age the correlation of strongyle eggs counts (*Nematodirus* excluded) and worm counts was low ($r = 0.23$), there was a reasonable correlation ($r = 0.74$) for a similar association when 190 separate lambs were studied. However, when egg counts from ewes and lambs were correlated with a total pathogenic index (T.P.I.) the association was almost good ($r = 0.69$) for both groups of animals. Consequently, a potential

diagnosis between sheep's FEC and its reliability in providing a strongyle worm count profile in young sheep flock could be assumed (McKenna, 1987) when at least 10 to 15 egg counts on randomly selected samples are taken (McKenna and Simpson, 1987).

However, these data are consistent with the view that there are inherent biological problems with FEC as an individual indicator for the quantitative assessment of the intensity of the infection (Anderson and Schad, 1985; Keymer and Hiorns, 1986; Ministry of Agriculture, Fisheries and Food, 1986; Cabaret *et al.*, 1998).

More recently, Bishop and Stear (2000) point out that both techniques are frequently only estimates of the true values because for parasites infecting sheep such as *Teladorsagia circumcincta* in the UK there is a convex relationship between worm burden and egg production, which suggests that between the number of nematodes within a host and their mean fecundity exists a strong density-dependant relationship in regulating the size of parasite populations.

Additionally, Keymer and Hiorns (1986) stated that through *post mortem* examinations, no information on the dynamics of mating can be obtained. Consequently, in nematodes for which copulation precludes simultaneous egg release this must exert a critical effect on the dynamics of egg production. Further, Keymer and Hiorns (1986) related that *post mortem* counts do not provide precise estimations of worm burdens because losses of parasites in sieving and in collection from the washed residues can occur, but also some worms that were exposed to chemotherapeutic agents may die and degenerate *in situ*.

1.4.4 Effect on nutrient metabolism

The effect of nematode parasites on animal metabolism has been widely recognised (Morris, 1998; Coop and Kyriazakis, 1999; Sykes and Coop, 2001). These include metabolic disturbances such as impaired acid secretion in the abomasum as well as impaired protein and energy metabolism reducing the feed conversion efficiency (Steel and Symons, 1979; Sykes and Poppi, 1982; Bown *et al.*, 1991).

The result is to produce a syndrome analogous to under-nutrition characterised by reduced immunological competence (Steel and Symons, 1979), haematopoiesis (Sykes and Poppi, 1982), reduction in VFI by up to 20% (Sykes and Coop, 1976, 1977; Steel *et al.*, 1980), reduced retention and metabolic flows of calcium and phosphorus (Bown *et al.*, 1991) with reduced skeletal growth and liveweight gain reductions of up to 50% (Sykes and Coop, 1976). Additionally, wool growth is impaired up to 26% (Steel *et al.*, 1980), and alterations of the structural characteristics of the wool (Steel and Symons, 1979) are present as well as increased dags (faecal material accumulating around the anus of sheep) and flystrike (Waghorn *et al.*, 1999)

In interpreting the metabolic disturbances Coop and Kyriazakis (1999) suggested that the interactions between the host and nutrition, and pathophysiology induced by parasitism must be considered in terms of resilience and resistance. Alberts *et al.* (1987) stated that in the face of a parasitic challenge a reasonable and sustained level of productivity can be considered as resilience. In contrast,

resistance is a measure of the host's ability to reduce persistence of a parasite population due to restrictions on its establishment, its maturity and or its fecundity (Coop and Kyriazakis, 1999).

1.4.5 Production losses

Gastro-intestinal nematodes in sheep pastoral grazing systems in New Zealand are responsible for a considerable economical loss due to the effects of subclinical infections (Howse *et al.*, 1992). Brunsdon (1988) stated that from an estimated sheep industry production value of \$ 950 million at that time, the economic impact of nematode parasitism could be estimated in losses of 16, 10, and 3% in wool, reproductive failure and meat production respectively. Additionally, costs of manual dag removal and chemotherapeutic compounds to drench growing animals were estimated as 4% of the New Zealand sheep pastoral value.

Further, Vlassof and McKenna (1994) stated that \$29.3M are spent approximately for nematode worm burdens control by farmers to avoid production penalties in their flocks and support sheep product export value of \$ 2193M, Free On Board (FOB).

1.4.6 Anthelmintic control

The aim of all nematode control programmes is to achieve economic production compatible with parasite populations (Vlassof *et al.*, 2001) by both reducing reliance on chemotherapy and the uptake of L₃ stages from pasture (Vlassoff and Brunsdon, 1981; Hein, *et al.*, 2001; Leathwick *et al.*, 1995).

Over the past decades parasite control for adult sheep and growing animals in New Zealand has been achieved under preventive, protective and curative drenching programmes (Ross, 1982). According to Vlassof and McKenna (1994) and Watson (1994) the use of broad-spectrum anthelmintics started in the 1960's with the introduction of benzimidazole and levamisole/morantel drench groups followed by avermectins in the 1980s and the milbamycin family in the 1990's.

More recently, in order to maintain sheep production at present levels, new chemotherapy options to control nematode parasites with long-acting activity have been released onto the market. These are controlled release capsules (CRCs), containing albendazole or ivermectin in the shape of intraruminal devices, releasing drug at constant rate for more than 3 months. Other drenches, such as moxidectin injection (second generation milbamycin) (Kempthorne *et al.*, 1996) and closantel assert high anthelmintic effects at the first time but decrease in logarithmic response the time (Leathwick *et al.*, 2001).

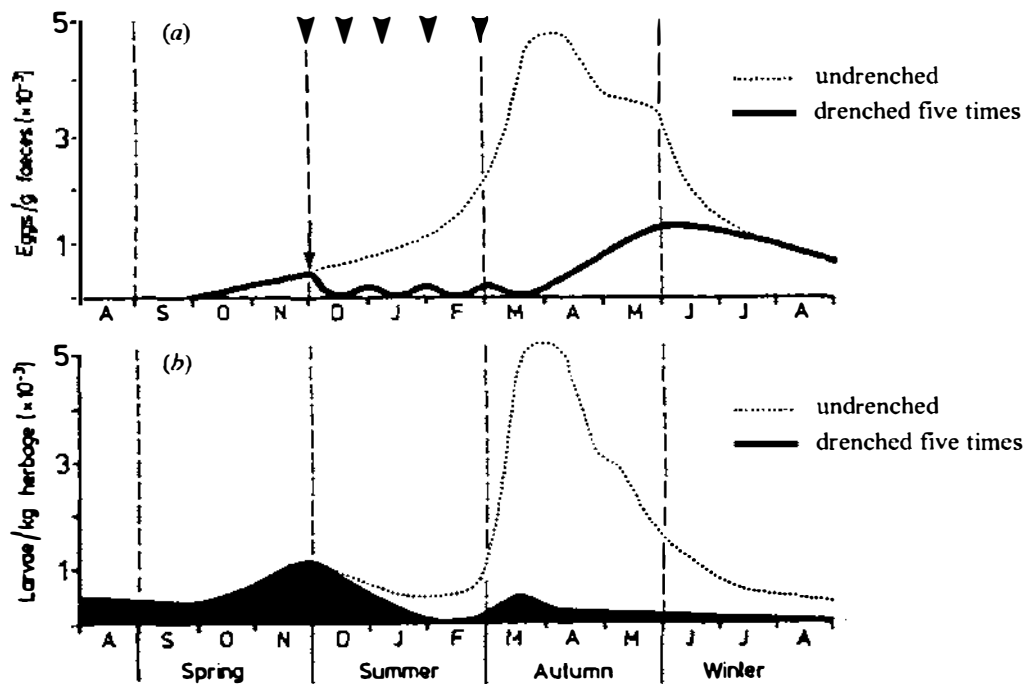


Figure 1.5. Faecal egg output (a) and pasture contamination with L_3 larvae (b) of undrenched ewes and lambs (...) and five times anthelmintic drenched lambs (–) grazing perennial ryegrass/white clover pasture during twelve months of the year. (Adapted from Brunsdon, 1981). The data refers to whole farm ewe and lamb systems, with the lambs either drenched or undrenched.

Anderson (1990) stated that the epidemiological knowledge of the time relationships between contamination of pastures and seasonal availability of infective larvae in different climatic regions has shown that preventive control is the best way to control gastro-intestinal parasitism. The information is consistent with the responses described by Brunsdon (1981) and Leathwick *et al.* (1995) who concluded that ideally using a basic five-drench programme for lambs and hoggets at 21 to 28-day intervals commencing at weaning in November resulted in far

fewer worms contaminating pasture with eggs in summer and virtually eliminated the autumn larval peak (Fig. 1.5).

Furthermore, a simulation model proposed by Leathwick *et al.* (1995) for mixed infections of gastrointestinal nematodes in lambs suggested that the addition of a post-lambing ewe drench was more effective than an extended drenching programme for lambs during the autumn to obtain a reduction in pasture contamination. In contrast Beckett (1993) stated that the five preventive drench programme starting at weaning ceases too early for hoggets to prevent the build up of *Haemonchus* and *Trichostrongylus* genera during late autumn and winter respectively on the East Coast of the North Island in New Zealand.

Consequently, there is evidence that more intensive farmer drenching practices looking for further increases in productivity are generally possible (Familton *et al.*, 1995; Sumner *et al.*, 1995; Vlassof *et al.*, 2001). Nevertheless, increased incidence in New Zealand of genetic resistance in nematodes to a level where treatment failure occurs in sheep farms has been reported (Watson, 1994; Macchi *et al.*, 1999; Leathwick *et al.*, 2000; Mason *et al.*, 2001; Leathwick *et al.*, 2001; Vickers *et al.*, 2001).

Therefore, the cost of low levels of the non-feeding infective L₃ on pasture generally results in high selection pressure for resistance (Sangster, 1999; Leathwick *et al.*, 2001). However, selection for resistance is not necessarily proportional to the frequency of treatment because not all drenches are equivalent in their ability to select for resistance (Leathwick *et al.*, 2001). Furthermore, it

seems unlikely from New Zealand results that selection for resistance to infection alone would result in lambs exposed to nematode challenge (i.e. resistance to the effects of infection) in increased production (Bisset *et al.*, 1994).

Thus, the development of sustainable strategies to current nematode parasite control practices is imperative for sheep grazing systems (Waller, 1992). There is also the need to meet the increasing market-pressure for chemical-free farming practices to allow access by New Zealand products to overseas markets without the effect of non-tariff trade barriers (Buddle, 2001).

Several alternative technological solutions to nematode control are reported by Waller (1992, 1998), Williams (1997), Sangster (1999), Hein *et al.* (2001), Sykes and Coop (2001) and Vlassoff *et al.* (2001), due to the emergence of anthelmintic resistance and the long-term economic effect for livestock productivity. These include integration of chemotherapy with grazing management, such as pasture spelling and renovation, integrated strategies involving alternate grazing with different stock classes, immunomodulants, vaccines, targeted silencing of genes regulating nematode development, biological control of nematode larvae, biological anthelmintics and the use of tannin-containing plants.

1.4.7 Condensed tannins and gastrointestinal parasites

Anthelmintic medication has its origin in the use of extracts and plant products. In animal health, the prospect for the use of plants with anthelmintic properties for controlling internal parasites is of great interest because of the continued growth of

organically produced livestock commodities to meet consumer preferences and animal rights issues (Waller, 1998).

Experimental evidence (Niezen *et al.*, 1995, 1998) suggests that CT has the potential value to control parasite infections of grazing ruminants. The data in Table 1.10 indicates that drenched lambs grazing CT-containing legumes (*Sulla* and *Lotus sp.*) grew at similar rate to lambs grazing non-CT-containing lucerne but faster than lambs grazing perennial ryegrass/white clover pasture. In contrast, daily gains were higher for un-drenched lambs grazing CT-containing legumes than when grazing either lucerne or pasture.

The data in Table 1.10 also shows that, although average faecal egg count values did not differ between lambs grazing CT-containing lotus species and non-CT-containing forages, lambs grazing *sulla* had the lowest faecal egg concentrations. Parasite burdens at slaughter were similar for lambs grazing *Lotus sp.* and pasture but were consistently lower for growing animals grazing *sulla*. The preceding information suggests that sheep grazing CT-containing legumes, especially *sulla*, may withstand the pathogenic effect of gastrointestinal parasites better than lambs grazing non-CT-containing forages.

Table 1.10. Arithmetic mean liveweight gains (LWG; g/day), faecal egg counts (FEC; eggs per gram fresh faeces) and total worm burden of anthelmintic drenched (D) and un-drenched (UD) lambs grazing sulla (*Hedysarum coronarium*), lucerne (*Medicago sativa*), *Lotus sp.* and perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture.

	Sulla		Lucerne		<i>Lotus corniculatus</i>		<i>Lotus pedunculatus</i>		Pasture	
	D	UD	D	UD	D	UD	D	UD	D	UD
Experiment 1										
Total condensed tannin (g/kg DM)	120		1							
LWG/day	302	206	244	50						
Average FEC	1,543		2,536							
Experiment 2										
Total condensed tannin (g/kg DM)	99		2							
LWG/day	200	129	184	-39						
Total worm burden	8,016		19,268							
Experiment 3										
LWG/day	226	175	243	121	208	86	232	160	166	84
Average FEC	1,538		2,199		2,571		2,864		2,109	
Total worm burden	13,090		18,084		22,990		23,665		15,806	

From Niezen et al. (1993, 1998).

Recent evidence (Molan *et al.*, 1999) estimates that the effect could be mediated in two ways. Firstly, CT increase the amino acid supply to the small intestine, overcoming the increased endogenous protein loss into the gastrointestinal tract (GIT) caused by parasite population, which in turn may improve the host immunological competence to withstand the effects of infestation and maintain a reasonable level of production (resilience). Secondly, CT may disrupt the nematode life cycle by reducing egg viability and larval development in the forage, and larval motility in the ruminant's digestive system (resistance). Consequently, use of CT-containing forages may increase animal productivity with a substantial reduction of anthelmintic drenching and so improve the quality of the final product in the market.

1.4.8 Dag formation and flystrike

Accumulation of faecal material over faeces already adhered to the wool around the tail and bellow to the sides has been described as dags in sheep flocks. Several factors associated with dag formation, such as parasitism (McEwan *et al.*, 1992; Larsen *et al.*, 1994, Scales *et al.*, 1995), genetic variation (Meyer *et al.*, 1983; Morris and Mackay, 2002), endophyte toxins ergovaline or lolitrem B (Fletcher *et al.*,1999), changes in mineral absorption (Reid and Cottle, 1999); faecal moisture content (Waghorn *et al.*, 1999) and CT-containing forages (Leathwick and Atkinson, 1995, 1998; Robertson *et al.*, 1995; Niezen *et al.*, 1995,1998; Ramirez-Restrepo *et al.*, 2002), has been mentioned. Nevertheless,

the specific mechanism involved in the incidence of dags is unclear (Waghorn *et al.*, 1999).

In practical terms, dags represents losses in productivity due to the costs involved in removing them, the protection against flystrike, with which, they are strongly correlated ($r = 0.97$) (Leathwick and Atkinson, 1995) and reduced wool returns (Larsen *et al.*, 1994). Meyer *et al.* (1983) estimated that 60 cents per animal plus labour in extra crutching are affecting the income of the farm by dags and flystrike.

Further, estimations from Heath and Bishop (1995) suggested that up to 5% of the national flock is affected by flystrike, with a cost of \$37M that includes production losses and treatment. More recently, Cole and Heath (1999) stated that around \$60M are spent annually by the New Zealand sheep farming industry to treat or prevent blowflies and lice.

In contrast, Waghorn *et al.* (1999) pointed out that, even though there are some estimations, the real cost to farming is unknown due to lack of nationwide statistics about the incidence and frequency of dagging. Furthermore, quantitative effects of dagging on performance, stress, labour input in checking stock, pesticide resistance, ethical costs as well as consequences of not dagging are not defined (Waghorn *et al.*, 1999).

Additionally, insecticide residue levels in greasy wool are a possible health risk for wool handlers and overseas consumer demands (Wakelin, 1994). Consequently, it is necessary to develop an integrated management system to consider both low or

non-chemical viable alternatives (Leathwick and Atkinson, 1996) and human and animal welfare issues (Morris and Mackay, 2002).

1.5 CONCLUSIONS AND NEEDS FOR FUTURE RESEARCH

The conclusions of this Literature Review can be summarised as follows:

- *Lotus corniculatus* is a crop adapted to different soil types and is particularly adapted to dry conditions and medium soil fertility, where productivity of other legumes is limited. Research in glasshouse environment and small plots show that birdsfoot trefoil maintains its quality during maturity and its greatest yield occurs during summer. It should be rotationally grazed, at not less than 4 week intervals, and to a residual height of approximately 10 cm. However, the effect of grazing management on plant production throughout consecutive years has not been investigated in dryland areas and research needs to be conducted in this area.
- Low concentrations of condensed tannins (20-40 g/kg DM) are present in *L. corniculatus*, which bind plant protein after chewing. Effects of CT upon nutritive value and animal production have been established through comparing unsupplemented sheep (CT-acting) with sheep supplemented orally and intraruminally with Polyethylene glycol (PEG; MW 3,350), which specifically binds and inactivates CT (CT-inactivated). Action of CT in birdsfoot trefoil reduces forage N degradation by rumen microorganisms, increases the flow of NAN at the duodenum / unit of N eaten and increases

the absorption of EAA from the small intestine, without depressing rumen carbohydrate digestion or voluntary feed intake.

- Wool growth is dependent upon the absorption of EAA from the small intestine and the availability of sulphur-containing amino acids (SAA). In grazing studies it has been shown that action of condensed tannins in *L. corniculatus* increases wool production (11%). Whilst lambs grazing birdsfoot trefoil grew faster than lambs grazing lucerne, PEG dosing studies showed that none of this increase could be attributed to the action of CT. Action of CT increased milk production (21%) and the net secretion of protein (14%) and lactose (12%), in ewes grazing *L. corniculatus*.
- Ovulation rate and lambing percentage of ewes grazing *L. corniculatus* for 6-8 week periods during mating was approx 25% higher than that of comparable ewes grazing perennial ryegrass/white clover pasture over this period. PEG supplementation studies have shown that approximately half of this increase was due to the action of CT, with the other half being due to the superior nutritive value of legumes, independent of CT. The beneficial effects of CT in increasing reproductive rate were associated with higher plasma concentration of branched chain amino acids (BCAA).
- Condensed tannins extracted from sulla, *Lotus spp.* and sainfoin (*Onobrychus viciifolia*) reduced the motility of infective L₃ internal parasite-larvae of both sheep and farmed deer. They also disrupted development of eggs and larval moults. In field studies, parasitised lambs grazing sulla or

chicory (*Cichorium intybus*) had reduced worm burdens and increased body growth relative to parasitised lambs grazing lucerne, so increasing resilience. Further research is needed to understand specific mechanisms of action and to reduce reliance on anthelmintic drenches in pastoral farming systems.

- All the results summarised above were obtained in separate feeding experiments, generally with only one aspect being investigated in each experiment. There is a need to investigate the integration of *L. corniculatus* into whole dryland sheep farming systems, where its use can be planned for the entire year and several aspects are measured in each experiment.
- Three priorities need to be investigated to increase whole year productivity in the whole farm system. First, use of *L. corniculatus* during mating for at least 6 weeks with lighter ewes to increase OR, lambing and weaning percentage. Second, feeding *L. corniculatus* to lactating ewes and their suckling lambs during spring to increase wool production and lamb weaning weight, reducing anthelmintic inputs and so increasing the proportion of lambs that can be drafted early for slaughter, before summer-dry conditions occur. Finally, evaluating *L. corniculatus* for increasing the growth of post-weaned lambs over the summer, to improve selling live weights in the market, with reduced reliance on anthelmintic options to control nematode parasites. These aspects are investigated in this thesis, through comparing sheep grazing swards of *L. corniculatus* and comparing them with similar sheep grazing conventional perennial ryegrass/white clover pasture.

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CHAPTER 2.

USE OF *LOTUS CORNICULATUS* CONTAINING CONDENSED TANNINS TO INCREASE LAMB AND WOOL PRODUCTION UNDER COMMERCIAL DRYLAND FARMING CONDITIONS WITHOUT THE USE OF ANTHELMINTICS



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ABSTRACT

Two grazing experiments were conducted for 12 and 13 weeks respectively over the spring periods of 2000 and 2001 at Massey University's Riverside farm, in the Wairarapa, New Zealand to compare the effects of grazing *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) dominant pasture during lactation on ewe and lamb live weight (LW), wool production, faecal nematode egg counts (FEC) and dag score. Ewes and their lambs (mainly twins) were rotationally grazed on *L. corniculatus* ($n = 50$) or pasture ($n = 50$) without any anthelmintic treatment at a herbage allowance of 6.5 and 8.0 kg green DM/ewe/day for Experiments 1 and 2 respectively. Total condensed tannins (CT) concentration in the diet selected was 24 to 27 g CT/kg DM for *L. corniculatus* and 1.4 to 1.5 g CT/kg DM for pasture. *In vitro* organic and estimated metabolisable energy (ME) concentration were higher for *L. corniculatus* than for pasture in both experiments, whilst the concentrations of neutral detergent fibre (NDF) was lower for *L. corniculatus* than for pasture. The LW gain, weaning LW and wool production were consistently greater ($P < 0.001$) for lambs grazing *L. corniculatus*, in both Experiment 1 (258 vs. 189 g/d; 36.1 vs. 30.1; 1.17 vs. 0.98 kg) and in Experiment 2 (247 vs. 162 g/d; 31.8 vs. 24.1; 1.17 vs. 0.81 kg), respectively. Ewe and lamb dag score were strongly and positively correlated with dag weight ($P < 0.001$) and generally increased with time in sheep grazing pasture, whilst grazing on lotus consistently reduced dag score. FEC in ewes grazing pasture showed a post-parturient rise (PPR) following lambing, whilst

ewes grazing *L. corniculatus* had a reduced PPR in FEC. Up to day 70, FEC in lambs grazing *L. corniculatus* was lower than that for lambs grazing pasture, but between day 70 and the end of both experiments (approximately day 90), FEC in lambs grazing *L. corniculatus* increased to similar values as for pasture-fed lambs. FEC was not correlated with dag score or dag weight in ewes or lambs grazing pasture, but these indices were weakly and positively correlated in ewes and lambs grazing *L. corniculatus*, suggesting that lowering FEC on *L. corniculatus* also reduced dag formation. It was concluded that under dryland farming conditions, the use of *L. corniculatus* (birdsfoot trefoil; cv. Grasslands Goldie) during the spring/early summer lactation period can be used to increase lamb growth and wool production, whilst eliminating the need for pre-lambing anthelmintic drenching and probably reducing the amount of insecticide needed to control flystrike. These effects are probably due to the CT in *L. corniculatus* reducing rumen protein degradability and controlling internal parasites and to the higher digestibility and voluntary feed intake (VFI) of *L. corniculatus* compared to perennial ryegrass/white clover pasture. The absence of endophyte in *L. corniculatus* may have also contributed to these effects.

Keywords: *Lotus corniculatus*, condensed tannins, dryland farming systems, oral anthelmintic.

Abbreviations: **BA**, break area; **BFT**, birdsfoot trefoil; **CFW**, clean fleece weight; **CT**, condensed tannins; **cv**, cultivar; **DM**, dry matter; **DOMD**, digestible organic matter in the dry matter (g)/kg DM; **EAA**, essential amino acid; **FA**, feed allowance; **FEC**, faecal nematode egg counts; **HM** herbage mass; **ILR**, irreversible loss rate;

LW, live weight; **LWG**, liveweight gain; **m**, metre; **ME**, metabolisable energy; **NDF**, neutral detergent fibre; **OMD**, organic matter digestibility; **PEG**, polyethylene glycol; **PPR**, post-partum rise; **SAA**, sulphur-containing amino acids; **SAS**, Statistical Analysis System; **VFI**, voluntary feed intake.

2.1 INTRODUCTION

Lotus corniculatus L. (birdsfoot trefoil) is a forage adapted to a variety of soil types (Seaney and Henson, 1970; Turkington and Franko, 1970). There is considerable interest in New Zealand on the pastoral value of birdsfoot trefoil (BFT) for dryland farming areas, where other legumes and grasses have reduced productivity in summer/autumn due to drought conditions and to reduced soil fertility in hill country (Bologna *et al.*, 1996; Waghorn *et al.*, 1998; Kemp *et al.*, 1999).

Birdsfoot trefoil contains a low concentration of condensed tannins (CT; 20 to 40 g/kg DM; Barry, 1989), which binds leaf protein after mastication (Jones and Mangan, 1977). Stability of the CT-protein complex is pH dependant, but it is insoluble at rumen pH (6.0 to 7.0) and releases protein at pH < 3.5, such as that in the abomasum (Jones and Mangan, 1977). This has increased the amount of essential amino acids (EAA) absorbed from the small intestine (Waghorn *et al.*, 1987) and improved animal productivity.

In grazing sheep, the action of the CT in BFT has increased wool growth by up to 11% during summer (Wang *et al.*, 1996b; Min *et al.*, 1998), milk yield (21%) during mid and late lactation (Wang *et al.*, 1996a) and both ovulation rate (27%) during mating, and lambing percentage (20%; Min *et al.*, 1999; Min *et al.*, 2001).

Experimental evidence (Niezen *et al.*, 1995, 1998) suggests that CT have the potential to control parasite infections and reduce dag formation (accumulation of faeces in the wool surrounding the anus; Leathwick and Atkinson, 1995), which could potentially lead to reduced use of anthelmintics to control parasites. Additionally, relative to perennial ryegrass, growth of sheep was higher (35%) when grazing BFT (Robertson *et al.* 1995). However, there was no evidence to support CT making a positive contribution to this effect, as judged by the level of response to polyethylene glycol (PEG) supplementation. To date, effects of CT upon animal productivity and parasitology have been determined in separate experiments

The objective of this study was to assess the effect of feeding *L. corniculatus* on sustainable productivity of lactating ewes in the spring under commercial dryland farming conditions without the use of anthelmintic drenches, using a systems approach, where effects on animal productivity and parasites were measured in the same experiment.

2.2 MATERIALS AND METHODS

2.2.1. Experimental design

Two rotational grazing experiments were carried out in the springs of 2000 and 2001 at Massey University's Riverside farm in the Wairarapa, New Zealand. This area is on the East Coast of the Southern North Island and experiences regular summer dry conditions. Experiment 1 was conducted from 18 October 2000 to 12 January 2001 (86 days). Experiment 2 commenced on 3 October 2001 and

finished on 2 January 2002 (91 days). Experiments 1 and 2 compared groups of 50 undrenched lactating Romney ewes and their lambs (mainly twins) grazing pure swards of *L. corniculatus* (cv. Grasslands Goldie; CT-containing) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture; non-CT-containing). Both forages were offered at the same dry matter (DM) allowance, in weekly breaks, with each break lasting 7 days. Under normal commercial farming, all ewes would receive a pre-lambing anthelmintic drench, but this was deliberately withheld in both years, in order to investigate if feeding *L. corniculatus* would reduce incidence of nematode parasitism.

Liveweight gain (LWG) of both ewes and lambs was determined, whilst wool production was measured by shearing at the end of both experiments. Rectal faecal samples for faecal nematode egg counts (FEC) were collected at intervals throughout the experiments from both ewes and lambs. Dag formation was assessed at intervals throughout the experiments, as the visual dag score, and by collecting all the crutching (i.e. dags) just prior to the main shearing. Dag measurements were done on all ewes and lambs. In commercial sheep farming, particularly in New Zealand and Australia, accumulation of dags in hot weather can lead to fly strike requiring insecticide treatment. No insecticides were used in these experiments to assess if it was feasible to produce weaned lambs without their use. Therefore, the use of *L. corniculatus* containing CT offers the possibility of reduced use of both anthelmintics and insecticide dips.

2.2.2. Forages

L. corniculatus L. (birdsfoot trefoil; cv. Grasslands Goldie) and perennial ryegrass-white clover (*Lolium perenne*/*Trifolium repens*) dominant pasture were grazed in the vegetative growth stage. Nine hectares of *L. corniculatus* was established for Experiment 1 and it was also used in Experiment 2. Surplus areas of legume and pasture were grazed by commercial flocks of sheep and cattle. Additionally, pasture paddocks were mechanically topped during spring to remove reproductive stem material to stimulate the vegetative growth stage. In winter, legume paddocks were sprayed with herbicides to control both grasses (Gallant[®] NF; Dow Elanco, NZ Ltd; 3 litres/ha) and broad-leaved weeds (Preeglone[®]; Zeneca Ltd; 3 litres/ha and Sencor[®] DF; Bayer, NZ; 1 kg/250 litres/ha).

Measurements of pre-grazing and post-grazing herbage mass and botanical composition were determined for each weekly break by cutting random quadrats (8 x 0.180 m²) per break of each forage to ground level. Samples were washed and dried overnight (16 h) in a forced-air oven (Contherm; Thermotec 2000; New Zealand) at 80 °C. Pre-grazing and post-grazing height, and pasture cover were measured for each weekly break using a sward stick and plate meter (Ashgrove; New Zealand) respectively.

Six wire mesh cages measuring about 1.4 x 0.9 m were placed in each break immediately before sheep were introduced for grazing. At the end of grazing that break, the cages were removed and the forage was hand plucked corresponding

to what the sheep were observed to be eating (diet selected). These samples were pooled and stored at -20°C for nutrient analysis.

2.2.3. Grazing management

Ewes and lambs were rotationally grazed in both experiments at a feed allowance of 6.5 kg green DM/ewe/day in Experiment 1 and 8.0 kg green DM/ewe/day in Experiment 2. Weekly breaks were used in both swards with front and back electric fences. The area of each weekly break was calculated as:

$$\text{BA} = \frac{7 \text{ days} \times n \times \text{FA}}{\text{HM}}$$

Where HM is herbage mass (kg DM/ha), BA is break area (ha), n is number of ewes, and FA is feed allowance per head per day (kg). This management ensured vegetative high quality forage at all time. Ewes and lambs had free access to water in both experiments.

2.2.4. Animal measurements

Liveweight gains were measured for both ewes and lambs at fortnightly periods using electronic scales (Tru-test, Auckland, NZ). Rectal faecal samples from 20 randomly selected ewes grazing both *L. corniculatus* and pasture were sampled at the beginning of the Experiment 1 (day 0) and on day 21 and at fortnightly intervals to estimate faecal nematode egg concentration (FEC). In Experiment 2, ewes were sampled on day 0 and at fortnightly periods. FEC was monitored from 30 randomly selected lambs grazing each sward in both experiments. In Experiment 1, faecal

samples were collected at 6 and 3 weeks before and at weaning. In Experiment 2, lambs were sampled at 7, 5 and 3 weeks before and at weaning. The same ewes and lambs were sampled on each occasion. Gastrointestinal larvae from ewes and lambs in Experiment 2 were cultured from faeces collected for FEC to estimate relative nematode populations.

Ewes and lambs in both experiments were scored on a scale 1 to 5 (1 = no dags, 5 = the highest incidence of dags) for dagginess at docking (the start of the experiments) and at two-week intervals. At the end of the trials, lambs and ewes were full leg crutched and crutchings were weighed, oven dried and re-weighed. Ewes and lambs were shorn at the end of the experiments and fleece weight recorded.

2.2.5. Laboratory analyses

2.2.5.1. Forages and faeces

All samples of feed offered and diet selected were stored at – 20° C and freeze-dried using a Cuddon 0610 freeze drier (W.G.G. Cuddon Ltd, Blenheim, New Zealand), and ground to pass a 1 mm diameter sieve (Wiley mill, Swedesboro, USA) before laboratory analysis. Total nitrogen (N) was determined by the Dumas principle (Leco CNS 2000 Analyser, Model 602 600 200, USA). Neutral detergent fibre (NDF) was determined by the detergent system of Robertson and Van Soest (1981), with alpha amylase (BDH, Poole, UK) being added during NDF extraction. Sodium sulphite was not added. Acetone/water-extractable, protein-bound and fibre-bound CT fractions in forages were determined using a butanol-HCL

colorimetric procedure (Terrill *et al.*, 1992b), with total CT concentration being reported.

All CT concentrations were determined using CT extracted from *Lotus pedunculatus* as a standard reference (Jackson *et al.*, 1996). *In vitro* organic matter digestibility (OMD) and digestible OM in the DM (DOMD) were measured using the enzymic procedure of Roughan and Holland (1977), with samples from *in vivo* digestibility trials used as standards, with pasture standards used for the *in vitro* determination of pasture samples, and *L. corniculatus* standards used for the *in vitro* determination of *L. corniculatus* samples. Faecal samples for FEC were refrigerated overnight (4° C) and FEC determined using a modified McMaster method (Stafford *et al.*, 1994) where each egg counted represented 50 eggs/g of wet faeces. Larval cultures were made from pooled faeces from each group mixed with vermiculite and water and cultured at 25° C for 10 days. Larvae were recovered using a Baermann technique (Ministry of Agriculture, Fisheries and Food, 1986).

2.2.5.2. Wool samples

Fleeces were weighed at shearing to determine greasy fleece weight. Wool samples (150 g) from both left and right mid-side areas were used for laboratory analysis. Clean fleece weight (CFW) and fleece yield (%) were determined using a standard greasy wool washing procedure described by Min *et al.* (1998). Wool staple length (cm) from each animal was determined by measuring the length of 10 randomly chosen unstretched staples along a ruler.

2.2.6. Statistical analyses

Differences in chemical forage composition, forage botanical composition, pre-grazing and post-grazing herbage mass, and VFI between pasture and *L. corniculatus* were assessed using the MIXED procedure of the SAS statistical package (SAS, 2001), with a linear model that included the effects of forage type (*L. corniculatus* or pasture).

Faecal nematode egg counts were analysed after square root transformation to normalise the data (Snedecor and Cochran, 1980). Data for LW, transformed FEC and dag score were analysed using the MIXED procedure of SAS (2001). The linear model included the fixed effects of day, forage type, forage type by day interaction, and the random effect of animal. Using the Akaike's information criterion, a compound symmetric error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals (Littel *et al.*, 1998). Daily LW gain, greasy fleece weight, clean fleece, yield and staple length were analysed using the PROC GLM (SAS, 2001) with the variable fitted being forage type.

Chi-square test was performed using PROC FREQ (SAS, 2001) to test for significant differences between pastures in the frequency of infective larvae in each of the parasite species. Correlations among dag weight, dag score and FEC were analysed using PROC CORR (SAS, 2001).

2.3 RESULTS

2.3.1. Forages and botanical composition

Pre-grazing herbage mass was similar between *L. corniculatus* and perennial ryegrass-white clover pasture in both experiments, but post-grazing herbage mass was higher for pasture than for *L. corniculatus* (Table 2.1). For both forages, the diet selected was predominantly leaf, with negligible amounts of stem being consumed. Only small amounts of white clover and of other species were consumed in both experiments.

2.3.2. Chemical composition

Total N concentration was higher for *L. corniculatus* ($P < 0.001$) than for the pasture in the diet selected in Experiment 2 (Table 2.2), whilst NDF concentration was lower ($P < 0.001$) in *L. corniculatus* than in pasture in both years. *L. corniculatus* contained approximately 25 g/CT/kg DM in the diet selected, with only trace amounts of total CT being detected in the pasture diet selected. Most CT in the *L. corniculatus* selected was readily extractable (68.2%), with much smaller amounts being protein-bound (28.0%) or fibre-bound (3.8%).

Table 2.1. Pre-grazing and post-grazing herbage mass (t DM/ha) and plant components of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture ^a.

	Experiment 1						Experiment 2					
	Pre-grazing			Post-grazing			Pre-grazing			Post-grazing		
	Pasture	<i>Lotus</i>	S.E.M	Pasture	<i>Lotus</i>	S.E.M	Pasture	<i>Lotus</i>	S.E.M	Pasture	<i>Lotus</i>	S.E.M
Herbage mass	3.82	3.94	0.25	2.49	1.77	0.13	3.13	3.37	0.25	2.23	1.78	0.13
Green DM	3.52	3.72	0.23	2.07	1.40	0.11	2.99	3.32	0.23	2.09	1.66	0.11
Dead matter	0.30	0.21	0.06	0.41	0.34	0.06	0.13	0.04	0.06	0.13	0.11	0.06
Leaves	1.95	1.49	0.12	0.95	0.15	0.06	1.51	1.62	0.12	0.82	0.33	0.06
Stems	0.41	0.90	0.10	0.62	0.76	0.10	0.76	1.02	0.10	0.78	1.04	0.10
White clover	0.59	0.53	0.07	0.16	0.19	0.02	0.43	0.47	0.07	0.14	0.21	0.02
Weeds	0.09	0.68	0.07	0.01	0.26	0.03	0.15	0.17	0.07	0.16	0.07	0.03

^a Means for 13 weekly breaks and their pooled standard errors.

Table 2.2. Total nitrogen (N), neutral detergent fibre (NDF), condensed tannin (CT), *in vitro* organic matter digestibility (OMD) and digestible organic matter in dry matter (DOMD), as well as estimated metabolisable energy concentration (ME, MJ/ kg DM), of the diet selected by sheep grazing perennial ryegrass/white clover (*Lolium perenne*/Trifolium repens) pasture or *Lotus corniculatus* L.

	Experiment 1				Experiment 2			
	Pasture	<i>Lotus</i>	<i>P</i>	S.E.M	Pasture	<i>Lotus</i>	<i>P</i>	S.E.M
	(<i>n</i> = 13)	(<i>n</i> = 13)			(<i>n</i> = 13)	(<i>n</i> = 13)		
Total N (g/kg DM)	29.7	30.3	NS	1.38	26.3	33.5	***	1.15
NDF (g/kg DM)	413.0	312.6	***	12.50	444.3	319.1	***	17.39
	(<i>n</i> = 4) ^a	(<i>n</i> = 4)			(<i>n</i> = 4)	(<i>n</i> = 4)		
Total CT (g/kg DM)	1.42	24.12	***	1.35	0.52	26.72	***	0.66
Bound CT (% CT) ^b	96	36	NA	NA	100	27	NA	NA
	(<i>n</i> = 12) ^c	(<i>n</i> = 12)			(<i>n</i> = 13)	(<i>n</i> = 13)		
<i>In vitro</i>								
OMD	0.65	0.70	***	0.006	0.69	0.71	NS	0.01
DOMD	0.60	0.65	***	0.004	0.62	0.65	*	0.008
ME ^d	9.80	10.60	***	0.078	10.24	10.69	*	0.001

^a Subsamples for CT analysis.

^b % Bound CT = ((protein-bound + fibre-bound CT)/total CT).

^c Subsamples for *in vitro* analysis.

^d ME = DOMD x 16.3

NA: not applicable.

NS: not significant ($P < 0.05$).

* ($P < 0.05$).

*** ($P < 0.001$).

2.3.3. Liveweight gain, wool production, wool characteristics and dag weight

Final LW ($P < 0.01$) and greasy fleece weight ($P < 0.001$) were higher in ewes grazing *L. corniculatus* than pasture in Experiment 2 (Table 2.3), whilst wool staple length was consistently longer for ewes grazing *L. corniculatus* in both experiments

($P < 0.01$). Relative to ewes that grazed pasture, grazing on *L. corniculatus* consistently reduced dag weight at shearing in both experiments ($P < 0.001$).

Table 2.3. Effect of grazing ewes on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. upon animal productivity, without use of anthelmintic drench input in dryland farming conditions during two consecutive years.

	Experiment 1			Experiment 2		
	Pasture	<i>Lotus</i>	<i>P</i>	Pasture	<i>Lotus</i>	<i>P</i>
	(<i>n</i> = 50)	(<i>n</i> = 50)		(<i>n</i> = 50)	(<i>n</i> = 50)	
Initial live weight (kg)	60.9 ± 0.92	61.4 ± 0.92	NS	55.2 ± 0.95	55.0 ± 0.95	NS
Final live weight (kg)	67.3 ± 0.92	69.0 ± 0.92	NS	58.5 ± 0.95	65.7 ± 0.95	**
Greasy fleece weight (kg)	4.07 ± 0.09	4.22 ± 0.09	NS	1.43 ± 0.04	1.69 ± 0.04	***
Clean fleece weight (kg)	3.17 ± 0.09	3.29 ± 0.07	NS	1.19 ± 0.04	1.40 ± 0.04	**
Fleece yield (%)	77.6 ± 0.55	77.9 ± 0.54	NS	82.3 ± 0.55	82.5 ± 0.54	NS
Staple length (cm)	14.45 ± 0.22	15.42 ± 0.22	**	7.15 ± 0.11	7.73 ± 0.11	**
Dag weight:						
Wet (kg)	0.391 ± 0.02	0.227 ± 0.02	***	0.332 ± 0.02	0.183 ± 0.02	***
Dry (kg)	0.269 ± 0.01	0.168 ± 0.01	***	0.176 ± 0.01	0.134 ± 0.01	•

NS: not significant ($P < 0.05$).

* ($P < 0.05$).

** ($P < 0.01$).

*** ($P < 0.001$).

The LW gain and wool production were greater ($P < 0.001$) in lambs grazing *L. corniculatus* than their counterparts grazing pasture (Table 2.4) in both experiments. Grazing on *L. corniculatus* increased LWG of predominantly twin lambs by 69 g/day (37%) in Experiment 1 and by 85 g/day (52%) in Experiment 2, increasing weaning weight by 6.0 kg (20%) and 7.7 kg (32%) respectively in Experiments 1 and 2. Increases in wool production from grazing lambs on *L.*

corniculatus were respectively 19% and 44% in Experiment 1 and 2, whilst wool staple length was increased in both experiments. Lower dag weights at shearing were recorded for lambs grazing *L. corniculatus* in Experiment 1, but in Experiment 2 the lambs grazing pasture had the lowest dag weight at shearing.

Table 2.4. Liveweight change (kg), wool production (kg) and dag weight (g) of undrenched lambs grazing on perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture or *Lotus corniculatus* L. over the springs 2000 and 2001.

	Experiment 1			Experiment 2		
	Pasture	<i>Lotus</i>	<i>P</i>	Pasture	<i>Lotus</i>	<i>P</i>
	(<i>n</i> = 78)	(<i>n</i> = 79)		(<i>n</i> = 92)	(<i>n</i> = 90)	
Initial live weight (kg)	13.7 ± 0.51	13.9 ± 0.53	NS	9.3 ± 0.45	9.3 ± 0.46	NS
Final live weight (kg)	30.1 ± 0.51	36.1 ± 0.53	***	24.1 ± 0.45	31.8 ± 0.46	***
Liveweight change (g/d)	189 ± 3.0	258 ± 3.0	***	162 ± 5.0	247 ± 5.0	***
Greasy fleece weight (kg)	0.98 ± 0.02	1.17 ± 0.02	***	0.81 ± 0.02	1.17 ± 0.02	***
Clean fleece weight (kg)	0.81 ± 0.02	0.97 ± 0.02	***	0.69 ± 0.02	0.98 ± 0.02	***
Fleece yield (%)	82.5 ± 0.35	82.6 ± 0.35	NS	84.5 ± 0.32	82.9 ± 0.31	***
Staple length (cm)	6.61 ± 0.12	7.64 ± 0.12	***	6.66 ± 0.19	7.87 ± 0.20	***
Dag weight:						
Wet (g)	131.63 ± 8.03	76.35 ± 7.98	***	82.22 ± 4.76	98.58 ± 4.70	*
Dry (g)	92.05 ± 4.29	62.92 ± 4.35	***	60.94 ± 2.65	79.20 ± 2.68	***

NS: not significant ($P < 0.05$).

* ($P < 0.05$).

*** ($P < 0.001$).

2.3.4. Dag score, faecal nematode egg counts and larval culture

The changes in dag score over the duration of the experiments are summarised in Fig. 2.1. Dag score generally increased with time in both ewes and lambs grazing pasture and was consistently lower in ewes grazing *L. corniculatus* in either

Experiment 1 ($P < 0.001$) or Experiment 2 ($P < 0.01$). Dag score of lambs grazing *L. corniculatus* was also lower than that of lambs grazing pasture, with the difference being more pronounced in Experiment 1 than in Experiment 2.

Ewes grazing pasture showed a post-partum rise (PPR) in FEC in both experiments (Fig. 2.2 and Fig. 2.3). Faecal nematode egg counts were lower ($P = 0.06$; Fig. 2.2) for ewes fed *L. corniculatus* than for ewes fed pasture in Experiment 1. Although FEC values of *L. corniculatus*-fed ewes were higher at the start of the Experiment 2, FEC thereafter remained lower ($P < 0.001$; Fig. 2.3) in ewes fed *L. corniculatus*, vs. in ewes fed pasture, throughout the experiment.

Lambs grazing *L. corniculatus* had lower FEC values at day 49 ($P < 0.001$) and day 70 ($P < 0.001$) than those grazing pasture in Experiment 1 (Fig. 2.4), but not at day 86. A similar trend was observed in lambs grazing *L. corniculatus* compared with their counterparts on pasture in Experiment 2, with *L. corniculatus* -fed lambs having lower FEC at 42, 56 and 70 days ($P < 0.001$; Fig. 2.5). At day 91 (weaning) FEC for *L. corniculatus*-fed lambs was higher than for pasture-fed lambs ($P < 0.01$).

After nematode larval incubation, *Trichostrongylus* plus *Ostertagia* species were predominant in ewes (67.7%) and lambs (81.9%) in Experiment 2 (Fig. 2.6). Relative to sheep grazing pasture (Fig. 2.6), grazing on *L. corniculatus* tended to reduce the proportion of *Trichostrongylus* and *Ostertagia*, and to increase the proportion of *Cooperia*, *Chabertia* and *Oesophagostomum*, with these effects being more pronounced in lambs than in ewes.

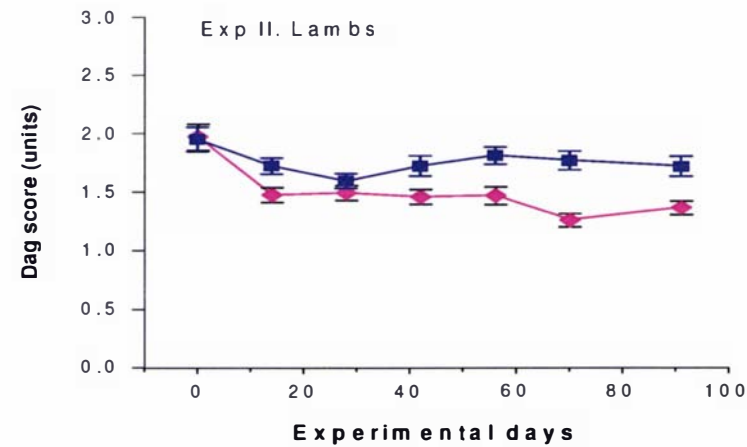
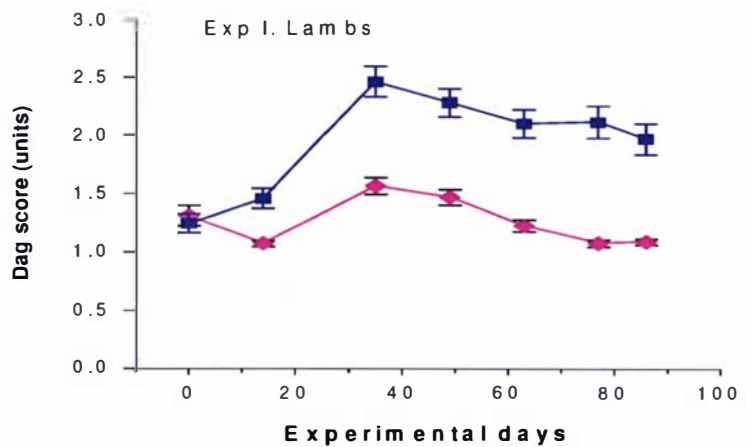
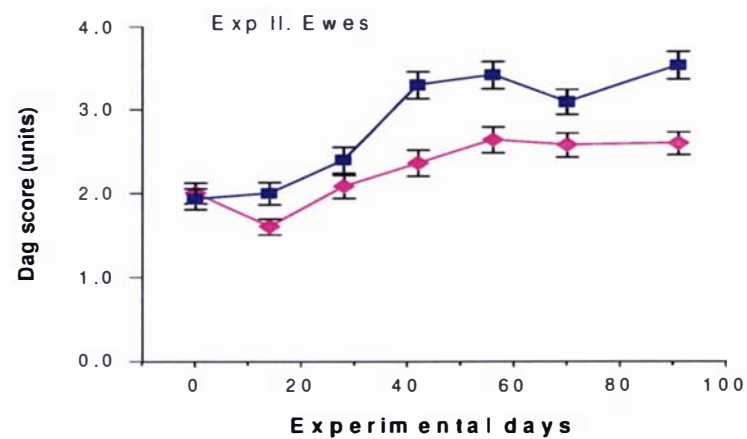
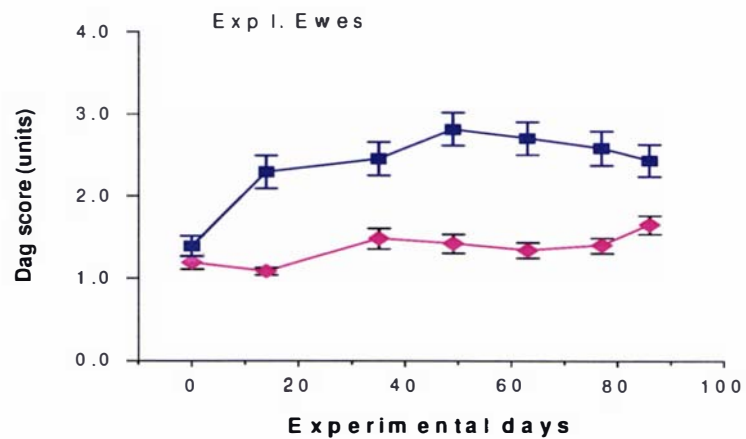


Figure 2.1. Comparative dag score of ewes and lambs grazing (◆) *Lotus corniculatus* L. (birdsfoot trefoil) and (■) perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture in two consecutive experiments.

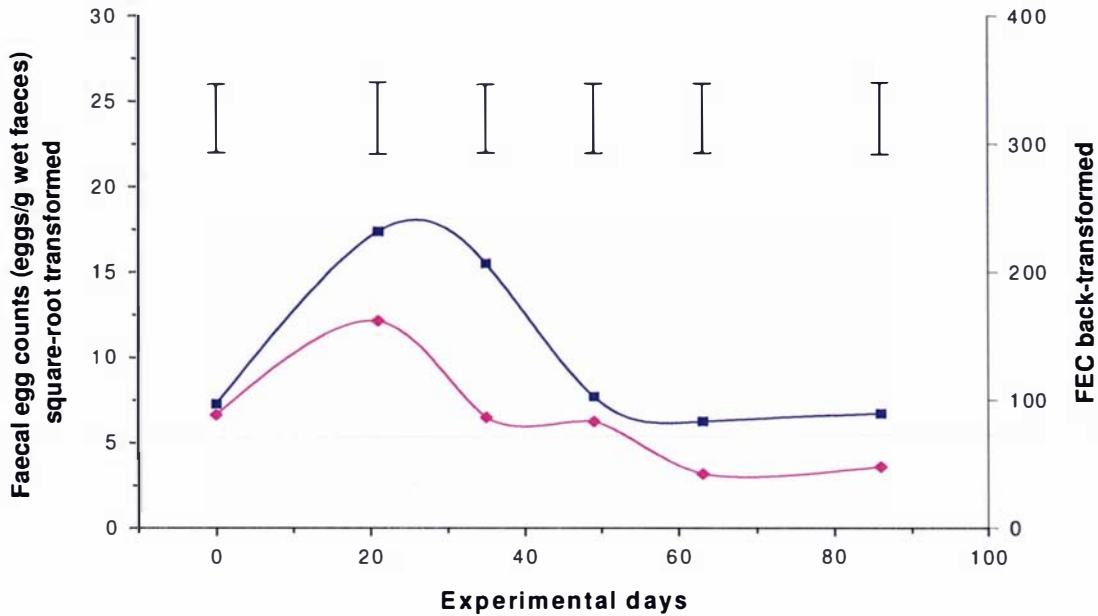


Figure 2.2. Experiment 1. Comparative least square means of faecal egg counts (FEC) (eggs g/wet faeces) of ewes grazing (♦) *Lotus corniculatus* or (■) perennial ryegrass/white clover pasture. Vertical bars show pooled standard error from square-root transformed data for clearer interpretation of trends.

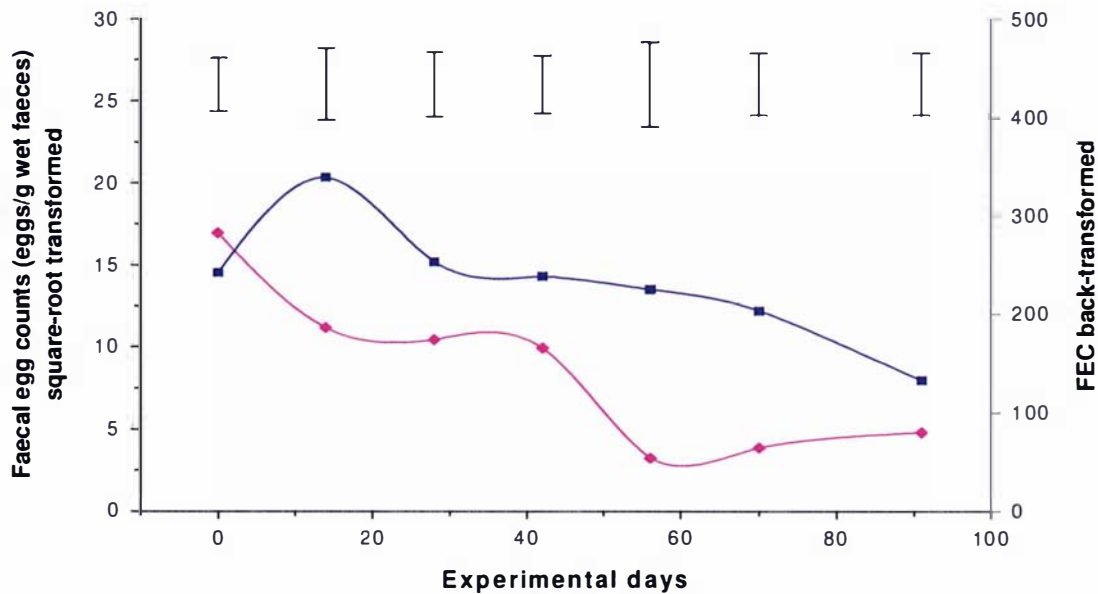


Figure 2.3. Experiment 2. Least square means of faecal egg counts (FEC) (eggs g/wet faeces) of ewes grazing *Lotus corniculatus* (♦) or perennial ryegrass/white clover pasture (■). I = pooled standard error from square-root transformed data for clearer interpretation of trends.

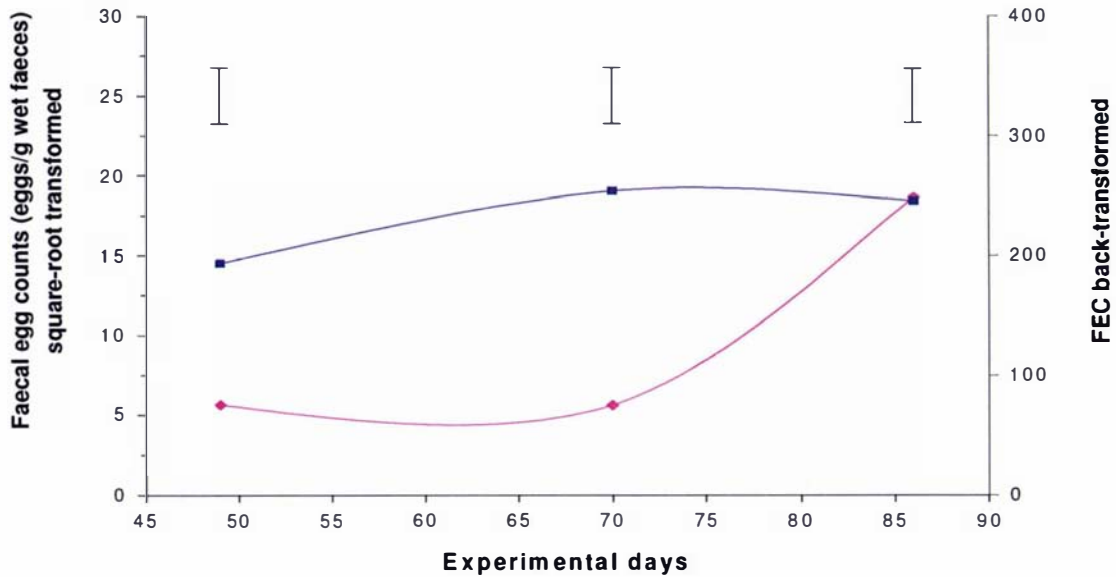


Figure 2.4. Experiment 1. Least square mean values of FEC (eggs g/wet faeces) in groups of lambs grazing (♦) *Lotus corniculatus* or (■) perennial ryegrass/white clover pasture. Bars represent pooled standard error from square-root transformed data for clearer interpretation of trends.

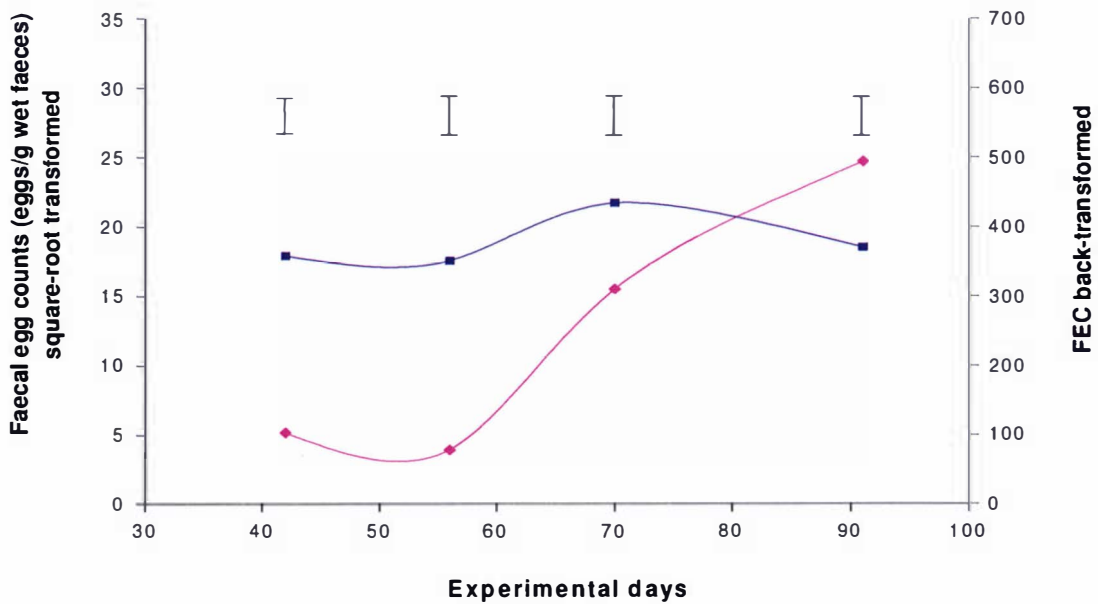


Figure 2.5. Experiment 2. Least square mean values of FEC (eggs g/wet faeces) in groups of lambs grazing *Lotus corniculatus* (♦) or perennial ryegrass/white clover pasture (■). I = pooled standard error from square-root transformed data for clearer interpretation of trends.

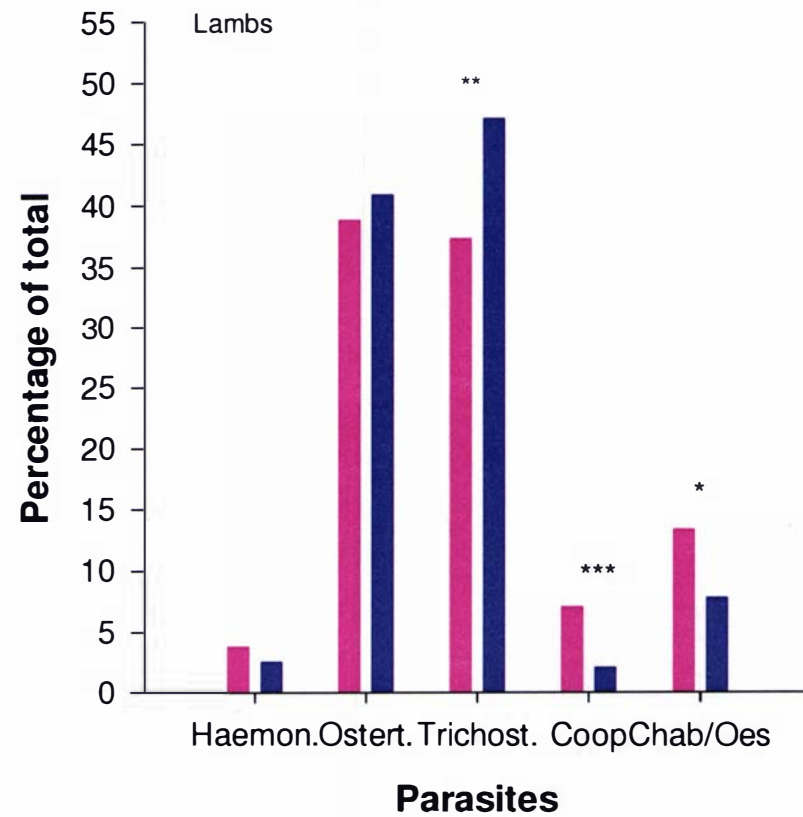
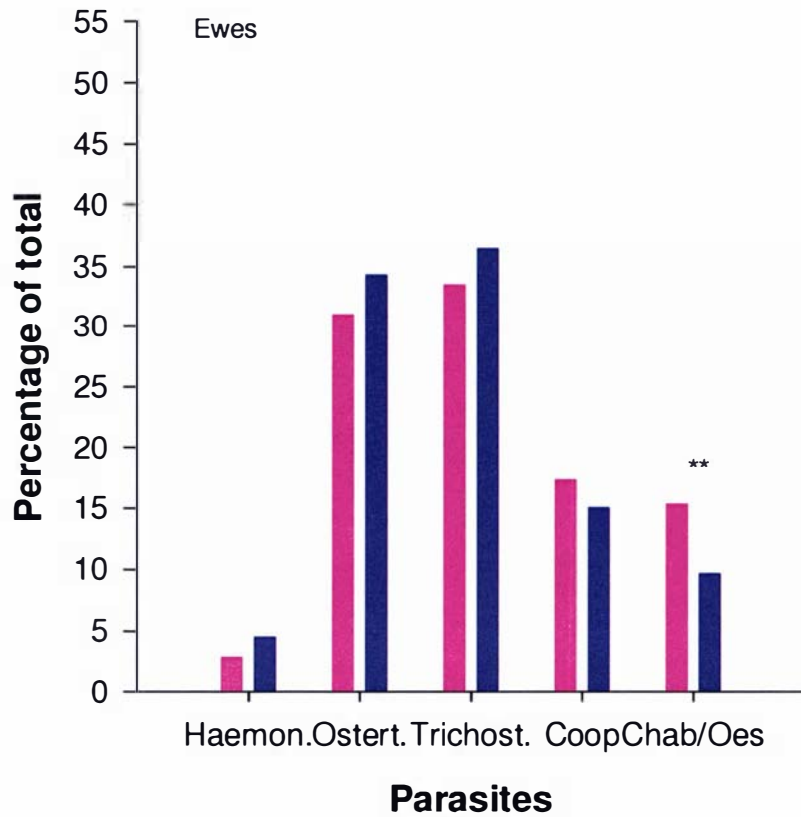


Figure 2.6. Experiment 2. Comparative proportions of infective gastrointestinal nematode larvae of ewes and lambs grazing (■) *Lotus corniculatus* L. (birdsfoot trefoil) and (■) perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture. Chab/Oes: *Chabertia* and *Oesophagostomum* species. * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$).

2.3.5. Correlations

There was a positive ($P < 0.001$) relationship between dag score and dag weight for both ewes and lambs grazing lotus or pasture in both years. Dag score was positively related to FEC in ewes fed lotus in Experiment 1 ($P < 0.05$) and dag weight was positively related to FEC in ewes fed *L. corniculatus* in Experiment 2 ($P < 0.05$) (Table 2.5). Dag score and FEC ($P < 0.05$), and dag weight and FEC ($P < 0.01$) were positively correlated for lambs grazing *L. corniculatus* in Experiment 1 (Table 2.5).

Table 2.5. Correlation coefficients between faecal egg counts (FEC), dag weight and dag score in undrenched ewes and lambs over the springs of 2000 (Exp. 1) and 2001 (Exp. 2).

	Experiment 1						Experiment 2					
	Pasture			Lotus			Pasture			Lotus		
	r	P	(n)	r	P	(n)	r	P	(n)	r	P	(n)
Ewes												
Dag score and wet dag weight	0.83	***	50	0.76	***	50	0.74	***	49	0.79	***	50
Dag score and dry dag weight	0.74	***	48	0.58	***	50	0.77	***	49	0.76	***	50
Dag score and FEC	0.15	NS	20	0.49	*	20	0.28	NS	20	0.35	NS	20
FEC and wet dag weight	-0.02	NS	20	0.28	NS	20	0.02	NS	19	0.45	*	20
FEC and dry dag weight	-0.20	NS	18	0.09	NS	19	0.005	NS	20	0.46	*	20
Lambs												
Dag score and wet dag weight	0.75	***	79	0.21	NS	80	0.53	***	85	0.38	***	86
Dag score and dry dag weight	0.65	***	78	0.11	NS	76	0.39	***	88	0.40	***	86
Dag score and FEC	0.06	NS	30	0.40	*	28	0.04	NS	30	-0.15	NS	30
FEC and wet dag weight	0.08	NS	30	0.49	**	29	0.22	NS	29	0.006	NS	30
FEC and dry dag weight	0.04	NS	30	0.50	**	29	0.008	NS	30	0.01	NS	30

NS: not significant ($P < 0.05$).

* ($P < 0.05$).

** ($P < 0.01$).

*** ($P < 0.001$).

2.4 DISCUSSION

The objective of these experiments was to assess the value of *L. corniculatus* for sustainable sheep production during spring lactation under commercial dryland farming conditions, with no anthelmintic drench input. The main findings were that relative to conventional perennial ryegrass/white clover pasture, *L. corniculatus* can be used to increase lamb growth, and both ewe and lamb wool production, whilst eliminating the need for pre-lambing anthelmintic drenching, as judged by the lower levels of FEC and of dag production.

The approximate voluntary feed intake (VFI) of grazing animals can be calculated from pre-grazing and post-grazing pasture DM masses, as the kg of DM utilised by each ewe and her lambs per day. This gives an average for each experiment and cannot be separated into that consumed by the ewes and that consumed by their lambs. However, even with these limitations, it is evident that VFI was higher ($P < 0.001$) for ewes grazing *L. corniculatus* vs. perennial ryegrass-based pasture both in Experiment 1 (3.73 vs. 2.51 kg DM/ewe/day) and in Experiment 2 (3.81 vs. 2.56 kg DM/ewe/day). The superior body and wool growth of lambs grazing *L. corniculatus* is therefore due to a combination of factors, including possibly higher VFI, higher OMD and metabolisable energy (ME) and probably by the improved efficiency of protein digestion caused by the CT in *L. corniculatus*.

Benefits of condensed tannins in *L. corniculatus* have been related to reduced forage N degradation by rumen microorganisms and to the increased absorption of EAA in the small intestine (Waghorn *et al.*, 1987). Results from Wang *et al.*

(1996a) indicated that CT in *L. corniculatus* increased ewe milk production and the secretion rates of protein and lactose by 21, 12 and 12% respectively during mid and late lactation. Wool growth is dependent upon absorption of EAA from the small intestine, specifically the availability of sulphur-containing amino acids (SAA; Reis, 1979). Wang *et al.* (1994) showed that CT in *L. corniculatus* reduced degradation of SAA in the rumen, increased irreversible loss (IRL) of cysteine from blood plasma and increased the flux of cysteine to body synthetic reactions. Hence, some of the greater response in lamb growth and both lamb and ewe wool production in sheep grazing lotus vs. their counterparts grazing pasture in the present study, was probably due to the effects of CT in reducing rumen protein degradability and in increasing absorption of EAA from the small intestine. These findings are consistent with the responses described previously by Douglas *et al.* (1995) and Wang *et al.* (1996a).

One of the objectives of this experimental programme was to determine if use of CT-containing *L. corniculatus* could result in less anthelmintic drench use. Hence, ewes and their lambs grazing *L. corniculatus* were compared with similar ewes grazing perennial ryegrass/white clover pasture, in the absence of pre-lambing anthelmintic drenching. In the case of ewes, this was successful, with ewes grazing *L. corniculatus* having consistently lower dag scores and a lower PPR in FEC than the ewes grazing pasture, giving lower levels of forage contamination. For lambs, the corresponding data was time-dependant; up to day 70, both dag score and FEC were consistently lower for the lambs grazing *L. corniculatus*, but after day 70 these values for *L. corniculatus*-fed lambs either approached those of

pasture-fed lambs (Experiment 1) or tended to surpass them (Experiment 2). As day 70 is close to weaning, it seems that lambs need to be changed to other CT-containing forages, or to plants containing other secondary compounds, for post-weaning growth if sustainable systems are to be developed with low anthelmintic drench input.

The physiology of nematode eggs and infectivity of nematode larvae (L₃ stage) that survived over winter could be disrupted by both the period of exposure to CT on *L. corniculatus* prior to the start of the study (Molan *et al.*, 2000) and by inhibition of larval motility in the digestive system after ingestion of the infective L₃ stage. Thus, the intake of the infective larvae derived in most part from the PPR of lactating ewes during the experimental grazing could have been less on *L. corniculatus* vs. pasture. *In vitro* data from Molan *et al.* (1999, 2000) showed that CT extracted from *L. pedunculatus*, *L. corniculatus*, sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychus viciifolia*) reduced the motility of infective L₃ internal parasite-larvae of both sheep and farmed deer and also disrupted development of eggs and larval moults.

It is also likely that ewes and growing lambs fed *L. corniculatus* could have had an improved immune response to parasite challenge due to the higher protein intake and the higher amount of EAA absorbed from the small intestine. The positive response of the immune system to protein intake has been shown in previous metabolism studies with ewes (Houdijk *et al.*, 2000) and lambs (Abbott *et al.*, 1988). Likewise, after mid lactation, the higher LWG in lambs grazing *L. corniculatus* showed that in spite of the increased FEC values, young lambs coped

with further infection by continuing to increase their growth, in contrast to the conventional perennial ryegrass/white clover pasture.

Table 2.6. Comparative performance of drenched (D) and undrenched (UD) lactating ewes and their lambs grazing *Lotus corniculatus* L., lucerne (*Medicago sativa*) or pasture (*Lolium perenne*/*Trifolium repens*).

	Plant fed						Reference
	<i>Lotus corniculatus</i>		Lucerne		Pasture		
	D	UD	D	UD	D	UD	
	Liveweight gain (d/day)						
Ewes			- 89		35		^a Rattray <i>et al.</i> (1982)
					-199		^b McCall <i>et al.</i> (1986)
	251		59				Douglas <i>et al.</i> (1995)
	67						Wang <i>et al.</i> (1996a)
					75		Litherland <i>et al.</i> (1999)
		87				74	^c Experiment 1
		117				36	^c Experiment 2
Lambs				210		210	^a Rattray <i>et al.</i> (1982)
						213	^b McCall <i>et al.</i> (1986)
		275		263			Douglas <i>et al.</i> (1995)
		231					Wang <i>et al.</i> (1996a)
						217	Litherland <i>et al.</i> (1999)
		258				189	^c Experiment 1
		247				162	^c Experiment 2
	Wool growth (mg/100 cm ² per day)						
Ewes	133		123				Douglas <i>et al.</i> (1995)
	132						Wang <i>et al.</i> (1996a)
	Greasy fleece weight (kg)						
Ewes			1.80		1.65		^a Rattray <i>et al.</i> (1982)
					1.65		^b McCall <i>et al.</i> (1986)
		4.22				4.07	^c Experiment 1
		1.69				1.43	^c Experiment 2
Lambs		1.17				0.98	^c Experiment 1
		1.17				0.81	^c Experiment 2

^a Mean values from two consecutive experimental years.

^b Mean values from a 2³ factorial experimental design.

^c Present study.

Table 2.6 highlights the elevated nutritive value of *L. corniculatus* on commercial dryland farming during ewe lactation. Therefore, use of CT-acting *L. corniculatus* during spring under dryland conditions may increase sheep productivity better than lambs grazing non-CT-containing forages, with a substantial reduction of the perceived risk of chemical residues and so improve the quality of the sheep meat in the market.

Grazing studies with the CT-containing legume sulla (Niezen *et al.*, 1998) and chicory (*Cichorium intybus* cv. Puna; Scales *et al.*, 1995), a herb that contains low concentrations of CT and other secondary compounds, including sesquiterpene lactones, flavonoids, coumarins and caffeic acid derivatives (Rees and Harborne, 1985), showed higher growth of post-weaned parasitized lambs and deer with reduced FEC values and reduced worm burdens compared to lambs and deer grazing other forages (Hoskin *et al.*, 1999; Barry, 1998; Barry *et al.*, 2002). Part of the response to chicory may be due to its taller plant morphology, leading to reduced ingestion of L₃ infective larvae (Moss and Vlassoff, 1993).

Quantitative assessment of the intensity of the parasite infection to trigger drenching has traditionally been based on FEC values and subjective visual dag score. Results from the present study showed good correlations between dag score and both wet dag and dry dag weight, for both ewes and lambs grazing both pasture and *L. corniculatus*, giving a high degree of confidence in the use of visual dag score as an index of dag weight. However, there were no relationships between FEC and any of the measurements of dag production for either ewes or lambs grazing pasture, suggesting that other factors may have influenced dag

formation in sheep consuming this forage. The most likely factors are the endophyte (*Neotyphodium lolii*) alkaloids produced in perennial ryegrass, especially ergovaline, which are known to increase both faeces moisture content and dag formation in sheep (Fletcher *et al.*, 1999). The concentration of endophyte alkaloids increases in perennial ryegrass during the spring and peak in summer, the same period that dag formation increased in both ewes and lambs grazing pasture in the present experiments.

Positive correlations between FEC and measurements of dag formation were significant, but weak, in three out of four data sets for sheep grazing *L. corniculatus*, suggesting that in this case reductions in FEC were more likely to lead to reductions in dag formation. Feeding experiments with parasitized sheep fed *L. corniculatus* or high endophyte ryegrass cv. Nui (Leathwick and Atkinson, 1995) and ryegrass/white clover (Leathwick and Atkinson, 1998; Niezen *et al.*, 1998; Robertson *et al.*, 1995) showed fewer dags on *L. corniculatus* vs. ryegrass. Similar results were reported by Niezen *et al.* (1995), when parasitized sheep were grazing sulla compared with their counterparts grazing lucerne.

Alternative approaches are necessary to develop parasite epidemiology in pastoral farming systems where forages containing CT and other secondary compounds are part of the grazing system. The primary interest during the last few decades has been to develop sustainable farming systems with low chemical inputs, environmental protection and competitive outputs to meet market opportunities. Results from our study show that the biological feasibility of *L. corniculatus* for sheep organic farming practices is related to the reduced anthelmintic drenching

use, although the reduced dag formation in sheep grazing *L. corniculatus* may also translate into less insecticide being needed to control fly strike. Consequently, there will be environmental, human health and economic benefits because clean animal production on *L. corniculatus* could allow New Zealand to increase financial returns on an estimated international lamb export market of NZ \$2.4 billion, whilst animal production systems from marginal areas in developing countries could be improved to reduce poverty levels through transferring this technology by collaborative scientific programmes.

These studies have shown that *L. corniculatus* can be used in dryland farming systems to increase lamb growth and wool production during the spring/early summer lactation period, in the absence of pre-lambing anthelmintic drenching, thereby increasing both lamb weaning weight and the proportion of lambs that can be drafted early for slaughter. Lower levels of dag formation in sheep grazing *L. corniculatus* may reduce the need for insecticide treatment to control fly strike.

As both FEC and dag scores were rising in *L. corniculatus*-fed lambs from day 70, the integration into the grazing system of the legume sulla (Terrill *et al.*, 1992a; Niezen *et al.*, 1995) and the herb chicory (Scales *et al.*, 1995; Barry, 1999) with high feeding value and greater opportunity to control gastrointestinal nematodes, needs to be considered for post-weaning nutrition to reduce the risk of anthelmintic drench resistance and to give more sustainable grazing systems.

Further work is required to understand the epidemiology of nematode infections of sheep grazing plants containing CT and other secondary compounds, to define

effects due to the secondary compounds and also those attribute to differences in plant morphology and density (Knapp, 1964). Additionally, further studies are needed in dryland farming conditions to compare the growth of weaned lambs grazing *L. corniculatus* and perennial ryegrass/white clover pasture at high allowances over the summer/autumn period, with reduced reliance on anthelmintic drenching, to study the possibilities of *L. corniculatus* as a specialist feed for a more ecologically sustainable production system under hotter conditions than used in the present studies.

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CHAPTER 3.

USE OF *LOTUS CORNICULATUS* CONTAINING CONDENSED TANNINS TO INCREASE REPRODUCTIVE EFFICIENCY IN EWES UNDER COMMERCIAL DRYLAND FARMING CONDITIONS



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ABSTRACT

Two grazing experiments were conducted over 9.5 month periods of 2001 and 2002 at Massey University's Riverside dryland farm, in the Wairarapa, New Zealand. Dry conditions occur during the summer-autumn and were more severe in Experiment 1 than in Experiment 2. The experiments compared effects of grazing ewes on *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) versus perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture for 9 weeks (Experiment 1) and 11 weeks (Experiment 2) during late summer/autumn, including the mating period. Experiment 2 also investigated the length of time (days) that ewes need to graze *L. corniculatus* before mating to maximise reproductive performance.

In Experiment 1, shorn mixed age Romney ewes in light condition (mean LW 56.2 kg) were rotationally grazed on *L. corniculatus* ($n = 100$) or pasture ($n = 100$) at a herbage allowance of 1.8 kg green DM/ewe/day for the first three weeks of feeding, increased to *ad libitum* (2.3 kg green DM/ewe/day) during the mating period for two cycles. In Experiment 2, groups of 75 ewes grazed *L. corniculatus* for 42, 21, 10 and 0 days before synchronised oestrous, with perennial ryegrass-white clover pasture being grazed for the balance of the 42 days. All *L. corniculatus* groups continued grazing *L. corniculatus* for a further 5 weeks. Feed allowance was initially 2.0 kg green DM/ewe/day, increased to 2.3 kg green DM/ewe/day during the mating period over two cycles. At the end of *L. corniculatus* feeding in both experiments the groups were combined and grazed on pasture during pregnancy and lactation until weaning.

Total condensed tannin (CT) concentration in the diet selected was 18 to 29 g CT/kg DM for lotus, with only trace amounts in pasture. *In vitro* organic matter digestibility (OMD), digestible organic matter in dry matter (DOMD), and estimated metabolisable energy concentration (ME) were higher for *L. corniculatus* than for pasture, whilst the concentration of neutral detergent fibre (NDF) was lower for *L. corniculatus* than in pasture, with these differences being greater in Experiment 1 than in Experiment 2.

In Experiment 1 mating ewes on *L. corniculatus* increased the number of lambs born and lambs weaned per ewe lambing by 16 and 32% units respectively ($P < 0.05$), due to more multiple and less single births ($P = 0.06$) and to reduced lamb mortality ($P < 0.05$). In Experiment 2, increased days of grazing *L. corniculatus* before ovulation (0, 10, 21, 42 days) linearly increased ovulation rate ($P < 0.05$), number of lambs born and lambs weaned by up to 16% units, but had no effect upon lamb mortality. Mating ewes on lotus increased wool production ($P < 0.01$) and fibre length ($P < 0.05$) in Experiment 1 but not in Experiment 2. Grazing *L. corniculatus* had no effect on lamb birth weight and only small positive effects on weaning weight.

It was concluded that, under commercial dryland farming conditions, the use of *L. corniculatus* during the mating season in late summer/autumn can be used to increase reproductive efficiency and wool production, with the largest responses in years with exceptionally dry autumn periods. These effects are probably due to the higher digestibility and estimated ME concentration of *L. corniculatus* than pasture

and to the CT in *L. corniculatus* improving both protein digestion and absorption. Effects of forage CT upon the uterine microenvironment at the time of conception, implantation and early foetal growth need to be investigated in future studies.

Keywords: *Lotus corniculatus*, condensed tannins, perennial ryegrass/white clover pasture, reproductive efficiency, lamb viability, dryland farming systems.

Abbreviations: **ANOVA**, analysis of variance; **BA**, break area; **BCAA**, branched chain amino acids; **CIDR**, controlled intravaginal release device; **CL**, corpora lutea; **CT**, condensed tannins; **cv**, cultivar; **DM**, dry matter; **DOMD**, digestible organic matter in the dry matter (g)/100 g DM; **EAA**, essential amino acid; **FA**, feed allowance; **HM** herbage mass; **LW**, live weight; **LWG**, liveweight gain; **m**, metre; **ME**, metabolisable energy; **MJ**, mega joules; **NDF**, neutral detergent fibre; **NL**, no **OMD** organic matter digestibility; **OR**, ovulation rate; **PA**, proanthocyanidins; **PEG**, polyethylene glycol; **pH**; acidic or alkali degree; **RDN**, rumen degradable nitrogen; **RDP**, rumen degradable protein; **SAA**, sulphur-containing amino acids; **SAS**, Statistical Analysis System; **VFI**, voluntary feed intake.

3.1 INTRODUCTION

Short periods of improved nutrient supply before and during mating and reproduction have been found to increase ovulation rate (OR) along with increased follicle size and/or number (Bellows *et al.*, 1963), reduce late follicular atresia (Haresign, 1981; Downing and Scaramuzzi, 1991), and alter plasma gonadotrophin concentration (Smith, 1988) and ovarian sensitivity to

gonadotrophins (Downing and Scaramuzzi, 1991). These effects probably occur as a result of changes in live weight and body condition (Allen and Lamming, 1961; Coop, 1962; Knight, 1980), energy and protein intake and protein absorption from the small intestine (Knight, 1980; Smith, 1985; Cruickshank *et al.*, 1988; Smith, 1991; Min *et al.*, 1999; 2001), plasma concentrations of essential amino acids (EAA), principally branched chain amino acids (BCAA) (Waghorn, 1986; Waghorn *et al.*, 1990; Downing *et al.*, 1995), and levels of plasma metabolic hormones (especially insulin; Downing and Scaramuzzi, 1991; Downing *et al.*, 1995).

Temperate forages are characterised by high concentrations of protein, high digestibility and a low concentration of soluble carbohydrates (Ulyatt, 1981; Waghorn and Barry, 1987). A large part of the dietary protein is hydrolysed in the rumen to ammonia, some of which is re-incorporated into microbial protein. Excess ammonia is absorbed from the rumen and metabolised to urea in the liver, leading to increased plasma ammonia and urea concentrations (Kenny *et al.*, 2000; O'Callaghan *et al.*, 2000; Min *et al.*, 2001). In other studies increased dietary rumen degradable nitrogen (RDN) intake has similarly increased plasma urea concentration, leading to increased concentrations of ammonia and urea in plasma in the utero-oviductal microenvironment (McEvoy *et al.*, 1997) and uterine secretions (Jordan *et al.*, 1983), decreased uterine pH (Elrod and Butler, 1993) impaired viability of sperm (Dasgupta *et al.*, 1971; Umezaki and Fordney-Settlage, 1975) and oocytes (Fahey *et al.*, 1998; O'Callaghan and Boland, 1999), decreased fertilization rate and reduced embryo survival and embryonic development in cows (Blanchard *et al.*, 1990) and ewes (McEvoy *et al.*, 1997; Fahey *et al.*, 1998).

Condensed tannins (CT) or proanthocyanidins (PA) are polyphenolic compounds, which are present in the leaves and stems of a number of forage plants, including *Lotus corniculatus* L. (birdsfoot trefoil), *L. pedunculatus* (big trefoil), *Hedysarum coronarium* (sulla), *Lespedeza cuneata* (sericea lespedeza) and *Onobrychis vicifolia* (sainfoin) (McLeod, 1974; Foo *et al.*, 1982; Barry, 1989). The reactivity of CT with proteins is based upon two mechanisms, hydrogen (H) bonding, which is reversible, and oxidative coupling, which is not reversible (McLeod, 1974; Swain, 1979). Most of the positive effects of CT in ruminant nutrition are associated with its great affinity for leaf protein after mastication (Jones and Mangan, 1977). The CT-protein complexes are stable at rumen pH (6.0-7.0) but then release protein at pH < 3.5 in the abomasum and small intestine for hydrolysis and absorption (Jones and Mangan, 1977).

Waghorn *et al.* (1987) showed that CT in *L. corniculatus* markedly decreased rumen protein degradability and ammonia formation, and increased both the flux of EAA (52%) through the abomasum and their absorption (62%) from the small intestine. Subsequent grazing experiments with sheep showed that CT in *L. corniculatus* increased both ovulation rate and lambing percentage (20-27%) (Min *et al.*, 1999, 2001), and wool growth by up to 11% during summer (Wang *et al.*, 1996; Min *et al.*, 1998), without affecting voluntary feed intake (VFI).

The objectives of the present study were to measure in two consecutive years (i) the effects of grazing ewes in a commercial dryland farming system on *L. corniculatus* over the mating period upon reproductive efficiency and (ii) to define the length of time (weeks) that ewes need to graze *L. corniculatus* before mating to

maximise reproductive performance. Effects on ewe wool production and liveweight gain of their lambs to weaning were also measured.

3.2 MATERIALS AND METHODS

3.2.1. *Experimental design*

Two grazing experiments were carried out over the seasonal production of 2001 and 2002 at Massey University's Riverside farm in the Wairarapa, on the East Coast of the North Island of New Zealand. Drought conditions normally occur on the farm during summer-autumn periods (Jan – March). Experiment 1 was conducted from 13 February 2001 to 19 November 2001 (279 days). Experiment 2 commenced on 1 February 2002 and finished on 13 November 2002 (285 days).

Experiment 1 compared groups of 100 shorn mixed-age Romney ewes in LW light condition grazing swards of *L. corniculatus* (cv. Grasslands Goldie) (CT-acting) (57.0 ± 0.63 kg) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture (non-CT-containing) (55.4 ± 0.63 kg) for four weeks prior to mating and for two oestrous cycles during mating (1:50, ram : ewe ratio), lasting for a total of 63 days. In Experiment 2, groups of 75 mixed-age oestrus synchronised Romney ewes grazed *L. corniculatus* swards for 42 (55.5 ± 0.84 kg), 21 (55.5 ± 0.87 kg) and 10 (55.3 ± 0.87 kg) days before mating and then for a further 33 days during mating (1:40, ram : ewe ratio), whilst 75 ewes (55.2 ± 0.89 kg) grazed pasture as a control group during the whole period. In both experiments ewes grazing each forage were fed at the same dry matter (DM) allowance in weekly breaks. At the end of *L. corniculatus* feeding, all the ewes were combined into a

single group and grazed on pasture until the end of the experiment at weaning in November.

Reproductive efficiency in Experiment 1 was defined as scanning rate (foetus/100 ewes mated), pregnancy rate (ewes pregnant/100 ewes mated) and fecundity (percentage proportion of lambs born/100 ewes lambing). In Experiment 2, reproductive efficiency was measured as ovulation rate (*corpora lutea (CL)*/100 ewes mated) in the first synchronised oestrous cycle using laparoscopy and as lambing percentage (lambs born/100 ewes lambing).

3.2.2. Climatic factors

Annual rainfall and seasonal variation in both rainfall and soil temperatures (at a depth of 10 cm) were recorded at the farm during the two consecutive years. Summer conditions were characterised by low rainfall and high temperatures during January – March in both years (Table 3.1), with conditions being hotter and drier in Experiment 1 than in Experiment 2.

Table 3.1. Annual rainfall (mm) and seasonal soil (10 cm) and air temperatures during two consecutive years at Massey University's Riverside farm, in the Wairarapa on the East Coast of the Southern North Island, New Zealand.

	Experiment 1 (2001)	Experiment 2 (2002)
Annual rainfall (mm/ m ²)	1005 ± 13.67	995 ± 11.71
Summer / autumn:		
Rainfall (mm/ m ²)	142 ± 10.02	222± 2.17
No of rain days	13	18
Mean daily max soil temp (° C)	22	21
Mean daily min soil temp (° C)	14	12
Mean daily max air temp (° C)	24.1	21.7
Mean daily min air temp (° C)	11.1	11.6

3.2.3. Forages

In both experiments pure vegetative *L. corniculatus* (birdsfoot trefoil) and perennial ryegrass-white clover (*Lolium perenne*/Trifolium *repens*) pasture were grazed. Nine hectares of *L. corniculatus* were used for Experiment 1, and an additional 5 ha were established for Experiment 2. Surplus areas of *L. corniculatus* and pasture were lightly grazed by commercial flocks of sheep and cattle or topped mechanically to stimulate vegetative growth and maintain quality.

Measurements of pre-grazing and post-grazing herbage mass and botanical composition were determined for each weekly break by cutting 8 random quadrats (0.180 m²) per break of each forage to ground level. Samples were washed and dried overnight (16 h) in a forced-air oven (Contherm; Thermotec 2000; New Zealand) at 80° C.

Six wire mesh cages measuring 1.4 x 0.9 m were placed in each break immediately before sheep were introduced for grazing. At the end of grazing each break, the cages were removed and the forage was hand plucked down to a level corresponding to that eaten by the animals (diet selected). These samples were pooled and stored at -20°C for nutritive value analysis.

3.2.4. Grazing management

3.2.4.1. Experiment 1

Ewes were rotationally grazed on *L. corniculatus* or pasture at a feed allowance of 1.8 kg green DM/ewe/day (green DM = total dry matter – dead matter) for the first three weeks. The allowance was then increased to *ad libitum* (2.3 kg green DM/ewe/day) during the mating period for two cycles. Weekly breaks were used in both swards, with front and back electric fences. The area of each weekly break was calculated as:

$$\text{BA} = \frac{7 \text{ days} \times n \times \text{FA}}{\text{HM}}$$

Where HM is herbage mass (kg DM/ha), BA is break area (ha), n is number of ewes, and FA is feed allowance per head per day (kg). This management was in order to provide vegetative, high quality forage at all times. Ewes and rams had free access to water.

3.2.4.2. Experiment 2

A group of 75 ewes grazed *L. corniculatus* for 42 days before mating and two groups each of 75 ewes grazed perennial ryegrass/white clover pasture for 21 and 32 days before being transferred to *L. corniculatus* (grazing 21 and 10 days on *L. corniculatus* before mating). Another group of 75 ewes grazed on pasture as a control group. Feed allowance was initially 2.0 kg green DM/ewe/day and increased to 2.3 kg green DM/ewe/day during the mating period over two cycles.

3.2.5. Animal measurements

3.2.5.1. Experiment 1

Ewes were weighed (Tru-test, Auckland, New Zealand) and condition scored (Jefferies, 1961) at the start of the experiment and at two-weekly intervals until the end of the mating season and then at monthly intervals until the end of the experiment. Mating was undertaken using harnessed rams, with ewes marked recorded at five-day intervals. All ewes were pregnancy diagnosed at 60 days after the mid point of the mating period by trans-abdominal ultrasonography using a 3.5 MHz sector scanner. Dam, birth weight and birth rank (single or multiple) were recorded at lambing. Number of lambs surviving at docking and at weaning, and weaning weights were also measured. Ewes were shorn at weaning in late November, and fleece weight, and staple length were recorded.

3.2.5.2. Experiment 2

All ewes were weighed and condition scored as in Experiment 1. Oestrus was synchronised using controlled intravaginal release devices (CIDR; Pharmacia & Upjohn; containing 0.3 g progesterone). CIDR's were inserted into all ewes for 12 days of the first ovulatory cycle prior to introduction of rams. Ewes were mated using harnessed rams, which remained out for two cycles. Ovulation rate was determined by counting *corpora lutea* during cycle one using laparoscopy (Kelly and Allison, 1976), seven days after the start of the oestrus period. Dam, birth weight, birth rank and lamb viability from lambing to weaning were recorded. Wool production from ewes and staple length were also measured at weaning in late November.

3.2.6. Laboratory analyses

3.2.6.1. Forages

All samples of diet selected were stored at -20°C and freeze-dried using a Cuddon 0610 freeze drier (W.G.G. Cuddon LTD, Blenheim, New Zealand), and ground to pass a 1 mm diameter sieve (Wiley mill, Swedesboro, USA) before laboratory analysis. Total nitrogen (N) was determined by the Dumas principle (Leco CNS 2000 Analyser, Model 602 600 200, USA). Neutral detergent fibre (NDF) was determined by the detergent system of Robertson and Van Soest (1981), with alpha amylase (BDH, Poole, UK) being added during NDF extraction. Sodium sulphite was not added. Acetone/water-extractable, protein-bound and fibre-bound CT fractions in forages were determined using a butanol-HCL

colorimetric procedure (Terrill *et al.*, 1992), with total CT concentration being reported. All CT concentrations were determined using CT extracted from *Lotus pedunculatus* as a standard reference (Jackson *et al.*, 1996). *In vitro* organic matter digestibility (OMD) and digestible OM in the DM (DOMD) were measured using the enzymic procedure of Roughan and Holland (1977), with samples from *in vivo* digestibility trials used as standards, with pasture standards used for the *in vitro* determination of pasture samples, and *L. corniculatus* standards used for the *in vitro* determination of *L. corniculatus* samples.

3.2.6.2. Wool samples

Fleece weight was recorded in both Experiments by shearing the ewes at weaning. Wool samples (40 g) from both left and right mid-side areas were used to measure staple length (cm) from each animal by measuring the length of 10 randomly chosen unstretched staples along a ruler.

3.2.7. Statistical analyses

In Experiment 1, differences in forage chemical composition, forage botanical composition, pre-grazing and post-grazing herbage mass, VFI, daily liveweight gain, change in condition score, greasy fleece weight and staple length between pasture and lotus were assessed using the MIXED procedure of the SAS statistical package (SAS, 2001). Repeated measurements of live weight (LW) and condition score in the same animal were analysed using PROC MIXED (SAS, 2001). The linear model included the fixed effects of day, forage type and the forage type by day interaction, and the random effect of animal. Using the Akaike's information

criterion, a compound symmetric error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals (Littel *et al.*, 1998).

Least square means and their standard errors for litter size at scanning and number of lambs born were obtained with the MIXED procedure in SAS (2001) with the variable fitted being forage type. Chi-square test was performed using PROC FREQ (SAS, 2001) to test for significant differences between forages in the frequency of lambs at docking and at weaning. Values for conception rate, fecundity (singles or multiples at lambing) and mortality at weaning were analysed using the PROC GENMOD (SAS, 2001) with logit transformation assuming a binomial distribution. The model included the fixed effects of forage type. Data for lamb birth weight and weaning weight were performed using PROC MIXED (SAS, 2001). The linear model included the fixed effects of forage, sex, birth rank and the interaction among them.

In Experiment 2, differences in forage chemical composition, forage botanical composition, pre-grazing and post-grazing herbage mass, VFI, daily liveweight gain, change in condition score, greasy fleece weight and staple length between treatments (0, 10, 21, 42 days grazing on *L. corniculatus* before ovulation) were assessed using the MIXED procedure of the SAS statistical package (SAS, 2001). Repeated measurements of live weights and condition scores on the same animal were analysed using the MIXED procedure of SAS (2001). The linear model included the fixed effects of treatment, day, breed, the interaction between treatment, breed and day interaction, and the random effect of animal. A

compound symmetric error structure was found to be more appropriate for the data.

Ovulation data analysed in terms of *CL* /ewes mated, litter size at lambing, survival at 24 hours, percentages of lambs at docking and at weaning, and reproductive wastages were performed using PROC MIXED (SAS, 2001). The linear model considered the fixed effects of treatment. Means and standard errors for mortality at weaning were estimated using the PROC GENMOD (SAS, 2001) with logit transformation assuming a binomial distribution with the variable fitted being treatment. Correlations between LW, daily liveweight gain, body condition score and OR (square-root transformed; Snedecor and Cochran, 1980); were analysed using PROC CORR (SAS, 2001).

3.3 RESULTS

3.3.1. Forages and botanical composition

Both *L. corniculatus* and pasture swards were in the vegetative growth stage throughout the experiments. Pre-grazing herbage mass was similar between treatments in Experiment 1, but higher for *L. corniculatus* than for pasture forage in Experiment 2. In contrast, post-grazing pasture mass was lower for *L. corniculatus* than for pasture in both Experiments (Table 3.2). Pre- and post-grazing dead matter content was consistently greater in pasture than in *L. corniculatus* at the same stage of both experiments. For both *L. corniculatus* and pasture swards, leaf was the main component of the diet selected in both experiments, with small amounts of stem and white clover being consumed.

Table 3.2. Pre-grazing and post-grazing herbage mass (t DM/ha) and plant components of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture that were grazed during mating in 2001 (Experiment 1) and in 2002 (Experiment 2) on the East Coast in New Zealand.

	Experiment 1				Experiment 2			
	Pasture		<i>Lotus</i>		Pasture		<i>Lotus</i>	
	Pre-grazing (<i>n</i> = 10)	Post-grazing	Pre-grazing (<i>n</i> = 9)	Post-grazing	Pre-grazing (<i>n</i> = 11)	Post-grazing	Pre-grazing (<i>n</i> = 11)	Post-grazing
Herbage mass	2.00 ± 0.28	1.14 ± 0.14	1.98 ± 0.30	0.90 ± 0.15	3.07 ± 0.27	1.60 ± 0.14	3.86 ± 0.27	1.34 ± 0.14
Green DM	1.27 ± 0.27	0.50 ± 0.12	1.67 ± 0.28	0.37 ± 0.13	2.55 ± 0.25	1.12 ± 0.12	3.50 ± 0.25	0.92 ± 0.12
Dead matter	0.72 ± 0.07	0.64 ± 0.75	0.30 ± 0.07	0.54 ± 0.79	0.52 ± 0.07	0.47 ± 0.71	0.36 ± 0.07	0.42 ± 0.71
†	36.0	56.1	15.0	60.0	16.9	29.4	9.3	31.3
Leaves	0.88 ± 0.14	0.29 ± 0.07	1.04 ± 0.14	0.03 ± 0.08	1.90 ± 0.13	0.66 ± 0.74	1.14 ± 0.13	0.09 ± 0.07
Stems	0.22 ± 0.11	0.15 ± 0.11	0.45 ± 0.12	0.28 ± 0.12	0.27 ± 0.11	0.33 ± 0.11	1.38 ± 0.11	0.74 ± 0.11
White clover	0.08 ± 0.09	0.03 ± 0.03	0.03 ± 0.09	0.03 ± 0.03	0.25 ± 0.08	0.08 ± 0.03	0.31 ± 0.08	0.05 ± 0.03
Weeds	NL	NL	0.01 ± 0.08	NL	0.06 ± 0.07	0.01 ± 0.03	0.09 ± 0.07	0.01 ± 0.03

(*n*) Means are for weekly breaks with their standard errors. (S.E.M).

NL; No LS means were obtained because no weeds were present.

† % of total herbage mass.

3.3.2. Chemical composition

Total N concentration was higher for *L. corniculatus* ($P < 0.001$) than for pasture in Experiment 1 (Table 3.3), whilst NDF concentration was consistently lower for *L. corniculatus* than for pasture in Experiment 1 ($P < 0.001$) and in Experiment 2 ($P < 0.05$). Total CT concentration in *L. corniculatus* swards was 18.4 g CT/kg DM in Experiment 1 and 28.6 g CT/kg DM in Experiment 2; in both experiments only trace amounts of total CT were detected in pasture. Most CT in the *L. corniculatus* in Experiment 1 (58.6%) and Experiment 2 (63.7%) was readily extractable, with much smaller amounts being protein-bound (33.8% and 29.2%) or fibre-bound (7.9% and 7%) respectively. *In vitro* OMD, DOMD and estimated metabolisable energy concentration (ME) were all higher ($P < 0.001$) for *L. corniculatus* than for pasture in both experiments (Table 3.3), with the differences being larger in Experiment 1.

3.3.3. Live weight, wool production and wool characteristics

In Experiment 1, grazing on *L. corniculatus* increased liveweight gain (LWG) over the mating period ($P < 0.001$) (Table 3.4) and reduced the loss in body condition score ($P < 0.01$) relative to ewes that grazed pasture. These differences persisted throughout pregnancy and up to the start of lambing, but had disappeared by weaning in mid November (Fig. 3.1). Greasy fleece weight ($P < 0.01$) and staple length ($P < 0.05$) were greater at weaning in ewes that had grazed *L. corniculatus* for nine weeks during the mating period (Table 3.4).

Table 3.3. Mean values of total nitrogen (N), neutral detergent fibre (NDF), condensed tannin (CT), *in vitro* organic matter digestibility (OMD) and digestible organic matter in dry matter (DOMD), and estimated metabolisable energy concentration (ME, MJ/ kg DM) of diet selected by sheep grazing perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. during the mating season in two consecutive years under dryland farming conditions. Mean values with S.E.M.

	Experiment 1			Experiment 2		
	Pasture	Lotus	P	Pasture	Lotus	P
	(n = 10)	(n = 9)		(n = 10)	(n = 10)	
Total N (g/kg DM)	18.4 ± 0.82	28.4 ± 0.82	***	25.5 ± 1.59	26.0 ± 1.59	NS
NDF (g/kg DM)	563.6 ± 9.09	306.2 ± 9.59	***	460.6 ± 18.99	397.4 ± 18.99	*
	(n = 3) ^a	(n = 3)		(n = 5)	(n = 5)	
Total CT (g/kg DM)	1.76 ± 1.33	18.43 ± 1.33	***	1.26 ± 2.26	28.62 ± 2.26	***
Bound CT (% total CT) ^b	94	42		70	36	
	(n = 10) ^c	(n = 9)		(n = 10)	(n = 10)	
<i>In vitro</i>						
OMD	0.54 ± 0.007	0.72 ± 0.007	***	0.61 ± 0.011	0.68 ± 0.011	***
DOMD	0.51 ± 0.007	0.67 ± 0.008	***	0.55 ± 0.008	0.63 ± 0.008	***
ME	8.32 ± 0.11	10.93 ± 0.12	***	9.10 ± 0.14	10.35 ± 0.14	***

^a Subsamples for CT analysis.

^b % Bound CT = ((protein-bound + fibre-bound CT)/total CT).

^c Subsamples for *in vitro* analysis.

ME = DOMD × 16.3

NS: not significant ($P < 0.05$).

• ($P < 0.05$).

** ($P < 0.01$).

*** ($P < 0.001$).

Table 3.4. Experiment 1. Effect of grazing ewes on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. upon ewe liveweight change and body condition score during mating (72 days) and upon wool production and fibre length at weaning during 2001 in a dryland pastoral system. Mean values with S.E.M.

	Pasture	<i>Lotus</i>	<i>P</i>
	During mating		
	(<i>n</i> = 100)	(<i>n</i> = 100)	
Initial live weight (kg)	55.4 ± 0.63	57.0 ± 0.63	NS
Final live weight (kg) ¹	55.1 ± 0.63	61.6 ± 0.63	***
Liveweight change (g/day) ²	- 5 ± 5.0	67 ± 5.0	***
Initial condition score	2.58 ± 0.06	2.72 ± 0.06	NS
Final condition score	1.81 ± 0.06	2.24 ± 0.06	***
Condition score change	- 0.76 ± 0.06	- 0.48 ± 0.06	**
	At weaning		
	(<i>n</i> = 82)	(<i>n</i> = 86)	
Greasy fleece weight (kg)	2.90 ± 0.06	3.14 ± 0.05	**
Staple length (cm)	11.69 ± 0.19	12.26 ± 0.19	*

NS: not significant ($P < 0.05$).

* ($P < 0.05$).

** ($P < 0.01$).

*** ($P < 0.001$).

¹ Measured 72 days after initial live weight.

² Calculated during days 1 to 72 for both groups.

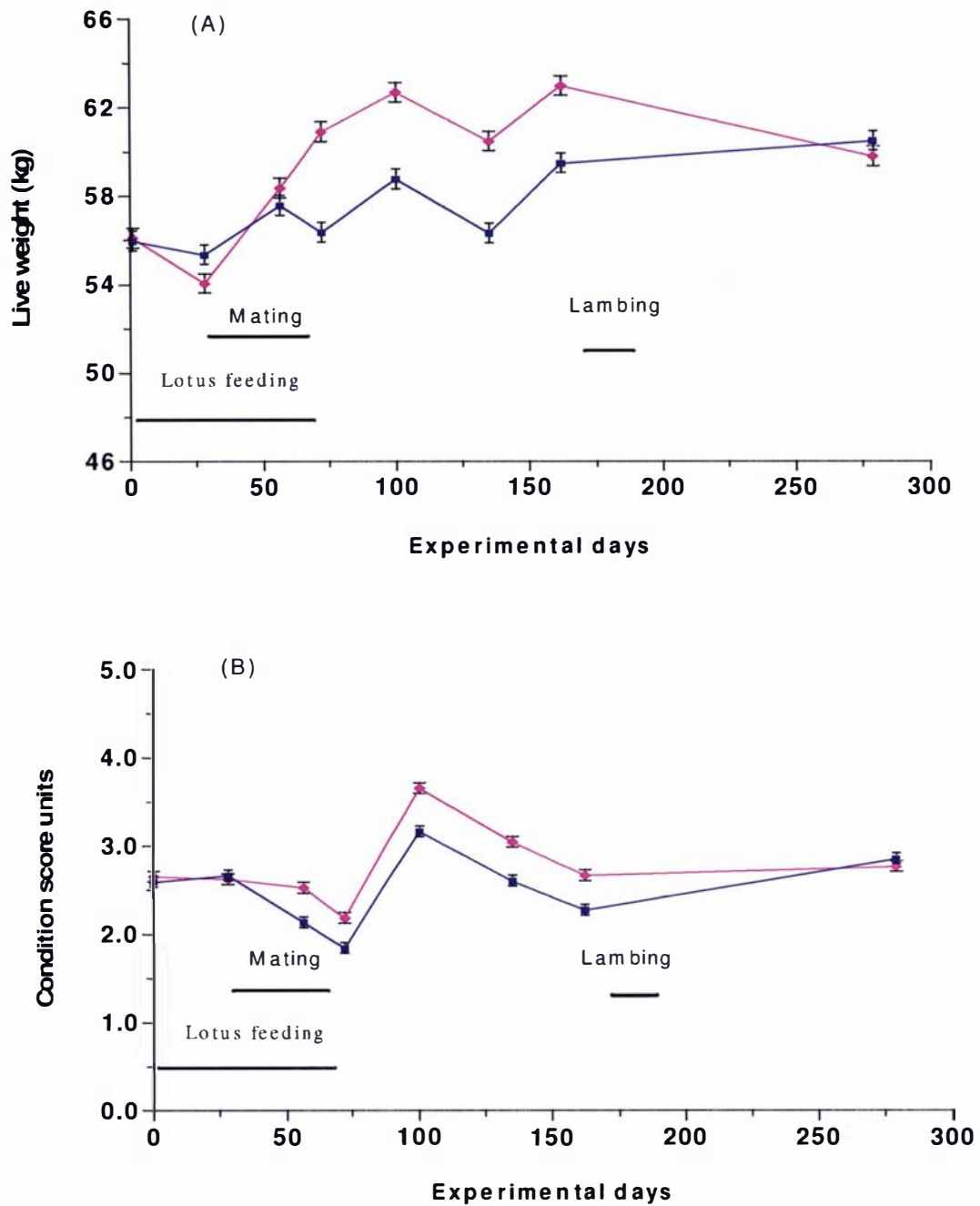


Figure 3.1. Experiment 1. (A) Mean live weight and (B) mean condition score of ewes fed (♦) *Lotus corniculatus* L. (birdsfoot trefoil) and (■) perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture over the mating period of 2001 and changes afterwards until weaning (I = S.E.M).

In Experiment 2, LW and body condition score increased during the mating season for all treatments (Table 3.5), with the increases being greater for ewes that were mated on *L. corniculatus* than on pasture ($P < 0.05$). The differences progressively decreased with time and had disappeared by weaning in mid November (Fig. 3.2). At shearing in late November, ewes that had grazed pasture only, or *L. corniculatus* during mating followed by pasture, had similar wool production and staple length (Table 3.5).

Table 3.5. Experiment 2. Comparative liveweight change, wool production, wool characteristics and condition score in 2002 of ewes fed perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture or *Lotus corniculatus* L. over the mating period (75 days). Mean values with S.E.M.

	Pasture	Days of <i>Lotus</i> feeding before cycle 1 mating		
	0	10	21	42
	(n = 75)	(n = 75)	(n = 75)	(n = 75)
Initial live weight (kg)	55.2 ± 0.89	55.3 ± 0.87	55.5 ± 0.87	55.5 ± 0.84
Final live weight (kg) ¹	59.1 ± 0.87 ^a	61.4 ± 0.87 ^b	60.8 ± 0.88	61.5 ± 0.85 ^b
Liveweight change (g/day) ²	56 ± 6.3 ^a	88 ± 6.2 ^b	77 ± 6.3 ^b	87 ± 6.1 ^b
Initial condition score	2.57 ± 0.08	2.57 ± 0.08	2.47 ± 0.08	2.62 ± 0.08
Final condition score	2.62 ± 0.08 ^a	2.83 ± 0.08	2.75 ± 0.08	2.96 ± 0.08 ^b
Condition score change	0.04 ± 0.05 ^a	0.23 ± 0.05 ^b	0.26 ± 0.05 ^b	0.32 ± 0.05 ^b
		At weaning		
	(n = 59)	(n = 61)	(n = 60)	(n = 61)
Greasy fleece weight (kg)	3.20 ± 0.08	3.25 ± 0.07	3.29 ± 0.07	3.21 ± 0.07
Staple length (cm)	11.67 ± 0.19	11.58 ± 0.19	11.96 ± 0.19	11.83 ± 0.19
Condition score	2.44 ± 0.08	2.54 ± 0.08	2.50 ± 0.09	2.49 ± 0.08

Means within the same row with differing superscripts are significantly different by analysis of variance. ($P < 0.05$).

¹ Measured 68 days after initial live weight.

² Calculated during days 1 to 68 for all groups.

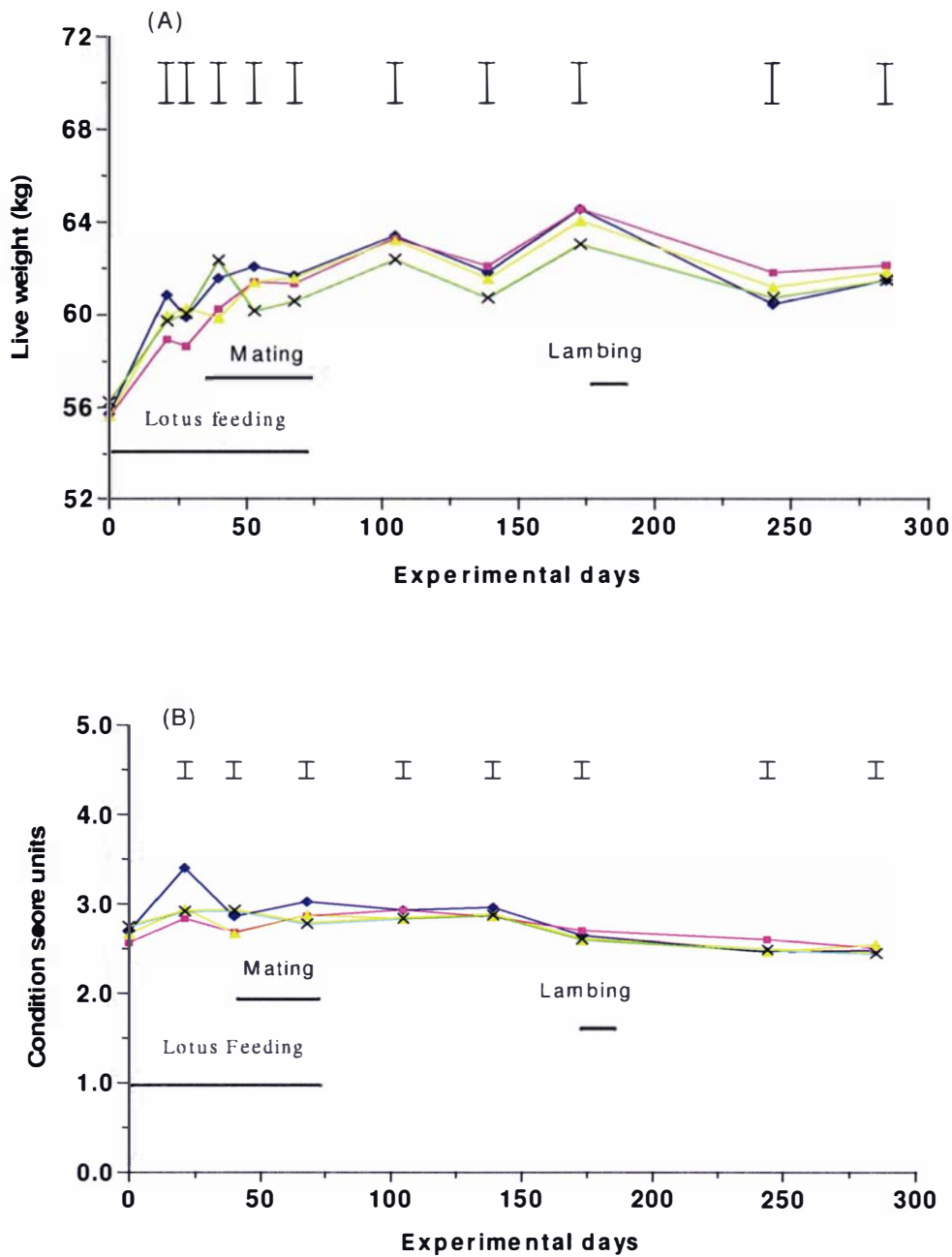


Figure 3.2. Experiment 2. (A) Comparative live weight and (B) condition score of ewes grazing (x) perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture and *Lotus corniculatus* L. (birdsfoot trefoil) over the mating period of 2002 and changes afterwards until weaning. Groups of ewes were grazed on *L. corniculatus* for (♦), 21 (■), 10 (▲) days before ovulation and continued on *L. corniculatus* during the mating. Vertical bars (I) represent pooled standard error for clearer interpretation of trends.

3.3.4. Reproductive rate, lamb survival and lamb body growth

In Experiment 1, there were no effects of forage type upon pregnancy rate (ewes pregnant/100 ewes mated). Ewes grazing *L. corniculatus* during the mating period for two cycles had greater reproductive efficiency at ultrasound scanning (foetus/100 ewes mated) and at lambing (lambs born/100 ewes lambing), compared to ewes grazing pasture ($P < 0.05$; Table 3.6). Also, the proportion of lambs surviving to weaning per lambs/100 ewes lambing were greater for ewes that were mated on *L. corniculatus* than for those mated on pasture ($P < 0.05$). The increased fecundity of ewes grazing *L. corniculatus* during mating is indicated by more ewes giving birth to multiple lambs ($P = 0.06$) and fewer ewes giving birth to one lamb ($P = 0.06$) than their counterparts grazing pasture (Table 3.6).

Birth weight and weaning weight were not affected by nutritional treatment and there were no interactions with birth rank at any stage. However, the percentage of lambs that survived from birth to weaning (Table 3.6) was greater ($P < 0.05$) in lambs that were conceived to dams which were grazed on *L. corniculatus* (88.3%) than those that were conceived to dams grazed on pasture (77.1%).

Table 3.6. Experiment 1. Effect of grazing ewes on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. on reproductive efficiency, lamb birth weight, lamb weaning weight and lamb mortality during 2001 in a dryland commercial pastoral system.

	Pasture	Lotus	P
<u>Reproductive efficiency</u>			
	(n = 93)	(n = 98)	
Scanning rate (foetus/ 100 ewes mated)	170	179	*
Pregnancy rate (ewes pregnant/ 100 ewes mated)	93 ± 40	98 ± 33	NS
	(n = 82)	(n = 87)	
Lambing (lambs born / 100 ewes lambing)	159	175	*
Fecundity (per 100 ewes/ lambing)			
Single bearing	40	26	P = 0.06
Multiple bearing	60	74	P = 0.06
(Confidence interval 95%)	0.49 to 0.70	0.63 to 0.82	
Docking (lambs/ 100 ewes lambing)	130	159	*
Weaning (lambs/ 100 ewes lambing)	123	155	*
<u>Lamb mortality</u>			
Birth – weaning (%)	22.9	11.7	*
<u>Birth weight</u>			
Singles male	6.43 ± 0.19	6.38 ± 0.37	NS
female	5.94 ± 0.22	5.91 ± 0.20	NS
Multiple male	4.93 ± 0.12	5.06 ± 0.09	NS
female	4.59 ± 0.11	4.60 ± 0.10	NS
<u>Weaning weight</u>			
Singles male	36.04 ± 1.29	38.56 ± 2.09	NS
female	35.32 ± 1.40	34.18 ± 1.16	NS
Twins male	29.75 ± 0.76	29.90 ± 0.58	NS
female	27.98 ± 0.75	27.03 ± 0.65	NS

NS: not significant ($P < 0.05$).

* ($P < 0.05$).

¹ Applies to multiple - bearing ewes.

In Experiment 2, during the first mating cycle (seven days after the start of mating), there was a linear relation between OR and the duration of grazing on *L. corniculatus* before mating ($P < 0.05$; Table 3.7). A similar trend was evident for lambs born and surviving to weaning, but this only attained statistical significance for lambs surviving 24 hours after birth ($P < 0.05$) and for lambs present at docking ($P < 0.10$). There were no significant differences between treatments in reproductive wastage and in lamb mortality from birth to weaning, even though this looked to decline with increased time of feeding on *L. corniculatus*.

Effects of the nutritional treatment upon mean birth weight with the interaction between birth rank and sex were not significant. By contrast, there were interactions between birth rank and sex upon weaning weight, with single male lambs conceived on *L. corniculatus* (10 days treatment; $P < 0.05$ and 21 days treatment; $P = 0.06$) being heavier than their counterparts conceived on pasture (Table 3.8). There were no treatment effects upon the weaning weight of multiple-born lambs.

3.3.5. Correlations.

There were weak positive relationships between ovulation rate and LW for ewes that were mated on *L. corniculatus*, but no such relationships for ewes that were mated on pasture (Table 3.9). There were no within-treatment relationships between OR and daily liveweight gain or between OR and body condition score.

Table 3.7. Experiment 2. Effect of grazing ewes on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. on reproductive efficiency for cycle one and lamb mortality during the productive season of 2002 in a dryland farming system.

	Pasture	Days of <i>Lotus</i> feeding before ovulation			Linear contrast
	0	10	21	42	
<u>Reproductive efficiency</u>					
	(n = 75)	(n = 75)	(n = 75)	(n = 75)	
Ovulation rate (<i>corpora lutea</i> / 100 ewes mated)	173 ± 7.89 ^a	182 ± 7.96	189 ± 7.75	200 ± 7.64 ^b	*
First cycle	(n = 51)	(n = 42)	(n = 50)	(n = 55)	
Lambing (lambs born/ 100 ewes lambing)	162 ± 8.34	171 ± 9.19	170 ± 8.42	176 ± 8.03	P = 0.28
Surviving after 24 hours (lambs / 100 ewes lambing)	131 ± 9.04 ^a	150 ± 9.96	158 ± 9.13 ^b	160 ± 8.70 ^b	*
Docking (lambs / 100 ewes lambing)	123 ± 9.08	130 ± 10.0	142 ± 9.17	141 ± 8.75	P = 0.10
Weaning (lambs / 100 ewes lambing)	123 ± 9.26	130 ± 10.2	136 ± 9.35	140 ± 8.91	P = 0.18
<u>Reproductive wastage</u>					
Ovulation – birth (%)	13.1 ± 3.1	14.5 ± 3.4	15.0 ± 3.1	13.4 ± 3.0	NS
Ovulation – weaning (%)	31.7 ± 4.7	32.9 ± 5.2	29.1 ± 4.8	30.0 ± 4.6	NS
<u>Lamb mortality</u>					
Birth – weaning (%)	24.1	22.5	19.5	20.6	NS

Number of ewes (n).

Means within the same row with differing superscripts are significantly different by analysis of variance (ANOVA). * (P < 0.05).

ANOVA also used for analyses of lamb mortality using logit-transformed data.

NS: no significance.

Table 3.8. Experiment 2. Comparative liveweight change of lambs conceived on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. over the productive season of 2002 in a commercial grazing dryland system on the East Coast of North Island of New Zealand. Mean values with S.E.M.

	Pasture	Days of <i>Lotus</i> feeding before mating		
	0	10	21	42
<u>Birth weight (kg)</u>				
Singles male	5.2 ± 0.33	5.6 ± 0.21	5.0 ± 0.29	5.3 ± 0.31
female	5.1 ± 0.25	4.9 ± 0.29	5.3 ± 0.26	5.2 ± 0.33
Multiple male	4.5 ± 0.15	4.4 ± 0.17	4.4 ± 0.14	4.3 ± 0.17
female	4.3 ± 0.14	4.5 ± 0.16	4.0 ± 0.17	4.3 ± 0.16
<u>Weaning weight (kg)</u>				
Singles male	26.3 ± 1.91 ^a	31.9 ± 1.18 ^b	31.2 ± 1.60	30.7 ± 1.86
female	28.5 ± 1.55	26.2 ± 1.74 ^a	30.9 ± 1.57 ^b	30.7 ± 1.90
Multiple male	25.0 ± 0.91	26.1 ± 0.99	27.3 ± 0.92	25.2 ± 0.96
female	23.9 ± 0.85	24.7 ± 0.95	24.3 ± 1.09	24.0 ± 0.90

Means within the same row with differing superscripts are significantly different by analysis of variance. ($P < 0.05$).

Table 3.9. Correlation coefficients between daily live weight gain, body condition score and ovulation rate (OR; square-root transformed) for cycle one in groups of ewes grazing perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L. (birdsfoot trefoil) over the mating season of 2002 in a commercial dryland farming system.

	Pasture			Days of <i>Lotus</i> feeding before mating								
	0			10			21			42		
	r	P	(n)	r	P	(n)	r	P	(n)	r	P	(n)
Live weight ¹	0.07	NS	69	0.23	0.06	70	0.36	0.05	72	0.21	0.08	74
Liveweight change (g/day) ²	0.07	NS	69	0.07	NS	70	0.14	NS	72	0.03	NS	74
Body condition score ³	0.13	NS	69	0.002	NS	70	0.09	NS	72	0.002	NS	74

(n) Number of animals for analysis.

¹ Measured at day 53 for all groups.

² Calculated between days 1 to 53 for all groups.

³ Measured at day 40 for all groups.

NS: no significance.

3.4 DISCUSSION

The objectives of these experiments were firstly to establish the effects of feeding *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) before and during mating upon reproductive efficiency in a commercial dryland farming system and to define the length of time that ewes need to graze *L. corniculatus* before mating to maximise reproductive performance. Secondary objectives were to establish the effect of feeding *L. corniculatus* during the mating period on wool production of the ewe and live weight to weaning of their lambs. The most significant findings were that grazing ewes on *L. corniculatus* rather than pasture before and over the mating period in summer-autumn increased the number of lambs born and weaned by 16 and 32% units (Experiment 1) and increased OR and number of lambs weaned by up to 16 and 14% units respectively (Experiment 2). The increase in reproductive efficiency was due to increases in ovulation rate in Experiment 2 and to possibly increases in fecundity in Experiment 1, with more multiple births and less single births for ewes that were mated on *L. corniculatus* ($P = 0.06$).

Responses in cycle one of Experiment 2 showed that increases in duration of grazing *L. corniculatus* before mating of up to 42 days increased OR. These results confirm the effects of mating upon *L. corniculatus* in increasing OR as summarised by Min *et al.* (2003) and extend the findings to commercial dryland farming. These studies have also opened up the possibility for the first time that mating ewes on *L. corniculatus* may reduce post-natal lamb mortality. Differences between Experiment 1 and 2 may be related to ewe numbers and to the length of

time that *L. corniculatus* was fed into pregnancy. Using the data generated in Experiments 1 and 2, the numbers of ewes/group needed to detect treatment differences in post-natal mortality at the 5% level of probability with a power of 80% can be calculated and are shown in Table 3.10. This shows that to have a reasonable probability of detecting treatment differences with two animal groups (control and *L. corniculatus*), the number of ewes per group needs to be dramatically increased. A minimum of 350 ewes per group is suggested to detect a 30% reduction in mortality.

An approximate measure of voluntary feed intake can be calculated from pre-grazing and post-grazing pasture masses and the areas of each feed that were grazed. The approximate VFI was similar for ewes mated on lotus or pasture in Experiment 1 (1.45 vs. 1.63 kg DM/ewe/day) and Experiment 2 (1.63 vs. 1.32 kg DM/ewe/day). Therefore, the greater LW and liveweight gain in sheep mated on *L. corniculatus* compared to sheep mated on pasture in both experiments can be explained by the higher OMD, DOMD and estimated ME values for *L. corniculatus*. However, the weak positive correlation between OR and live weight for ewes mated on *L. corniculatus* and the absence of a relationship between OR and live weight for ewes mated on pasture, suggests that the higher LW of ewes grazing on *L. corniculatus* was not a major factor explaining their greater reproductive rate. Similarly, as there was no relationship between OR and liveweight change and OR and body condition score, it is also evident that the higher values of both of these for *L. corniculatus*-fed ewes does not explain their greater reproductive rate.

Table 3.10. Estimated number of lambs and ewes needed to detect treatment differences in lamb mortality between birth and weaning at the 5% level of probability, based upon variation in the lamb mortality data generated between birth and weaning in Experiments 1 and 2.

Reduction in post-natal lamb mortality (birth to weaning)	Experiment 1 ^a data		Experiment 2 ^b data	
	(%)	Lambs/treatment	Ewes/treatment	Lambs/treatment
10	5092	2909	4767	2771
20	1221	698	1144	665
30	518	296	485	282
40	276	158	259	151
50	166	95	156	91

^a Based on mortality of 22.9% for lambs born to control ewes in Experiment 1.

^b Based on mortality of 24.1% for lambs born to control ewes in Experiment 2.

Thus, relative to ewes grazed on pasture, the increased reproductive efficiency of ewes mated on *L. corniculatus* in both experiments suggests that the effect was mediated by the CT in *L. corniculatus*, (Min *et al.*, 1999, 2001), which reduces proteolysis of forage protein in the rumen (Jones and Mangan, 1977), reduces rumen and plasma ammonia concentrations, reduces blood plasma urea concentration (Min *et al.*, 1999, 2001) and increases the net absorption of EAA (Waghorn *et al.*, 1987) especially branched chain amino acids (Min *et al.*, 1999) from the small intestine. These metabolic changes may promote events such as folliculogenesis (i.e. from final stages to ovulation), conception, attachment, embryo survival, foetal growth and lamb viability.

Smith *et al.* (1983) found that, although *ad libitum* pasture-feeding of ewes for up to six weeks prior to mating (flushing) linearly increased OR in lighter ewes, there was no increase in the proportion of ewes lambing multiples after three weeks of flushing, due to increased embryonic losses in groups flushed for four to six weeks. Possible reasons for this difference are that even though the protein availability in the diet increases OR through increased blood concentration of BCAA (Waghorn *et al.*, 1990; Downing and Scaramuzzi, 1991; Downing *et al.*, 1995), the high intake of dietary soluble protein may have led to increased embryonic mortality (Visek, 1984; Butler, 1998).

McRae (1984) postulated that the uterine environment (luminal and/or endometrial) is regulated by a blood-uterine lumen barrier, which regulates complex processes of permeability (influx and efflux), secretion and reabsorption. Feeding excess

crude protein (CP; 17 to 19%) diets in cattle (Buttler, 2000) during the luteal phase decreases uterine pH (Elrod and Buttler, 1993; Elrod *et al.*, 1993), alters the concentration of other ions in uterine secretions (Buttler, 2000), and reduces the survival of ova, sperm, embryos and uterine receptivity (Blanchard *et al.*, 1990). In more recent studies (McEvoy *et al.*, 1997) stated that feeding a surplus of rumen degradable protein (RDP) to sheep for nine weeks increases ammonia and urea concentration in both blood plasma and the utero-oviductal microenvironment, with the concentration of ammonia and urea in the utero lumen being correlated with urea concentration in blood plasma. In consequence, embryo survival three days after insemination is decreased due to embryotoxic substances that disrupt the oviduct environment (Fahey *et al.*, 1998; McEvoy, *et al.*, 2001) and alter the ovine embryo critical fourth cell cycle (Findlay *et al.*, 1990; Fahey *et al.*, 1998). Furthermore, in the long term, non-lethal reprogramming of the embryo with altered foetal growth and delayed parturition may occur (McEvoy *et al.*, 1997).

This, therefore, suggests that the linear effect of the duration of grazing *L. corniculatus* before mating on OR (Experiment 2) is probably due to the effect of CT reducing rumen protein degradation to ammonia and increasing EAA absorption, and thus reducing early embryonic losses (Min *et al.*, 2001) and increasing both neonatal viability and weaning percentages (Experiment 1) under some circumstances. It is likely that these effects are mediated possibly through changes in uterine environment. Nevertheless, it also has to be recognised that in the studies conducted to date, effects upon embryonic and post-natal survival have not always been repeatable from trial to trial, probably because of the disparity of

the duration of *L. corniculatus* feeding regimes between experiments. Thus, as experiments have concentrated on feeding *L. corniculatus* before and during mating, the length of feeding it on into early pregnancy has not been well defined. This may explain the difference between Experiment 1 and Experiment 2. Experiment 1 provided ewes with *L. corniculatus* well into pregnancy, but the emphasis in Experiment 2 on extended pre-mating feeding of *L. corniculatus* reduced the duration of its availability after mating. Further studies are required to define the length of time that is necessary to graze *L. corniculatus* after conception, relative to control ewes grazing perennial/white clover pasture, to investigate whether a reduction in reproductive wastage from ovulation to weaning under dryland farming conditions occurs.

Wool growth is dependent upon the availability and absorption of EAA especially sulphur-containing amino acids from the small intestine (Reis, 1979). Ewes grazing *L. corniculatus* during the mating season produced more wool than their pasture-fed counterparts in Experiment 1, but not in Experiment 2; a difference that appears to correspond to lower summer/autumn rainfall in 2001 than in 2002 (Table 3.1). Therefore, perhaps a short period of feeding *L. corniculatus* during mating may benefit wool production more during drought years than in non-drought years.

The greater reproductive efficiency in ewes mated on *L. corniculatus* relative to ewes mated on pasture in both experiments confirms earlier findings that mating on *L. corniculatus* increases OR (Min *et al.*, 2003) and may also increase lamb survival, although further experimentation is necessary to investigate this. The

larger responses in both reproductive rate and wool production in Experiment 1 (a drought year) than in Experiment 2 (a non-drought year) suggests that in dryland farming best responses to inputs of *L. corniculatus* can be expected in drought seasons.

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CHAPTER 4.

USE OF *LOTUS CORNICULATUS* CONTAINING CONDENSED TANNINS TO INCREASE LAMB GROWTH OVER THE SUMMER UNDER COMMERCIAL DRYLAND FARMING CONDITIONS WITH MINIMAL ANTHELMINTIC DRENCH INPUT



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ABSTRACT

A rotational grazing experiment was conducted for 95 days in the summer of 2002/2003 under dryland farming conditions to compare the effects of grazing *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture on live weight and carcass gain, and dynamics of nematode parasite infection, in Suffolk x Romney weaned lambs fed *ad libitum*. Half of the lambs ($n = 30$) grazing either *L. corniculatus* or pasture received oral anthelmintic at the start and at monthly intervals (regular-drenched groups), whilst the remaining 30 lambs in each treatment only received oral anthelmintic when mean faecal nematode egg counts (FECs) exceed 1,000 eggs/g wet faeces (trigger-drenched groups) and liveweight gains were depressed. This occurred on day 58 only for both groups. Trigger and regular-drench lambs grazed separate areas.

Total condensed tannin (CT) concentration in the diet selected was 31 to 40 g CT/kg DM for *L. corniculatus*, with only trace amounts in pasture. *In vitro* organic matter digestibility (OMD), digestible organic matter in dry matter (DOMD), and estimated metabolisable energy concentration (ME) were higher for *L. corniculatus* than for pasture and declined less under drought conditions than the grass-based pasture.

Regular-drenched lambs grazing *L. corniculatus* had significantly higher liveweight gains (LWG; 298 g/day) and carcass weight gains (133 g/day) than all the other groups, whilst trigger-drenched lambs grazing *L. corniculatus* had significantly

greater LWG (228 g/day) and carcass gains (99 g/day) than regular-drenched (200; 66 g/day) and trigger-drenched (187; 63 g/day) lambs grazing pasture. Carcass fatness was significantly lower for trigger-drenched lambs than for regular-drenched lambs, when fed either *L. corniculatus* or pasture. Dag score was consistently lower for regular-drenched lambs grazing *L. corniculatus* than pasture; trigger-drenched lambs showed similar effects up to day 48, with no differences between the two groups thereafter. Regular anthelmintic treatment maintained FECs at low values, while trigger-drenched lambs on *L. corniculatus* tended to have higher FECs than pasture-fed lambs. Relative to trigger-drenched lambs that grazed pasture, grazing on *L. corniculatus* reduced significantly the worm burdens at slaughter of *Haemochus contortus*, *Teladorsagia spp.*, *Nematodirus spp.* and *Cooperia spp.*, but greater burdens of *Trichostrongylus spp.*, *Chabertia ovina*, *Oesophagostomum spp.* and *Trichuris ovis* were present in *L. corniculatus*-fed lambs.

It was concluded that grazing *L. corniculatus* (birdsfoot trefoil cv. Grasslands Goldie) under dryland farming conditions can increase LWG and carcass gain of weaned lambs, whilst reducing reliance on anthelmintic drenches to control parasites. These effects are probably due to increased protein supply from the action of CT enabling the lambs to have a higher LWG when carrying a parasite burden, and to *L. corniculatus* maintaining its high ME value under drought conditions. Using *L. corniculatus* to finish weaned lambs without anthelmintic drenches for a seven-week period is proposed.

Keywords: *Lotus corniculatus*, perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture, condensed tannins, oral anthelmintic, dryland farming systems.

Abbreviations: **BA**, break area; **CT**, condensed tannins; [¹⁴C]CT, ¹⁴carbon-labelled CT; **cv**, cultivar; **CW**, carcass weight; **CWG**, carcass weight gain; **DM**, dry matter; **cm**, centimetre; **DOMD**, digestible organic matter in the dry matter (g)/100 g DM; **EAA**, essential amino acid; **FA**, feed allowance; **FCW**, final carcass weight; **FECs**, faecal egg counts; **GR**, carcass fatness; **HM** herbage mass; **LW**, live weight; **Ltd**, limited; **LWG**, liveweight gain; **ME**, metabolisable energy; **MJ**, mega joules; **µg**, micro; **NDF**, neutral detergent fibre; **NZ**; New Zealand, **OMD** organic matter digestibility; ®; Registered; **SAS**, Statistical Analysis System; **VFI**, voluntary feed intake; **WC**, worm counts.

4.1 INTRODUCTION

A New Zealand (NZ) sheep industry target is to achieve lamb liveweight gains of 400 g/day under pastoral conditions to meet increased market demands and to maintain their position in the international meat industry (The New Zealand Sheep Council, 2000). Premium prices for lamb could be paid if systems could be developed using specialist forages with a higher feeding value than perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture, whilst also reducing the internal parasite problems using low-chemical inputs to prevent anthelmintic drench resistance, improve feed conversion efficiency and decrease the risk of chemical residues in lamb meat to the final consumer.

Condensed tannins (CT) are plant secondary compounds that bind strongly with leaf protein after chewing (Jones and Mangan, 1977), reducing protein degradation in the rumen at pH (6.0-7.0); the CT-protein complex then dissociates at pH < 3.5 in the abomasum (Jones and Mangan, 1977) increasing essential amino acid (EAA) absorption in the small intestine in sheep fed *L. corniculatus* (Waghorn *et al.*, 1987). Ramírez-Restrepo *et al.* (2002, 2004a) showed that sustainable farming systems may be developed in dryland areas during the spring/early summer lactation period with no pre-lambing anthelmintic drenching, using the CT-containing legume *Lotus corniculatus* fed to ewes and lambs between birth and weaning. These effects were probably due to the CT in lotus reducing the motility of infective L₃ internal parasite-larvae and disrupting the development of eggs and larval moults (Molan *et al.*, 1999, 2000a) and to the increased EAA absorption enabling the animal to develop increased tolerance to worm burdens, with a substantial increased sheep productivity. Therefore, use of CT-containing *L. corniculatus* as a specialist feed may contribute to low chemical input sheep farming systems through simultaneously increasing lamb growth and reducing anthelmintic drenching requirements between weaning and slaughter.

The first objective of this experiment was to compare the growth of weaned lambs grazing *Lotus corniculatus* (Birdsfoot trefoil cv. Grasslands Goldie) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) at high allowances, such that leaf only was consumed, with the objective of allowing a liveweight gain (LWG) of 400 g/day as possible. The second objective was to achieve this with the least possible amount of anthelmintic drench, using a systems approach, where

plant production, animal performance and parasite epidemiology were measured at the same time.

4.2 MATERIALS AND METHODS

4.2.1. *Experimental design*

A rotational grazing experiment was conducted from 18 November 2002 to 21 February 2003 (95 days) at Massey's Riverside farm in the Wairarapa, on the East Coast of the Southern North Island, New Zealand. Following weaning in spring 2002, 120 Suffolk x Romney weaned male lambs were selected from a pool of 344 lambs that were conceived and developed early embryonic stages when their dams grazed on either *L. corniculatus* or perennial ryegrass/white clover pasture (Ramírez-Restrepo *et al.*, 2004b). Lambs were balanced for live weight and conception treatment and randomly allocated to *L. corniculatus* ($n = 60$) or perennial ryegrass/white clover pasture ($n = 60$). Half of each group ($n = 30$) was drenched at the start of the experiment with ivermectin (Ivomec[®]; Merk, Sharp and Dohme, NZ Ltd) and regularly drenched at approximately four-weekly intervals to control internal nematode parasites. The other half ($n = 30$) were not treated at the beginning of the experiment and were only drenched with anthelmintic when the group mean faecal nematode egg counts (FECs) exceed 1,000 eggs/g wet faeces (trigger-drenched groups) and lambs experienced reduced mean liveweight gains. Trigger drench was administered to both the *L. corniculatus* and pasture groups at the same time. Regular-drenched lambs grazed on separate replicates of each forage from trigger-drenched lambs.

Variables investigated were LWG, the incidence of dags (accumulation of faeces in the wool surrounding the anus), FECs, cultured larvae counts, carcass characteristics and worm counts (WC) in the digestive tract at slaughter.

4.2.2. Forages

Pure vegetative swards of *L. corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture were grazed in weekly breaks by the lambs. After grazing, areas of legume and pasture were lightly grazed by commercial flocks of sheep and cattle or topped mechanically to reduce the proportion of stem and remove any flowering tissue, to stimulate vegetative growth and provide high-quality forage in both swards.

Measurements of pre-grazing and post-grazing herbage mass and botanical composition were determined for each weekly break by cutting 8 random quadrats (0.180 m²) per break of each forage to ground level. Samples were washed and dried overnight (16 h) in a forced-air oven (Contherm; Thermotec 2000; New Zealand) at 80° C.

Six wire mesh cages measuring 1.4 x 0.9 m were placed in each break immediately before animals were introduced for grazing. At the end of grazing that break, the cages were removed and the forage was hand plucked corresponding to what animals were observed to be eating (diet selected). These samples were pooled and stored at – 20° C for nutritive value analysis.

4.2.3. Grazing management

Lambs were rotationally grazed on *L. corniculatus* or pasture at a feed allowance of 6.0 kg green DM/lamb/day (total DM – dead DM) for the first 8 weeks of feeding and increased to 8.0 kg green DM/lamb/day from ninth week until the end the experiment (13th week). Weekly breaks were used, with front and back electric fences. Area of each weekly break for regular drenched and restricted drench lambs in both swards were calculated as:

$$BA = \frac{7 \text{ days} \times n \times FA}{HM}$$

Where HM is herbage mass (kg DM/ha), BA is break area (ha), n is number of lambs, and FA is feed allowance per head per day (kg). This management was in order to provide vegetative high quality forage at all times. Lambs had free access to water.

4.2.4. Animal measurements

Live weight was measured at days 0, 16, 30, 51, 58, 65, 79, 95 using electronic scales (Tru-test, Auckland, New Zealand). Rectal faecal samples were collected on day 0, 14, 28, 49, 63, 77 and 93 to estimate FECs from all trigger-drenched lambs and from 18 regular-drenched randomly selected lambs grazing each sward. The same lambs were sampled on each occasion. Dag score was performed as described by Ramírez-Restrepo *et al.* (2004a) for all lambs in each group.

Initial carcass weight was estimated through slaughter of 20-non experimental lambs at the start of the experiment, to determine the ratio of carcass weight (CW) to live weight (LW; 0.47). This was then applied to the initial LW of the experimental lambs to provide an estimate of the initial carcass weight of each lamb used in this trial.

At the end of the trial, all lambs were weighed on the farm and slaughtered at a commercial abattoir. Values of CW and carcass fatness (GR; a measurement of subcutaneous fat depth measured as soft tissue depth over the 12th rib at a point 11 cm from the dorsal midline; Kirton, 1989) were recorded for all lambs. Abomasal, and small and large intestines for WC were collected from twelve regular-drenched and twelve trigger-drenched randomly selected lambs grazing each sward and stored at – 20° C.

4.2.5. Laboratory analyses

4.2.5.1. Forages

All samples of diet selected were stored at – 20° C and freeze-dried using a Cuddon 0610 freeze drier (W.G.G. Cuddon LTD, Blenheim, New Zealand), and ground to pass a 1 mm diameter sieve (Wiley mill, Swedesboro, USA) before laboratory analysis. Total nitrogen (N) was determined by the Dumas principle (Leco CNS 2000 Analyser, Model 602 600 200, USA). Neutral detergent fibre (NDF) was determined by the detergent system of Robertson and Van Soest (1981), with alpha amylase (BDH, Poole, UK) being added during NDF extraction. Sodium sulphite was not added. Acetone/water-extractable, protein-bound and

fibre-bound CT fractions in forages were determined using a butanol-HCL colorimetric procedure (Terrill *et al.*, 1992), with total CT concentration being reported. All CT concentrations were determined using CT extracted from *Lotus pedunculatus* as a standard reference (Jackson *et al.*, 1996). Phenolic glycosides were determined using the high-performance liquid chromatographic (HPLC) procedure of Meier *et al.* (1988). *In vitro* organic matter digestibility (OMD) and digestible OM in the DM (DOMD) were measured using the enzymic procedure of Roughan and Holland (1977), with samples from *in vivo* digestibility trials used as standards, with pasture standards used for the *in vitro* determination of pasture samples, and *L. corniculatus* standards used for the *in vitro* determination of *L. corniculatus* samples.

4.2.5.2. Parasitological techniques

Faecal samples for FECs were refrigerated overnight (4° C) and FECs determined using a modified McMaster method (Stafford *et al.*, 1994) where each egg counted represented 50 eggs/g of wet faeces. Larval cultures were made from pooled faeces from each group mixed with vermiculite and water and cultured at 25° C for 10 days. Larvae were recovered using a Baermann technique (Ministry of Agriculture, Fisheries and Food, 1986). Worm burdens were estimated from counts in 5% aliquots (Wood *et al.*, 1995). Up to 50 male nematodes from each genus were collected for speciation (Uriarte *et al.*, 2003).

4.2.6. Statistical analyses

Differences in forage chemical composition, forage botanical composition, pre-grazing and post-grazing herbage mass were assessed using the MIXED procedure of the SAS statistical package (SAS, 2001), with a linear model that included the effects of forage type (*L. corniculatus* or pasture), anthelmintic treatment and the interaction between them as factors. Data for LW, LWG, carcass weight gain (CWG), CW and carcass fatness were analysed using the MIXED procedure of SAS (2001). The linear model included the fixed effects of conception treatment (i.e. lambs conceived when dams grazing *L. corniculatus* or pasture), forage type, anthelmintic treatment and their interactions. Litter size at birth and the initial LW for each period were included as covariate for LWG, initial carcass weight was included as covariable for CWG and final carcass weight was included as covariable for carcass fatness.

Faecal egg count data were normalised after square root transformation (Snedecor and Cochran, 1980). Repeated measurements of LW, FECs and dag score were then analysed using PROC MIXED (SAS, 2001), with a linear model that included the effects of conception treatment, forage type, anthelmintic treatment, day, forage type by anthelmintic treatment interaction and the interactions amongst forage type, anthelmintic treatment and day. Using the Akaike's information criterion, a compound symmetric error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals for LW, transformed FECs and dag score (Littel *et al.*, 1998). Correlations

between dag score and FECs (square-root transformed; Snedecor and Cochran, 1980); were analysed using PROC CORR (SAS, 2001).

Significant differences between treatments in the frequency of infective larvae of each parasite genus in lamb faeces were performed using the PROC GENMOD (SAS, 2001) with logit transformation, assuming a binomial distribution. The linear model included only the fixed effects of treatment. Worm counts at slaughter for each parasite species were analysed with a linear model that included the effect of forage type using PROC GENMOD (SAS, 2001), assuming a Poisson distribution and therefore a log link function was declared. A second analysis considered the effect of sex of parasite within each combination of parasite species and forage type. The linear model considered the fixed effects of forage type, parasite species and their interaction. Differences between species for abomasal, small and large intestine parasites were performed using the PROC GENMOD (SAS, 2001), assuming a Poisson distribution and therefore a log link function was declared. The linear model considered the fixed effects of forage type, parasite species and their interaction.

4.3 RESULTS

4.3.1. Forages and botanical composition

Both *L. corniculatus* and perennial ryegrass/white clover pasture swards were in the vegetative growth stage throughout most the experiment, with some reproductive growth caused by the hot summer conditions. Pre-grazing and post-grazing herbage mass were higher for *L. corniculatus* than for pasture (Table 4.1),

with pre-grazing and post-grazing dead matter content being consistently greater for pasture than for *L. corniculatus* especially from week six to the end of the experiment (Fig. 4.1). For *L. corniculatus*, diet selected was predominantly leaf, with negligible amounts of stem or white clover being consumed. In contrast, the grass stem and white clover components of pasture swards accounted for 20 and 17% respectively of the diet selected, with grass leaves accounting for approximately 60% of the diet selected.

Table 4.1. Pre-grazing and post-grazing herbage mass (t DM/ha) and plant component of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) over the spring/summer season of 2002 and 2003 in a commercial farm on the East Coast in New Zealand.

	<i>Lotus</i>				Pasture			
	Regular-drenched		Trigger-drenched		Regular drenched		Trigger-drenched	
	Pre-grazing (<i>n</i> = 13)	Post-grazing	Pre-grazing (<i>n</i> = 13)	Post-grazing	Pre-grazing (<i>n</i> = 12)	Post-grazing	Pre-grazing (<i>n</i> = 12)	Post-grazing
Herbage mass	3.28 ± 0.27	2.26 ± 0.23	3.24 ± 0.27	2.20 ± 0.23	2.68 ± 0.28	1.74 ± 0.23	2.51 ± 0.28	1.79 ± 0.23
Green DM	3.20 ± 0.28	2.07 ± 0.23	3.07 ± 0.28	2.01 ± 0.23	2.36 ± 0.29	1.42 ± 0.23	2.20 ± 0.29	1.42 ± 0.23
Dead matter	0.07 ± 0.04	0.18 ± 0.06	0.16 ± 0.04	0.18 ± 0.06	0.28 ± 0.04	0.30 ± 0.06	0.23 ± 0.04	0.35 ± 0.06
†	2.1	7.9	4.9	8.1	10.4	17.2	9.1	19.5
Leaves	1.17 ± 0.14	0.51 ± 0.09	1.26 ± 0.14	0.56 ± 0.09	1.36 ± 0.14	0.73 ± 0.09	1.39 ± 0.14	0.82 ± 0.09
Stems	1.11 ± 0.12	1.11 ± 0.12	1.14 ± 0.12	0.97 ± 0.12	0.44 ± 0.13	0.18 ± 0.13	0.35 ± 0.13	0.21 ± 0.13
White clover	0.35 ± 0.08	0.26 ± 0.06	0.38 ± 0.08	0.31 ± 0.06	0.31 ± 0.08	0.08 ± 0.06	0.29 ± 0.08	0.18 ± 0.06
Weeds	0.16 ± 0.06	0.15 ± 0.06	0.19 ± 0.06	0.16 ± 0.06	0.18 ± 0.06	0.28 ± 0.07	0.24 ± 0.06	0.17 ± 0.07

(*n*) Means are for weekly breaks with their standard errors (S.E.M.).

† Percentage dead matter of total herbage mass.

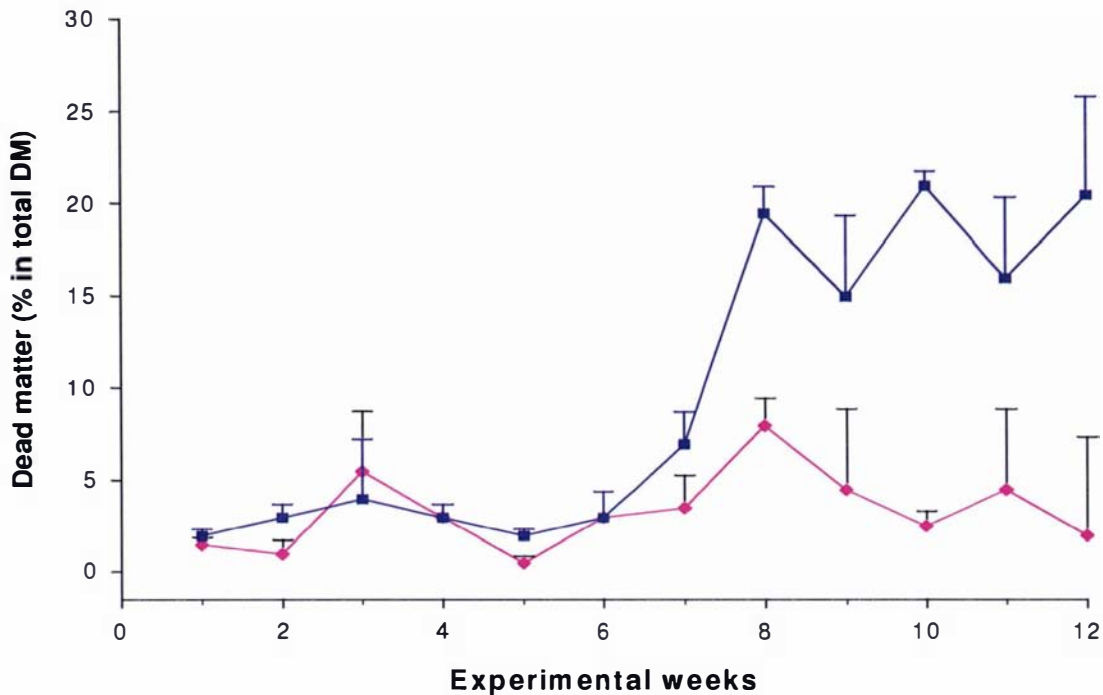


Figure 4.1. Pre-grazing dead matter in areas of *Lotus corniculatus* (◆) and perennial ryegrass/white clover pasture (■) grazed by groups or weaned lambs regularly anthelmintic treated or trigger-drenched over the spring/summer season of 2002 and 2003 in a commercial dryland system on the East Coast of New Zealand. Vertical bars represent one standard error of the mean.

4.3.2. Chemical composition

Total N concentration was slightly higher for *L. corniculatus* than for pasture in the diet selected (Table 4.2), whilst NDF concentration was consistently lower ($P < 0.001$) in lotus than in pasture. Total CT concentration in lotus swards was 35.6 g CT/kg DM. Only trace amounts of total CT were detected in the pasture. Most CT in the *L. corniculatus* selected was readily extractable (71.2%), with much smaller amounts being protein-bound (24.3%) or fibre-bound (4.5%). *In vitro* OMD, DOMD and estimated metabolisable energy concentration (ME) were all higher ($P <$

0.001) for *L. corniculatus* than for pasture (Table 4.2). In both swards OMD and estimated ME concentration declined slowly throughout the experiment from late spring to late summer (Fig. 4.2), with the decline for pasture being more pronounced from week 6 onwards.

4.3.3. Conception treatment

There were no conception or birth rank treatment effects on final live weight.

4.3.4. Live weight, liveweight gain, carcass weight and fatness values

Final live weight was greater ($P < 0.001$) for regular-drenched lambs than for trigger-drenched lambs grazing *L. corniculatus*, but was similar for both groups of lambs grazing pasture (Table 4.3). Trigger-drenched lambs grazing *L. corniculatus* were heavier than regular-drenched ($P < 0.054$) and trigger-drenched lambs grazing pasture ($P < 0.05$). The average LWG adjusted to birth rank and initial LW was significantly greater for *L. corniculatus*-fed regular-drenched group than that of the other three groups ($P < 0.001$) (Table 4.3), with the differences between the other three groups being similar to that of final LW.

Carcass weight and CWG were greater for regular-drenched than for *L. corniculatus*-fed trigger-drenched lambs ($P < 0.01$) and were also greater for *L. corniculatus*-fed trigger-drenched lambs than either regular-drenched or the pasture-fed trigger-drenched lambs ($P < 0.001$) (Table 4.3). Carcass fatness was significantly lower for trigger-drenched lambs than for regular-drenched lambs, when grazing either *L. corniculatus* or pasture ($P < 0.05$).

Table 4.2. Mean values of total nitrogen (N), neutral detergent fibre (NDF), *in vitro* organic matter digestibility (OMD) and digestible organic matter in dry matter (DOMD), estimated metabolisable energy concentration (ME, MJ/ kg DM), condensed tannin (CT) and phenolic fractions of diet selected by treated or trigger-treated lambs grazing *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) or perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture.

	<i>Lotus</i>		Pasture	
	Regular-drenched (<i>n</i> = 6)	Trigger-drenched (<i>n</i> = 6)	Regular-drenched (<i>n</i> = 6)	Trigger-drenched (<i>n</i> = 6)
Total N (g/kg DM)	27.1 ± 1.68	27.7 ± 1.68	26.5 ± 1.68	25.8 ± 1.68
NDF (g/kg DM)	343.6 ± 11.79 ^a	351.9 ± 11.79 ^a	462.9 ± 11.79 ^b	464.1 ± 11.79 ^b
	(<i>n</i> = 12)	(<i>n</i> = 12)	(<i>n</i> = 12)	(<i>n</i> = 12)
<i>In vitro</i>				
OMD	0.70 ± 0.007 ^a	0.69 ± 0.007 ^a	0.65 ± 0.007 ^b	0.64 ± 0.007 ^b
DOMD	0.65 ± 0.006 ^a	0.64 ± 0.006 ^a	0.59 ± 0.006 ^b	0.58 ± 0.006 ^b
ME	10.64 ± 0.0009 ^a	10.58 ± 0.0009 ^a	9.64 ± 0.0009 ^b	9.54 ± 0.0009 ^b
Secondary compounds (g/kg DM)	(<i>n</i> = 4)	(<i>n</i> = 4)	(<i>n</i> = 4)	(<i>n</i> = 4)
Total CT ¹ (g/kg DM)	39.87 ± 1.39 ^a	31.32 ± 1.39 ^b	1.57 ± 1.39 ^c	1.20 ± 1.39 ^c
Catechin + epicatechin	0.31 ± 0.03 ^a	NA	0.07 ± 0.03 ^b	NA
Other flavanoid monomers	11.63 ± 0.98 ^a	NA	6.23 ± 0.98 ^b	NA
Total phenolic glycosides	10.70 ± 2.09	NA	10.74 ± 2.09	NA
Chlorogenic acid	0.08 ± 0.08 ^a	NA	0.39 ± 0.08 ^b	NA

(*n*) Samples for analysis. ¹ Extractable + protein-bound + fibre-bound. ME = DOMD x 16.3.

Means within the same row with differing superscripts are significantly different by analysis of variance (^{abcd}; *P* < 0.05).

NA; not analysed.

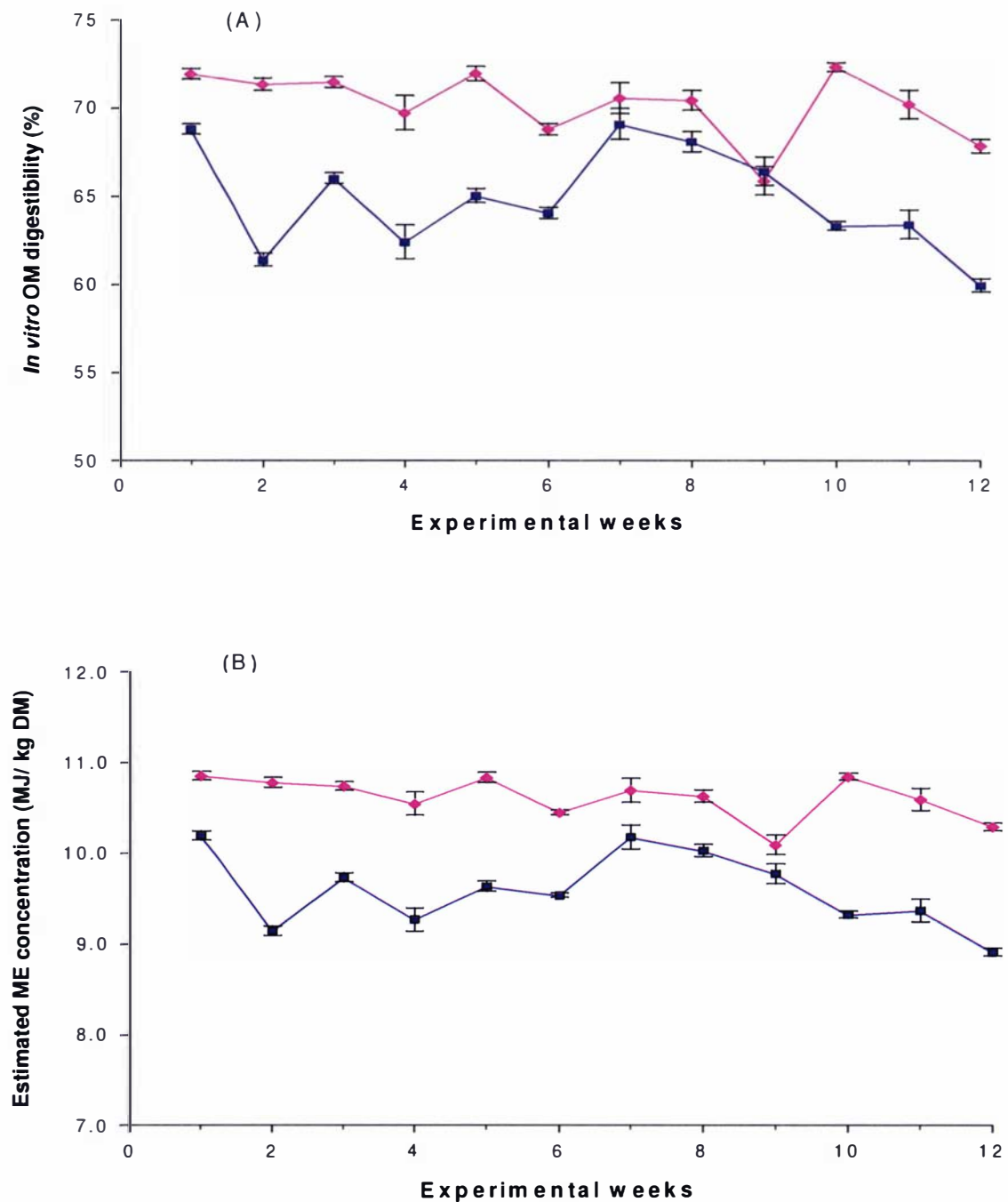


Figure 4.2. (A) Mean values of *in vitro* organic matter digestibility and (B) estimated metabolisable energy concentration (ME, MJ/ kg DM) of diet selected by treated and trigger treated lambs grazing *Lotus corniculatus* L. (birdsfoot trefoil; ◇) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture (■) over the summer finishing season of 2002-2003 in a dryland pastoral system (I = S.E.M.).

Table 4.3. Effect of grazing weaned lambs on *Lotus corniculatus* L. or perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture upon animal productivity with regular and trigger anthelmintic drench input in a dryland farming system.

	<i>Lotus</i>		Pasture	
	Regular-drenched	Trigger-drenched	Regular-drenched	Trigger-drenched
	(n = 30) ¹	(n = 30)	(n = 30)	(n = 30)
Initial live weight (kg)	28.7 ± 0.96	28.5 ± 0.91	29.1 ± 0.96	29.6 ± 1.00
Final live weight (kg)	57.2 ± 0.96 ^a	50.1 ± 0.91 ^b	47.7 ± 0.96 ^c	47.5 ± 1.00 ^c
Liveweight gain (g/d)	298 ± 7.8 ^a	227 ± 7.7 ^b	200 ± 7.9 ^c	186 ± 8.1 ^c
Carcass weight (kg)	26.8 ± 0.66 ^a	23.6 ± 0.66 ^b	20.3 ± 0.66 ^c	19.9 ± 0.66 ^c
Carcass weight gain (g/d) ²	133 ± 4.3 ^a	99 ± 4.3 ^b	66 ± 4.3 ^c	63 ± 4.3 ^c
Carcass fatness (GR, mm) ³	18.2 ± 0.7 ^a	16.2 ± 0.6 ^b	16.0 ± 0.6 ^b	13.7 ± 0.6 ^c

¹ Number of lambs per treatment group.

² Adjusted to equal initial carcass weight.

³ Adjusted to equal final carcass weight.

Means within the same row with different superscripts are significantly different (^{abcd}, $P < 0.05$).

When expressed in consecutive 4 week periods, lamb LWG generally followed the trend of being higher for the *L. corniculatus* regular-drenched group, least for the two pasture groups which were not significantly different and intermediate for the *L. corniculatus* trigger-drenched group (Table 4.4). All LWGs declined over time as herbage nutritive value declined due to high temperatures (Figs 4.1 and 4.2), with the decline being most pronounced for both trigger-drenched groups in period 2, prior to drenching on day 58. *Lotus corniculatus* regular-drenched lambs showed the least decline in growth with time and in period 1 approached the NZ Sheep Industry target of 400 g/day.

Table 4.4. Comparative liveweight gain (g/day) of grazing weaned lambs on *Lotus corniculatus* L. or perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture with regular and restricted anthelmintic drench input in a dryland pastoral system in the Wairarapa on the East Coast of North Island, New Zealand.

	<i>Lotus</i>		Pasture	
	Regular-drenched	Trigger-drenched	Regular drenched	Trigger-drenched
	(n = 30) ¹	(n = 30)	(n = 30)	(n = 30)
Weeks 1 – 4 (30 days)	350 ± 9.8 ^a	323 ± 9.7 ^b	286 ± 10.0 ^c	280 ± 10.1 ^c
Weeks 4 – 9 (35 days)	271 ± 13.1 ^a	136 ± 12.9 ^b	132 ± 13.2 ^b	114 ± 13.4 ^b
Weeks 9– 12 (30 days)	261 ± 12.9 ^a	238 ± 11.5 ^b	197 ± 11.9 ^c	181 ± 12.1 ^c
Week 1-7 (51 days) ²	353 ± 9.6 ^a	251 ± 9.5 ^b	210 ± 9.8 ^c	194 ± 9.9 ^c

¹ Number of lambs per treatment group.

² First drenching practice to restricted treatments.

Means within the same row with different superscripts are significantly different (^{abcd}; $P < 0.05$).

4.3.5. Dag score, faecal egg counts and gastrointestinal nematode burdens

Changes in dag score and FECs values over the duration of the experiment for regular-drenched and trigger-drenched lambs grazing *L. corniculatus* and pasture are summarised in Figure 4.3 and Figure 4.4. The *L. corniculatus* regular-drenched group had consistently a lower dag score than the pasture regular-drenched group ($P < 0.05$), whilst there were no significant differences in the trigger-drenched groups between *L. corniculatus* and pasture.

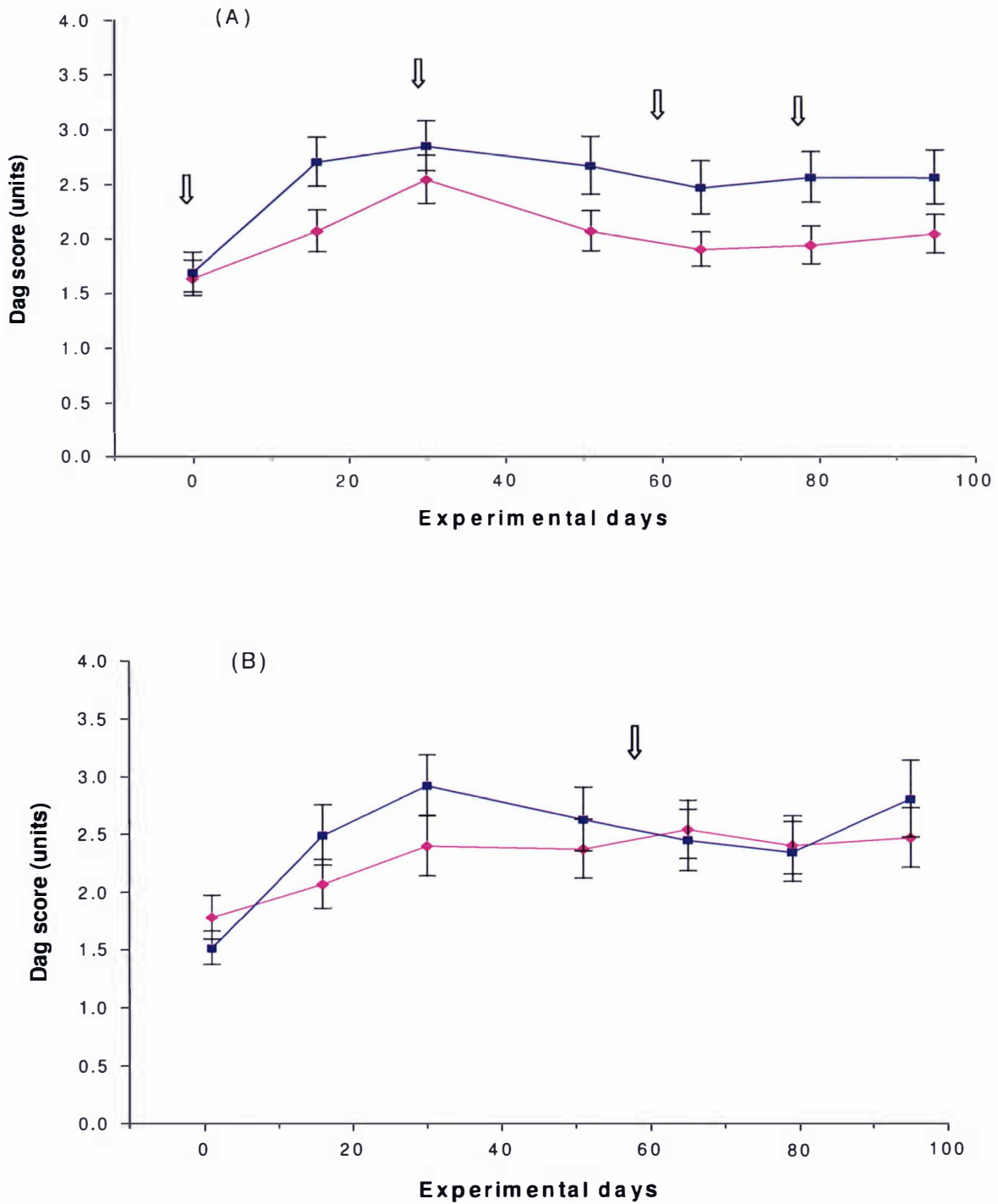


Figure 4.3. Mean dag score of groups (A) regularly treated (at four weeks intervals) or (B) trigger-drenched lambs grazing *Lotus corniculatus* L. (birdsfoot trefoil; ♦) and perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture (■). ↓ Indicates oral anthelmintic given. Vertical bars represent standard error of the mean.

On the *L. corniculatus* and pasture swards administration of regular anthelmintic treatment maintained similar and relatively low values of FECs (Fig. 4.4). In the trigger-drenched groups, FECs remained relatively constant with time for the lambs grazing pasture, but increased in lambs grazing *L. corniculatus* up to the time of drenching on day 58, with the value at day 49 being greater than for lambs grazing pasture ($P < 0.001$). Following drenching, FECs continued to increase again in the lambs grazing *L. corniculatus*.

After nematode larval incubation, relative to lambs that grazed on pasture, lambs grazed on *L. corniculatus* had consistently reduced proportions of *Trichostrongylus* larvae that hatched from faeces (Fig. 4.5).

Trigger-drenched *L. corniculatus*-fed lambs had significantly lower ($P < 0.001$) *Haemochus contortus*, *Teladorsagia* spp, *Nematodirus* spp. and *Cooperia* spp. worm burdens at slaughter than trigger-drenched lambs grazing pasture, but greater burdens of *Trichostrongylus* spp., *Chabertia ovina*, *Oesophagostomum* spp. and *Trichuris ovis* ($P < 0.001$) (Table 4.5). Relative to pasture-fed lambs, grazing on *L. corniculatus* also caused small changes in the relative proportion of individual sub-species within *Teladorsagia*, *Nematodirus* and *Trichostrongylus* species (Table 4.5). There were consistently higher female than male populations for abomasal and small intestine parasite species recovered from lambs grazing both *L. corniculatus* and pasture ($P < 0.001$), whilst in the large intestine differences were less notable (Table 4.6). Generally, the effects of forage type upon worm burdens were similar for male and female parasites, except for *Cooperia* spp.

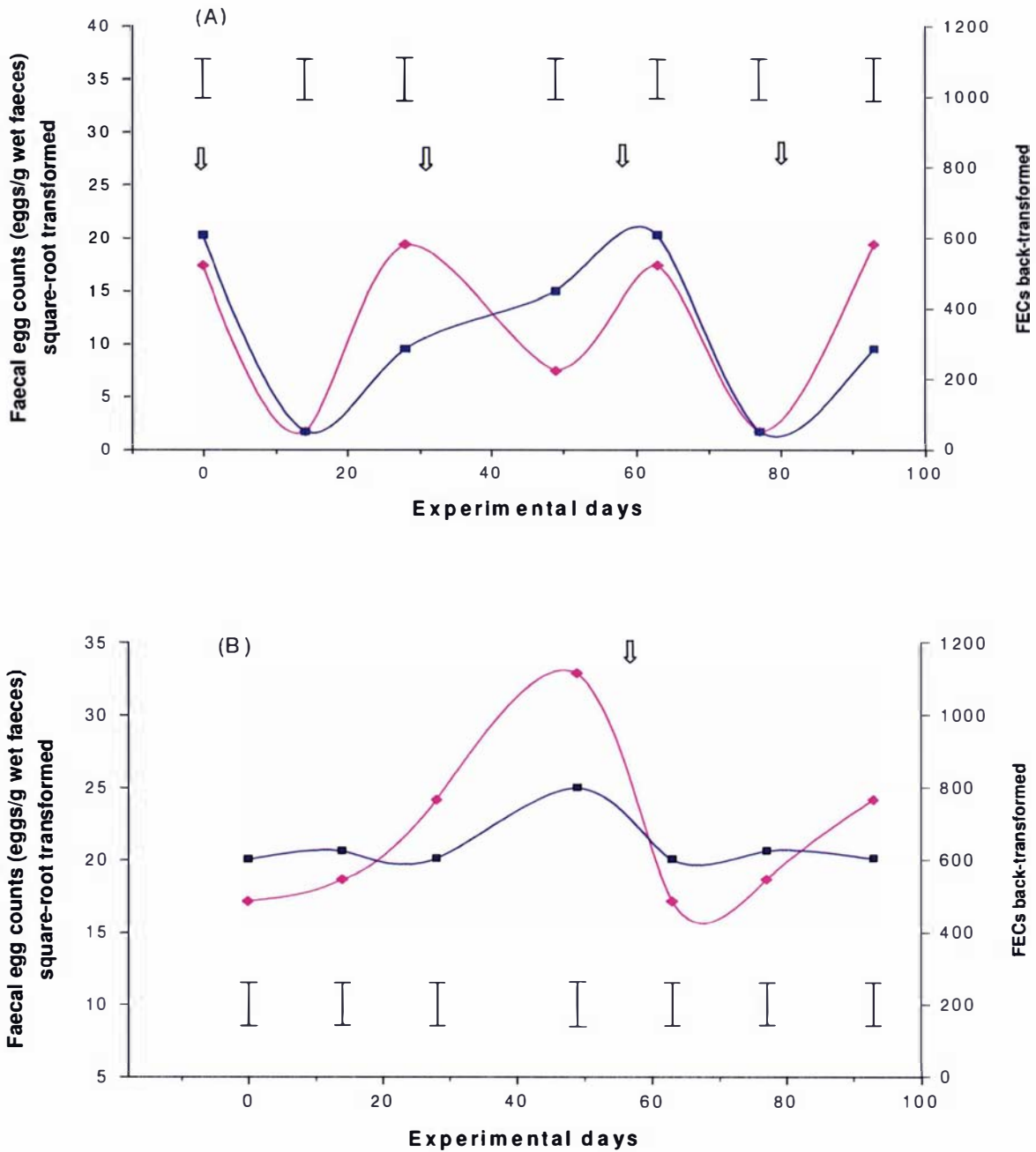


Figure 4.4. Least square mean values of FECs (eggs g/wet faeces) in (A) groups regularly anthelmintic treated (at four weeks intervals) or (B) trigger-drenched lambs (one drench) grazing *Lotus corniculatus* (♦) or perennial ryegrass/white clover pasture (■). ↓ Indicates oral anthelmintic given. Bars show pooled standard error from square-root transformed data for clearer interpretation of trends.

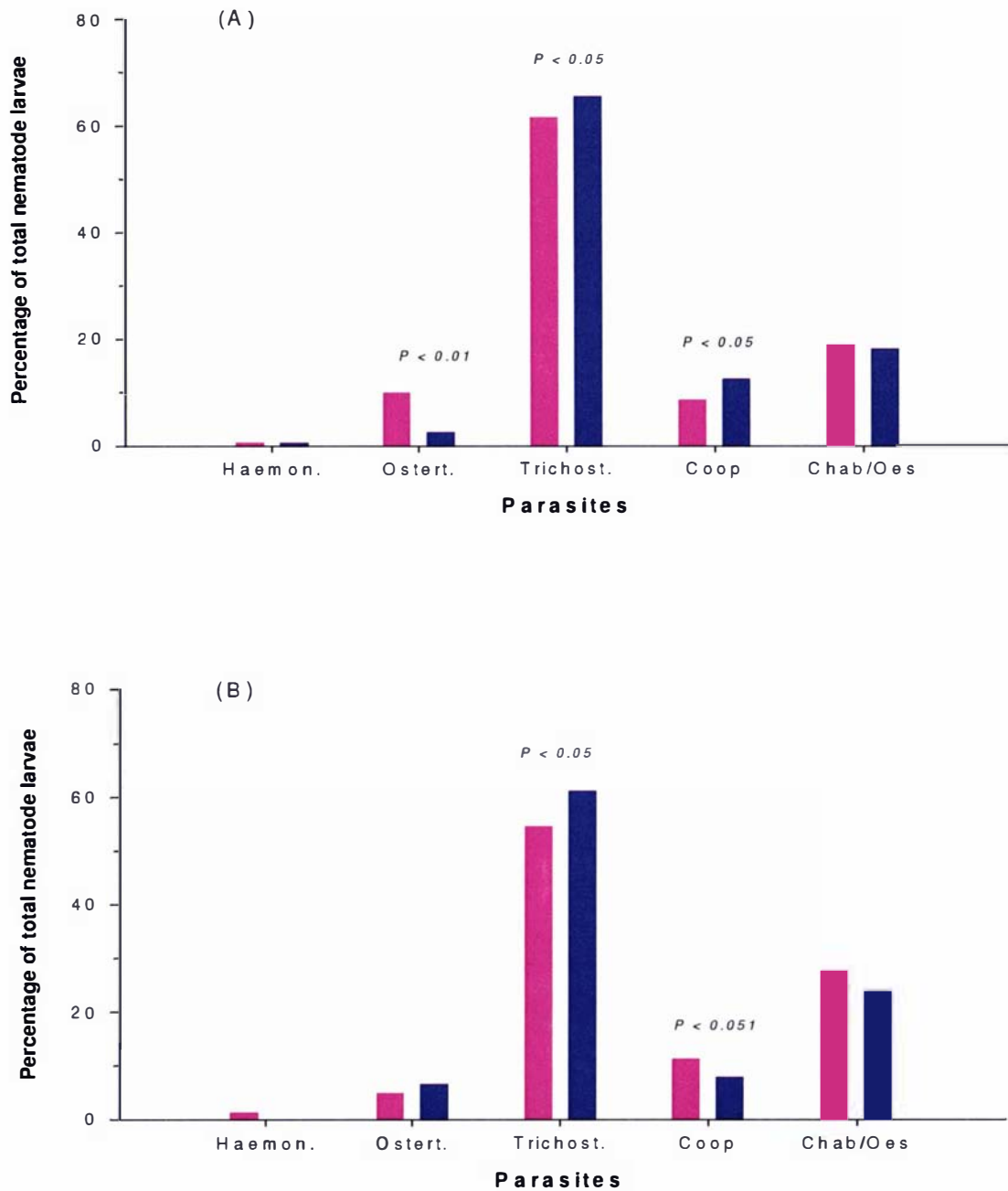


Figure 4.5. Comparative proportions of infective gastrointestinal nematode larvae hatched from 10 days incubation at 25°C of in (A) groups regularly anthelmintic treated (at four weeks intervals) or (B) trigger-drenched lambs (one drench) grazing *Lotus corniculatus* L. (birdsfoot trefoil) (■) or perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture (■). Chab/Oes: *Chabertia* and *Oesophagostomum* species.

Table 4.5. Arithmetic means and least square means of natural log transformed worm counts data (\pm S.E.M.) in groups of trigger-drenched lambs grazing *Lotus corniculatus* L. (birdsfoot trefoil) or perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture over the spring/summer autumn season of 2002-2003 in dryland farming conditions on the East Coast of the lower North Island, New Zealand.

	Abomasum			Small intestine			Large intestine		
	<i>Haemonchus</i>	<i>Teladosarg.</i>	<i>T. axei</i>	<i>Nematodirus</i>	<i>Trichostrongylus</i>	<i>Cooperia</i>	<i>Chabertia</i>	<i>Oesophag.</i>	<i>Trichuris</i>
<i>L. corniculatus</i>	17	2023 ¹	350	3025 ³	10840 ⁵	177 ⁷	111	262	7
	2.83 \pm 0.06	7.61 \pm 0.006	5.85 \pm 0.01	8.01 \pm 0.005	9.29 \pm 0.002	5.17 \pm 0.02	4.70 \pm 0.02	5.56 \pm 0.01	1.90 \pm 0.11
Perennial ryegrass/white clover	30	3975 ²	298	3823 ⁴	1458 ⁶	198 ⁷	22	56	3
	3.40 \pm 0.05	8.28 \pm 0.004	5.69 \pm 0.01	8.24 \pm 0.004	7.28 \pm 0.007	5.29 \pm 0.02	3.10 \pm 0.06	4.03 \pm 0.03	1.05 \pm 0.17
Significance ^a	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

^a Comparison between sward types within parasite species.

¹ 0.90 identified as *Teladosargia circumcincta* and 0.10 as *Ostertagia trifurcata*; ² 0.81 identified as *T. circumcincta* and 0.19 as *O. trifurcata*.

³ 0.95 identified as *Nematodirus spathiger* and 0.05 as *N. filicolis*; ⁴ 0.87 identified as *N. spathiger* and 0.13 as *N. filicolis*.

⁵ 0.74 identified as *Trichostrongylus vitrinus* and 0.26 as *T. colubriformis*; ⁶ 0.67 identified as *T. vitrinus* and 0.33 as *T. colubriformis*.

⁷ Identified as *Cooperia curticei*.

Table 4.6. Arithmetic means and least square means of natural log transformed data and their standard errors of male and female worm counts in groups of trigger-drenched lambs grazing *Lotus corniculatus* L. (birdsfoot trefoil) or perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture over the spring/summer autumn season of 2002-2003 in dryland farming conditions in the Wairarapa on the East Coast of New Zealand.

	<i>Lotus</i>		Pasture	
	Trigger-drenched		Trigger-drenched	
	male	female	male	female
<u>Abomasum</u>				
<i>Teladorsagia</i> spp.	580 (6.36 ± 0.012) ^{a1}	1443 (7.27 ± 0.007) ^{a2}	872 (6.77 ± 0.009) ^{b1}	3103 (8.04 ± 0.005) ^{b2}
<u>Small intestine</u>				
<i>Nematodirus</i> spp.	272 (5.60 ± 0.017) ^{a1}	2753 (7.29 ± 0.005) ^{a2}	522 (6.25 ± 0.012) ^{b1}	3302 (8.10 ± 0.005) ^{b2}
<i>Trichostrongylus</i> spp.	867 (6.76 ± 0.009) ^{a1}	9973 (9.20 ± 0.002) ^{a2}	357 (5.87 ± 0.015) ^{b1}	1100 (7.00 ± 0.008) ^{b2}
<i>Cooperia</i> spp.	55 (4.00 ± 0.038) ^{a1}	122 (4.80 ± 0.026) ^{a2}	50 (3.91 ± 0.040) ^{a1}	148 (5.00 ± 0.023) ^{b2}
<u>Large intestine</u>				
<i>Chabertia ovina</i>	58 (4.05 ± 0.037) ^a	53 (3.97 ± 0.039) ^a	11 (2.40 ± 0.086) ^b	11 (2.41 ± 0.086) ^b
<i>Oesophagostomum</i> spp.	147 (4.99 ± 0.023) ^{a1}	115 (4.74 ± 0.027) ^{a2}	26 (3.26 ± 0.056) ^b	30 (3.40 ± 0.052) ^b
<i>Trichuris ovis</i>	2 (0.75 ± 0.198) ^{a1}	5 (1.54 ± 0.133) ^{a2}	1 (-0.08 ± 0.301) ^{b1}	2 (0.82 ± 0.190) ^{b2}

Means between same genders within the same row with differing superscripts letter are significantly different (^{ab}; $P < 0.05$).

Means between genders within the same treatment with differing superscripts number are significantly different (¹²; $P < 0.05$).

4.3.6. Correlations

There were no significant positive relationships at any time of the experiment between dag score and FECs values for both drenching practices in lambs grazing either *L. corniculatus* or pasture.

4.4 DISCUSSION

The objectives of this experiment were firstly to produce high growth rates of weaned male lambs over summer in a commercial dryland finishing system, grazing *L. corniculatus* or perennial ryegrass/white clover pasture at high DM allowances. The second objective was to achieve this with reduced reliance on anthelmintic treatment to control nematode parasites. The main finding was that over the late spring/summer regular-drenched lambs grazing *L. corniculatus* produced the highest growth rates of approx 300 g/day over the 14 week period, which in spring were (350 g/day) close to the NZ Sheep Industry target of 400 g/day (The New Zealand Sheep Council, 2000). Trigger-drenched lambs grazing *L. corniculatus* produced a lower mean liveweight gain of approximately 230 g/day but higher than that of either group grazing perennial ryegrass/white clover pasture (200-190 g/day). As carcass-weight gain of trigger-drenched lambs grazing *L. corniculatus* was exactly half way between that of regular-drenched lambs grazing *L. corniculatus* and pasture, it seems that anthelmintic use can be restricted in weaned lambs grazing *L. corniculatus* but this will result in some loss of productivity relative to regular-drenched lambs on *L. corniculatus*. Whilst anthelmintic drench could be restricted without loss of productivity for lambs

grazing pasture in this experiment, a summary of similar experiments conducted in New Zealand has shown this to be a high risk strategy, which dramatically reduced lamb LWGs in half of the experiments (Ramírez-Restrepo *et al.*, unpublished).

From measurements of pre- and post-grazing herbage mass, it can be calculated that the approximate voluntary feed intake (VFI) was similar for lambs fed *L. corniculatus* and pasture in the regular-drenched groups (2.67 ± 0.45 vs. 3.06 ± 0.47 kg DM/lamb/day) and in the trigger-drenched groups (2.56 ± 0.44 vs. 2.59 ± 0.50 kg DM/lamb/day); the greater productivity of *L. corniculatus*-fed lambs can therefore be explained by combined effects of higher OMD, DOMD and estimated ME values for *L. corniculatus*, by both the improved efficiency of protein digestion (Waghorn *et al.*, 1987) and by improved tolerance of a parasite burden in the case of trigger-drenched lambs.

The CT-protein complex during digestion is stable in the rumen, reducing forage protein degradation in the rumen, but releases protein in the abomasum (Jones and Mangan 1977) due to pH dependant reactivity, which increases the absorption of EAA from the small intestine (Waghorn *et al.*, 1987) and improves nutritional status and sheep productivity (Ramírez-Restrepo *et al.*, 2002, 2004ab). Increased immune response to parasite challenge in ewes (Houdijk *et al.*, 2000) and lambs (Abbott *et al.*, 1988) has been related to the ingestion of high protein diets.

There are several ways in which grazing *L. corniculatus* could reduce parasite problems in lambs. These include a taller growth habit than perennial ryegrass-based pastures, reducing the ingestion of infective L₃ larvae and both direct effects

of CT in inhibiting larval motility (Molan *et al.*, 1999) and possible indirect effects of increased protein absorption from CT stimulating the immune system. From Figure 4.4 B and Table 4.5 grazing on *L. corniculatus* was not particularly effective in reducing egg output and parasite infection in the restricted drench lambs. By comparison with grazing ryegrass based pasture, grazing on *L. corniculatus* did reduce the establishment of *Haemonchus contortus*, *Teladorsagia spp.*, *Nematodirus spp.* and *Cooperia spp.* in trigger-drenched lambs but this was more than compensated for a large increase in the establishment of *Trichostrongylus spp.*. Thus, the main attribute of *L. corniculatus* appears be the increased ability of lambs to grow when carrying a parasite burden, probably due to the increased absorption of EAA.

The mechanism of how this occurs is unclear. The lowered of the population of *Haemonchus contortus*, *Teladorsagia spp.*, *Nematodirus spp.* and *Cooperia spp.* at slaughter *L. corniculatus*-fed versus pasture-fed lambs could involve resistance and the associated production of antibodies, but this would seem unlikely as the population of *Trichostrongylus spp.* increased dramatically in lambs grazing *L. corniculatus*. Resilience is characterised by a lack of immune response and stable parasite populations in infected animals, resulting in reduced dagginess (W.C. McNabb, personal communication). Results of the present experiment do not fully fit a resilience definition either, as FECs and established populations of *Trichostrongylus spp.* increased in lambs grazing *L. corniculatus*, although dagginess was markedly reduced. The development of resistance to internal parasites is usually associated with reduced animal productivity, which does not

occur in the case of resilience (W.C. McNabb, personal communication). The results of this experiment cannot be fully explained by the above definitions of either resistance or resilience; one possibility is that reactions occurred in the gut between digesta constituents (including CT) and parasites and that these affected some parasite species more than others (Niezen *et al.*, 1995; Hoskin *et al.*, 2000).

Epidemiological patterns of strongyloid nematodes after larval incubation (Fig. 4.5) illustrated that grazing on *L. corniculatus* during summer consistently reduced the proportions of *Trichostrongylus* larvae that hatched from lamb faeces, relative to lambs that grazed pasture. These results are similar to those of Ramírez-Restrepo *et al.* (2004a) who found that during spring, relative to parasited sheep grazing pasture, ewes and lambs grazing on *L. corniculatus* tended to reduce the proportion of *Trichostrongylus* larvae hatching after incubation, with these effects being more pronounced in lambs than in ewes. However, *Trichostrongylus* numbers at slaughter in trigger-drenched lambs accounted for 67% of total worm counts on *L. corniculatus* to 18% on pasture. This suggests that, although grazing on *L. corniculatus* did not reduce the establishment of *Trichostrongylus* worms in lambs, it might have reduced either egg laying or egg hatching or both. The most likely cause of this is the CT content of *L. corniculatus*. The *in vitro* data from Molan *et al.* (1999, 2000a) suggested that extracted CT has an inhibitory activity against sheep *Trichostrongylus colubriformis* nematode parasites, and specifically reduced egg hatching, the development of eggs to L₃ larvae and the motility of L₃ larvae, as demonstrated for CT extracted from *Lotus pedunculatus*, *L.*

corniculatus, sulla, sainfoin (*Onobrychus viciifolia*) and chicory (*Chicorium intybus*) (Molan *et al.*, 2000b).

Previous grazing studies (Niezen *et al.*, 1998) indicated that lambs on *L. corniculatus* had higher *Trichostrongylus* populations than lambs that grazed other forages. However, it is interesting to note that, whilst trigger-drenched lambs fed *L. corniculatus* had significantly more *T. axei* and *T. colubriformis* worm burdens compared with trigger-drenched lambs fed pasture, a different effect was reported by Niezen *et al.* (1995) who found that undrenched lambs fed sulla (*Hedysarum coronarium*) had lower numbers of these nematode species than those which fed lucerne (*Medicago sativa*). These results may be explained by differences in CT concentration, chemical structure and reactivity between these forage legumes, which has previously been described (Barry and McNabb, 1999).

The effects of grazing *L. corniculatus* on increasing the populations of some parasites at slaughter and decreasing the populations of others are probably due to direct or cumulative effects of CT on nematode cuticle, control and development of the ecdysis (i.e. moulting) process, or the neuromuscular activity in the nematode species. However, such a hypothesis needs further investigation. An alternative and complementary explanation is that changes in the CT molecule during digestion after the protein is released in the abomasum could reduce the inhibitory effect of CT on nematode parasites as digesta passes down the digestive tract. Effects of CT extracted from chicory on the motility of nematode larvae (L₃ stage) of gastrointestinal nematodes *in vitro* showed that the inhibitory activity of CT is reduced by 12 % and 5% from rumen to abomasal fluid at

concentrations of 100 or 1000 µg chicory CT/ml of fluid respectively (Molan *et al.*, 2003). Secondly, Terrill *et al.* (1994) found that, although [¹⁴C]CT appeared not to be absorbed from the small and large intestines, the recovery of CT from digesta decreased as it flowed from the abomasum to the rectum, suggesting that it was being changed to other compounds. The effectiveness of *L. corniculatus* feeding in reducing worm populations (Table 4.5) also decreases from the abomasum to the small intestine to the large intestine, and it is possible that declining effective CT concentrations are involved in this transition. Further studies are required to understand specific mechanisms of CT in *L. corniculatus* to limit nematode epidemiology under grazing conditions and also in the digestive tract.

The present data confirmed the suggestion by Ramírez-Restrepo *et al.* (2004a) that dag score compared to FECs is not a strong indicator to trigger drenching, but also that FECs are a time-dependant condition in parasitised lambs grazing *L. corniculatus*. Faecal nematode egg counts increased in restricted drench lambs on *L. corniculatus* up to day 49 and a similar increase was observed after anthelmintic treatment (day 58). As trigger-drenched lambs grazing *L. corniculatus* gained 12.8 kg live weight from weaning to day 51, compared with 8.8 kg from day 51 to day 95, it seems that, if sustainable finishing systems are to be developed on CT-containing *L. corniculatus* in the absence of anthelmintic drenching, lambs could be drafted for slaughter at about day 51. This early decision would avoid grading penalties due to excess carcass fat, as there was a tendency towards over fatness of regular-drenched lambs grazing *L. corniculatus*. Secondly, the farming system might meet both human health and animal welfare regulations; it would eliminate

the need for a withholding period before slaughter. Furthermore, as *L. corniculatus* produced similar dry matter yield to perennial ryegrass/white clover pasture during spring under dryland farming and is more productive during summer/autumn (Ramírez-Restrepo *et al.*, 2003), the finishing regimen suggested could allow lambs to be sold for slaughter early, thus ensuring there is sufficient *L. corniculatus* to feed ewes during mating in autumn, so that their reproductive performance is increased compared to ewes mated on pasture (Ramírez-Restrepo *et al.*, 2004b). This proposed system would have extra advantages in very dry summers, as digestibility and estimated ME concentration decreased less for *L. corniculatus* than for pasture under drought conditions (Fig. 4.2).

In a recent review Rattray (2003) estimated that internal parasites cost the NZ Sheep Industry around \$ 300 million per annum (including drench costs), whilst drench resistance is estimated to cost \$ 20 million annually, but predicted to reach \$ 60 million per year by 2022. It is therefore quite feasible that, in commercial dryland pastoral systems, the greater feeding value of CT-containing *L. corniculatus* relative to pasture, allows weaned lambs to enhance their ability to tolerate worm burdens, whilst maintaining or increasing productivity, delaying the development of drench resistance in some parasite populations and reducing the requirement for broad-spectrum anthelmintics to suppress nematode infections.

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CHAPTER 5.

PRODUCTION OF *LOTUS CORNICULATUS* UNDER GRAZING IN A DRYLAND ENVIRONMENT



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ABSTRACT

A three-year experiment (from November 2000 to October 2003) was conducted at Massey University's Riverside farm, in the Wairarapa on the East Coast of the lower North Island, New Zealand to compare, under grazing conditions, seasonal and annual grazed net herbage accumulation rate and seasonal dynamics of undisturbed net herbage accumulation rate of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) relative to perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) dominant pasture. Prediction equations to estimate standing DM in *L. corniculatus* and pasture from the rising plate meter (RPM) and sward surface height were also generated.

L. corniculatus and pasture growing in a moderate fertility and low-pH soil accumulated similar total net herbage mass accumulation (24.3 vs. 24.1 t DM/ha) over the three years, with the DM production being greater for *L. corniculatus* than for pasture during 2000-2001, producing more DM during summer/autumn drought conditions. The net herbage accumulation rates from undisturbed areas of *L. corniculatus* and pasture were similar. During the spring/summer period Pasture and *L. corniculatus* undisturbed net herbage accumulation rate was at maximum at a gross herbage yield of 9.9 and 5.8 t DM/ha respectively. The undisturbed net herbage accumulation rate was at its lowest, possibly due to severe moisture deficits, during the summer season. Seasonal variation in the calibration regressions fitted to estimate herbage mass of *L. corniculatus* non-destructively,

suggested a combination of destructive and non-destructive methods are needed to assess herbage mass.

It was concluded that *L. corniculatus* has the potential to increase the performance of a perennial ryegrass/white clover pasture-based sheep farming system due to its ability to grow in acidic soils, its tolerance of drought conditions during summer/autumn and its seasonality of feed supply. Its high feeding value and both the moderate and beneficial concentration of condensed tannins (CT) have been identified in other studies.

Keywords: *Lotus corniculatus*, perennial ryegrass/white clover pasture, herbage mass, sward surface height, dryland farming systems.

Abbreviations: **CEC**, cation exchange capacity; **CT**, condensed tannins; **cv**, cultivar; **DM**, dry matter; **K**, potassium; **Kh**, Kohinui soil series; **m**, meter; **me**, milliequivalents; **N**, nitrogen; **p**, phosphate; **PPD**, plant population density; ®; Registered; **RLA**, residual leaf area; **RPM**, rising plate mater; **RSD**, residual standard deviation; **SO₄**, sulfate; **SS**, sward stick.

5.1 INTRODUCTION

Efficient pastoral farming systems combine the practice of maintaining, or increasing pasture production, crop growth and animal performance within the constraints of socio-economic and biophysical environments. Nevertheless, biological sustainability and performance of dryland farming systems will depend on the high stability of the system components, such as forage production, especially during periods of soil moisture deficit (Squires, 1991).

Successful dryland farming systems in the North and South Island of New Zealand over the last 50 years have focused on the introduction of ryegrass and clover varieties (Brown and Green, 2003). However, pastoral farming systems in the dryland regions have been not able to cope with animal demands, especially during summer/autumn conditions, due to the depression in production and persistence of white clover (*Trifolium repens*), or even lucerne (*Medicago sativa*), when used as the predominant forage legumes within a perennial pasture based system (Brown and Green, 2003; Brook *et al.*, 2003; Moot *et al.*, 2003). This has resulted in an oscillation in feed quality and dry matter (DM) production, and considerable variability in animal production.

The use of alternative deep tap-rooted perennial legume species, such as *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie), relative to perennial ryegrass (*Lolium perenne*)/white clover pasture, has been suggested as way to increase the current year round sheep productivity in dryland farming environments (Ramírez-Restrepo *et al.*, 2002, 2004abc). The advantages of *L. corniculatus* are its better seasonality of feed supply, its high feed value, its moderate concentration of condensed tannins (CT; Barry *et al.*, 2003), its ability to grow in low fertility soils, and tolerance of soil acidity and impeded drainage (Heinrichs, 1970; Douglas and Foote 1993), and summer drought conditions (Ramírez-Restrepo *et al.*, 2004b). However, to date there has been a lack of research that compares, under grazing conditions, the patterns of net herbage accumulation and seasonal dynamics of net herbage accumulation rate of *L. corniculatus* relative to perennial ryegrass/white clover pasture in a dryland farming system in New Zealand.

Additionally, practical guidelines to estimate farm average *L. corniculatus* cover by the use of rising plate meter units (RPM) and the sward stick (SS; sward surface height, cm) are not available.

The first objective of this study was to determine seasonal and annual grazed net herbage accumulation rate of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) relative to perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture over three consecutive years in a dryland commercial environment using a systems approach, where plant production and effects upon animal productivity (Ramírez-Restrepo *et al.*, 2002, 2004abc) were measured simultaneously. The second objective was to assess the seasonal dynamics of un-grazed net herbage accumulation rate of *L. corniculatus* and pasture. The third objective was to develop equations to estimate *L. corniculatus* herbage mass by the use of the rising plate meter and the sward stick.

5.2 MATERIALS AND METHODS

5.2.1. Location and experimental site

The evaluation was carried out at Massey University's Riverside farm located approximately 15 km North of Masterton, in the Wairarapa (grid reference; 307374 NZMS 260T26) on the East Coast in the lower North Island of New Zealand (40° 50' 40" S, 175° 73' 00" E) from October 2000 to October 2003. Riverside farm experiences a mean rainfall of 1230 mm (recorded at Riverside from 1989 to 2002) with summer/autumn periods (December–April) historically characterised by low rainfall rates and high evapotranspiration (Salinger, 2003). The site was located on

an aggradational terrace system characterised by a Kohinui soil series (Kh; Pollok *et al.*, 1994). The soil was a medium-textured stony soil, 40 to 50 cm deep, free draining (Pollok *et al.*, 1994) with a pH of 5.35. Soil analyses were Olsen phosphate (P) of 31.8 $\mu\text{g P/g}$, sulfate (SO_4) 9.9 $\mu\text{g/g}$ and cations, potassium (K) 0.40 and CEC 0.83 me/100g.

5.2.2. Establishment and grazing management

Nine hectares (ha) of *L. corniculatus* were established as a monoculture in March/2000. Sowing rate was 20 kg/ha of coated and inoculated seed and the seed was sown 10 mm deep and in rows 150 mm apart with a cone-type plot seeder equipped with a double disc. During the first wet season, the legume was sprayed with herbicide to remove invading grasses (haloxyfop 300 g a.i./ha (Gallant[®] NF). Broad-leaved weeds were sprayed during the second winter with 375 g a.i./ha paraquat plus 225 g a.i./ha diquat (Preeglone^{TM®}) and metribuzin 700 g a.i./ha (Sencor[®]). No fertiliser was applied on the crop at any stage during the evaluation, except for 18 kg N/ha, as urea, during August 2000. Similar areas of adjoining perennial ryegrass/white clover pasture were used as the control.

Areas of *L. corniculatus* and pasture were rotationally grazed with sheep (Ramírez-Restrepo *et al.*, 2002, 2004abc) except during winter and early spring (i.e. May-August). There were between seven to eight grazings per year with a rest during winter. Surplus areas of legume and pasture were grazed by commercial groups of sheep and cattle. Additionally, pasture paddocks were mechanically topped during

summer to remove reproductive stem material to stimulate the vegetative growth stage.

5.2.3. Plant measurements

The net herbage accumulation rate (kg DM/ha/day) in grazed areas of both *L. corniculatus* and pasture was measured at monthly intervals over 36 months using two methods simultaneously. Firstly, eight random quadrats (0.18 m²) were selected, marked and cut to ground level using a portable electric shearing hand-piece in a specific area to be grazed in approximately 30 days. On the same another set of eight random quadrats were selected, marked and protected with wire mesh enclosure cages measuring (1.4 x 0.9 m) in a nearby area to be also sampled 30 days later to give an estimate of growth over a fixed period. For the second method, the residue after grazing was cut to ground level from eight quadrats and pasture mass estimated. This area was allowed to regrow and was sampled again from eight different enclosure areas 30 days later. These two methods gave the net herbage accumulation rate for forage in the 30 days post-grazing, and the net herbage accumulation rate for forage in a spelled area that was to be grazed in 30 days time. The initial dates of sampling were staggered so that growth was studied in successive overlapping periods of 30 days (Davies, 1993).

Un-grazed net herbage accumulation rate (kg DM/ha/day) was measured fortnightly throughout four experimental seasons. At the start of each evaluation, six large wire mesh exclusion cages (2 m x 0.5 m) were placed permanently on

both *L. corniculatus* and pasture. Evaluation 1 was conducted from 21/10/00 to 12/01/01 (83 days; 42 quadrats/forage). Evaluation 2 commenced on 16/02/01 and finished on 27/04/01 (70 days; 48 quadrats/forage). Evaluation 3 began on 5/02/02 and finished on 15/04/02 (69 days; 36 quadrats/forage). Evaluation 4 was carried out between 7/10/02 and 10/02/03 (126 days; 60 quadrats/forage).

For all evaluations, quadrats (0.18 m²) were positioned in rows within each cage. Harvests from Evaluation 1 to Evaluation 3 were cut to ground level. Plant samples during the fourth evaluation were harvested from initial pre-trimmed areas of *L. corniculatus* (50 mm sward surface height) and pasture (30 mm sward surface height) to check that ground levels cuts were not affecting regrowth. Both forages were harvested to a similar mean sward surface height at each sampling to ensure optimum plant regrowth. Thus, the estimate profile of un-grazed net herbage accumulation rate was constructed throughout the seasons by dividing difference in net accumulated herbage mass between samplings by the number of days of the regrowth period. All plant material was washed to remove soil and faeces, dried overnight (16 h) in a forced-air oven (Contherm; Thermotec 2000; New Zealand) at 80° C and weighed individually.

Prediction equations to estimate herbage mass (kg DM/ha) were calculated from a pool of 6520 quadrat (0.18 m²) cuts made in different transects to estimate pre- and post-grazing experimental herbages masses (Ramírez-Restrepo *et al.*, 2002, 2004abc). Each quadrat was measured in the centre of its undisturbed sward with both the SS and the RPM before the sward was cut to ground level. There were 3281 quadrats in *L. corniculatus* and 3239 quadrats in pasture.

After the determination of DM harvested, herbage mass (y) per unit of RPM (units, x) or sward surface height of SS (cm, x) were calculated monthly as the slope component (b) of a linear equation ($y = a + bx$) from a simple linear regression without an intercept (a) for both *L. corniculatus* and pasture. Therefore, given the average RPM reading or sward surface height and the month of that reading the average herbage mass could be calculated.

Prediction equations to reduce the main source of variation due to seasonal changes in swards characteristics were also calculated from pooled data across years during early spring (August-September), late spring (October-November), summer (December-February), autumn (March-April) and winter (May-July) since this provided more observations, especially over winter, to convert both the plate meter reading and sward surface height measurements in *L. corniculatus* and pasture to herbage mass. Monthly and seasonal regressions were compared between forages.

5.2.4. Climatic conditions

Annual and seasonal rainfall to compare with 50-year values (New Zealand Metereological Service, 1983), and diurnal soil temperatures (at a depth of 10 cm) were recorded at Riverside farm throughout the three consecutive years.

5.2.5. Calculation of data and statistical analyses

Data for annual and seasonal precipitation and soil temperature were analysed using arithmetic mean values. Data of herbage accumulation in each year was

analysed using the MIXED procedure of SAS (2001). The linear model included the effects of forage type (*L. corniculatus* or pasture), month, their interaction and the residual error. Multiple comparison of least square means for each forage in each month were performed.

The significance of the differences between forage type was not calculated because the study was a systems experiment without true replication. However, significant differences between means (using forage type as replicates) of annual and seasonal grazed net herbage accumulation rate were analysed using the PROC MIXED (SAS, 2001) with a linear model that included the effect of forage type. Correlations between grazed net herbage accumulation rate and soil temperature were analysed using PROC CORR (SAS, 2001).

Seasonal patterns of un-grazed net herbage accumulation rate were analysed using the MIXED procedure of SAS (2001), with a linear model for repeated measurements that included the effect of forage type, date and their interaction. Using the Akaike's information criterion, a compound symmetric error structure was determined as the most appropriate residual covariance structure for repeated measures over time (Littel *et al.*, 1998).

Regression analysis for the prediction of herbage mass by the use of RPM or SS calibration equations was performed. Fitness of the model was determined by the coefficient of variation (CV) associated with the calibration equation. The CV was calculated as residual standard deviation (RSD)/mean of herbage mass.

Comparison of regression slopes between *L. corniculatus* and pasture were performed using the PROC GLM (SAS, 2001).

5.3 RESULTS

5.3.1. Rainfall and soil temperature

Annual rainfall (November-October) was higher than average in 2001-2002, but 2000-2001 and 2002-2003 were close to the 50-year average (New Zealand Metereological Service, 1983; Table 5.1). The summer/autumn season (December-April) in 2002-2003 was drier than the 50-year average and the other two years (Table 5.1). Annual soil temperature was higher in 2000-2001 than the other years, and also the summer/autumn mean soil temperature was higher than the other two years.

5.3.2. Annual and seasonal dry matter production

Total DM production that is net herbage mass accumulation over three years was similar for *L. corniculatus* and pasture (Table 5.2). Annual DM production of *L. corniculatus* was greater than pasture in 2000-2001, but was lower than pasture in 2002-2003 (Table 5.2). The highest values of total seasonal DM production for *L. corniculatus* and pasture were in summer/autumn and spring with the lowest DM production in winter (Table 5.2).

Table 5.1. Total and seasonal rainfall values compared with the 50-year average values, and mean diurnal soil temperature (10 cm depth) over three consecutive years at Massey University's Riverside farm, in the Wairarapa on the East Coast of the lower North Island, New Zealand.

Year/month	Rainfall (mm)	Soil temperature (°C)
<i>2000-2001</i>	967	15.8
Summer/autumn ¹	305	17.2
Winter ²	307	10.9
Spring ³	356	16.8
<i>2001-2002</i>	1072	12.9
Summer/autumn	444	16.8
Winter	355	8.8
Spring	273	11.4
<i>2002-2003</i>	991	13.9
Summer/autumn	230	14.7
Winter	339	9.3
Spring	423	16.5
<i>50-year values⁴</i>	971	NA
Summer/autumn	341	NA
Winter	306	NA
Spring	324	NA

¹ Calculated from December to April, May to July² and August to November³.

⁴ Data from New Zealand Metereological Service 1983 recorded at East Taratahi (Wairarapa) metereological station 20 km south of the site.

NA; no apply.

Table 5.2. Annual and seasonal dry matter production (t DM/ha) of perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or *Lotus corniculatus* L.(birdsfoot trefoil; cv. Grasslands Goldie) averaged over three consecutive years in a commercial dryland farming system on the East Coast in New Zealand. Mean values with standard error (S.E.M).

	Pasture	Lotus	P
<i>Annual production</i> ¹			
2000-2001	7.03	8.46	
2001-2002	9.99	10.5	
2002-2003	7.06	5.30	
<i>Seasonal production</i>			
<i>2000-2001</i>			
Summer/autumn ²	1.98	4.48	
Winter ³	0.22	0.06	
Spring ⁴	4.82	3.91	
<i>2001-2002</i>			
Summer/autumn	4.19	3.70	
Winter	0.81	0.95	
Spring	4.97	5.93	
<i>2002-2003</i>			
Summer/autumn	3.64	1.56	
Winter	0.00	0.13	
Spring	3.41	3.60	
<i>Total production</i>			
Annual	8.02 ± 0.98	8.08 ± 1.51	NS
Summer/autumn	3.27 ± 0.87	3.24 ± 0.66	NS
Winter	0.34 ± 0.24	0.34 ± 0.24	NS
Spring	4.40 ± 0.49	4.48 ± 0.73	NS

¹ Calculated from November to October.

² Estimated from December to April, May to July³ and August to November⁴.
NS; no significance.

5.3.3. Grazed net herbage accumulation rate

Patterns of grazed net herbage accumulation rate for both *L. corniculatus* and pasture were similar in 2000-2001 (*L. corniculatus*; 21.87 ± 1.85 kg DM/ha/day vs. pasture; 17.67 ± 1.88 kg DM/ha/day) and 2001-2002 (*L. corniculatus*; 26.08 ± 1.57 kg DM/ha/day vs. pasture; 25.83 ± 1.59 kg DM/ha/day), but lower for *L. corniculatus* (8.00 ± 1.53 kg DM/ha/day) than for pasture (15.44 ± 1.53 kg DM/ha/day; $P < 0.001$) in 2002-2003. The grazed net herbage accumulation rate of *L. corniculatus* was greater than for pasture in December ($P < 0.001$), January ($P < 0.05$), February ($P < 0.001$) and September ($P < 0.01$) in the lower rainfall of 2000-2001 rather than in the wetter conditions of either 2001-2002 or 2002-2003 (Fig. 5.1). The negative grazed net herbage accumulation rates for some periods in late autumn and winter (Fig. 5.1) were associated with low soil temperatures in the range 7.0 to 12.3° C resulting in *L. corniculatus* ($P < 0.05$; $r = 0.35$) and pasture growth ($P = 0.06$; $r = 0.33$) that occurred more slowly than the rate of senescence.

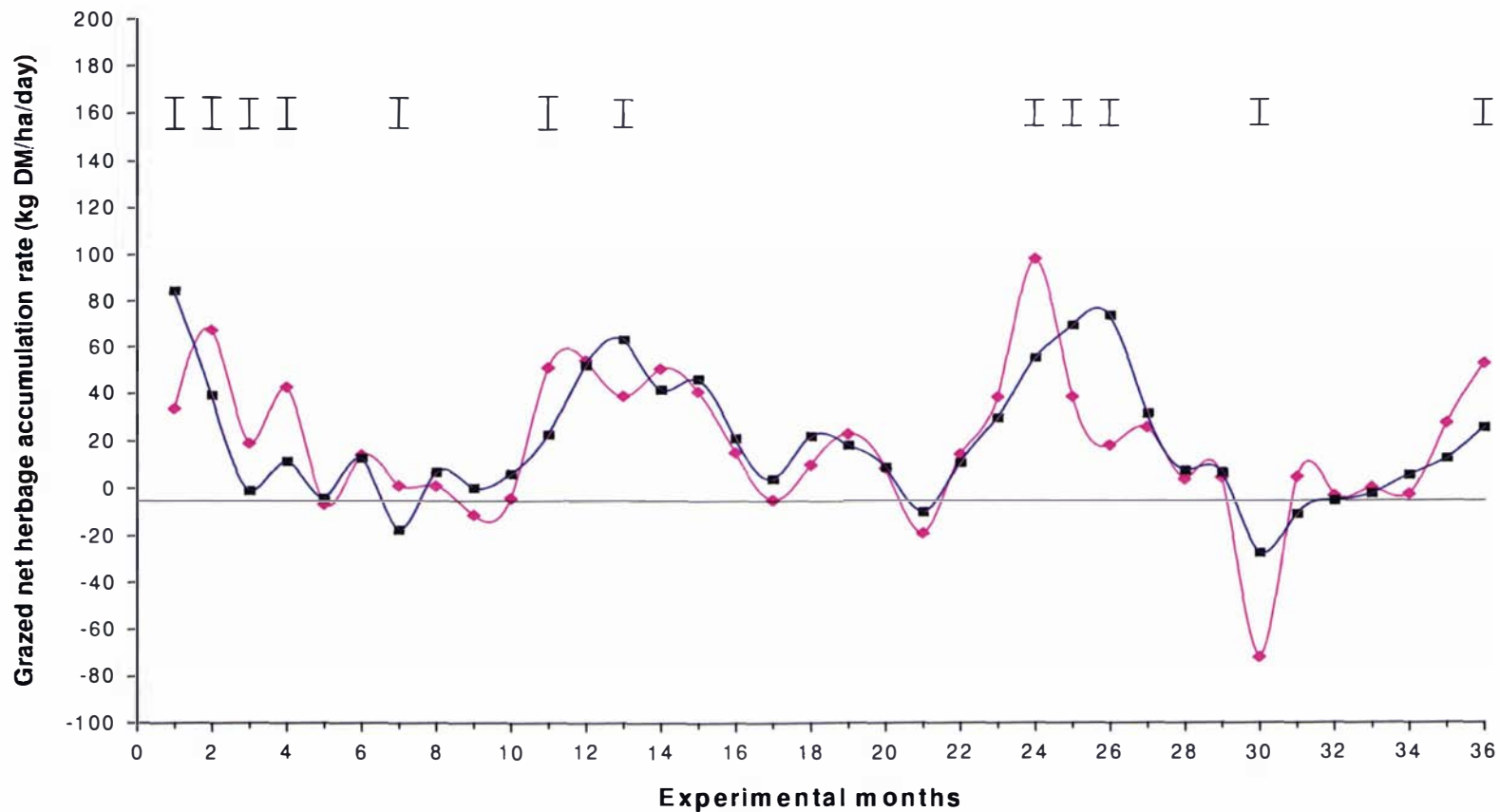


Figure 5.1. Grazed net herbage accumulation rate of (■) perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture and (◆) *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) grown in the Wairarapa on the East Coast of the North Island, New Zealand. Data collected from November 2000 to October 2003. Bars (I) indicate pooled standard error for clearer interpretation of trends when forages significantly different ($P < 0.05$).

5.3.4. Un-grazed net herbage accumulation rate

In Evaluation 1, the highest net herbage accumulation rate, in un-grazed pasture, was observed during late spring and early summer at a herbage mass of 6007 and 9982 kg DM/ha respectively, but from late spring (4053 kg DM/ha) to the end of the evaluation (mid summer; 5280 kg DM/ha) *L. corniculatus* accumulated less herbage mass than pasture (Fig. 5.2A). A similar trend from initial pre-trimmed areas was found in Evaluation 4, with the highest un-grazed net herbage accumulation rate for both *L. corniculatus* and pasture occurring during late spring at a herbage mass of 1766 and 1956 kg DM/ha respectively, but from mid November onwards, *L. corniculatus* also accumulated less herbage mass than did pasture (Fig. 5.2B).

In Evaluations 2 and 3 over the summer/autumn periods minor differences in the dynamics of un-grazed net herbage accumulation rate between species were detected (Fig. 5.3), with both species having a negative un-grazed net herbage accumulation rate for part of the period.

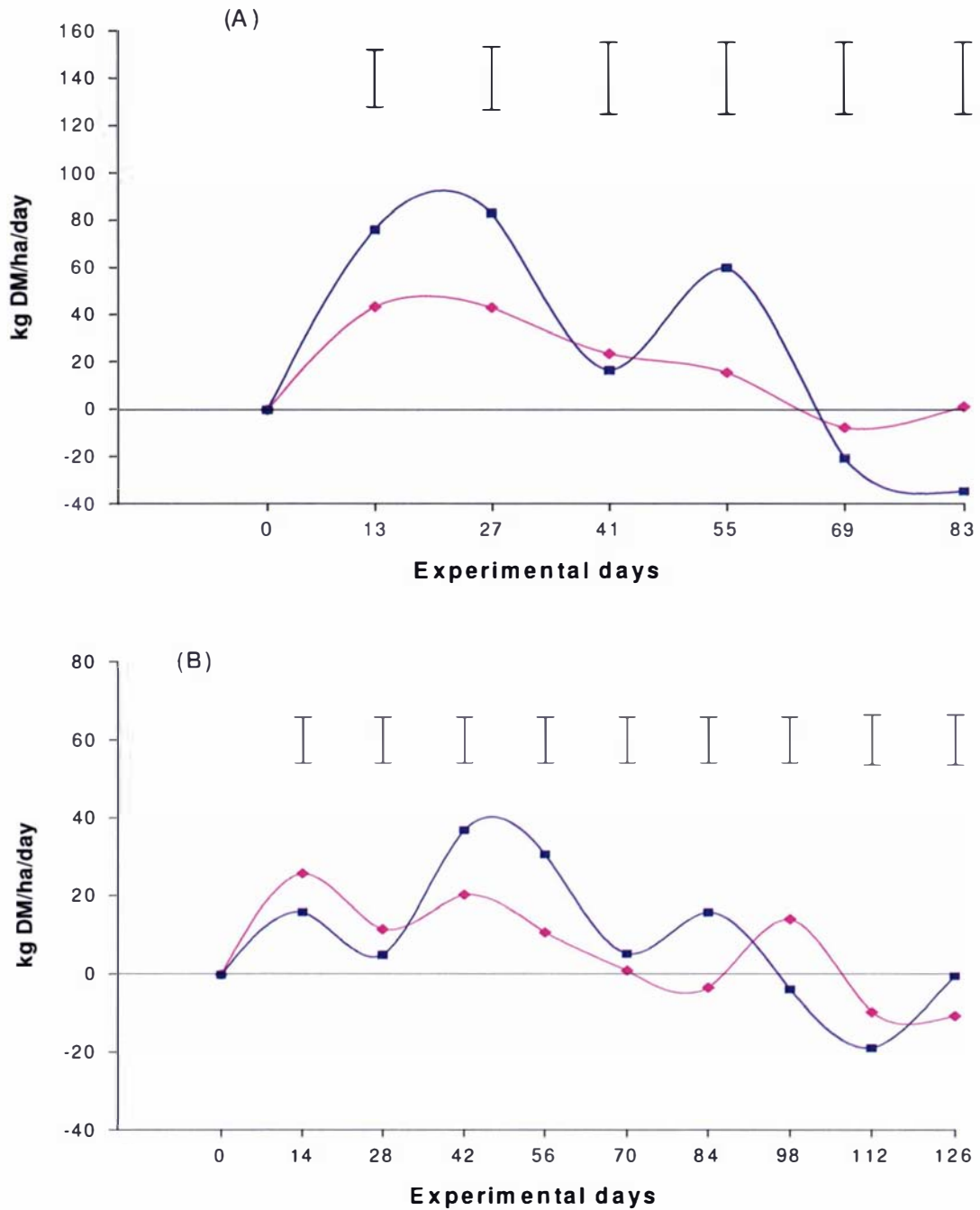


Figure 5.2. Comparative un-grazed net herbage accumulation rate (kg DM/ha/day) for spring/summer of (■) perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture and (◆) *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie). Measured from (A) 21/10/00 and 12/01/01 and (B) 7/10/02 to 10/02/03 in a commercial dryland pastoral system in the Wairarapa on the East Coast of the southern North Island, New Zealand. Vertical bars (I) indicate pooled standard error for clearer interpretation of trends.

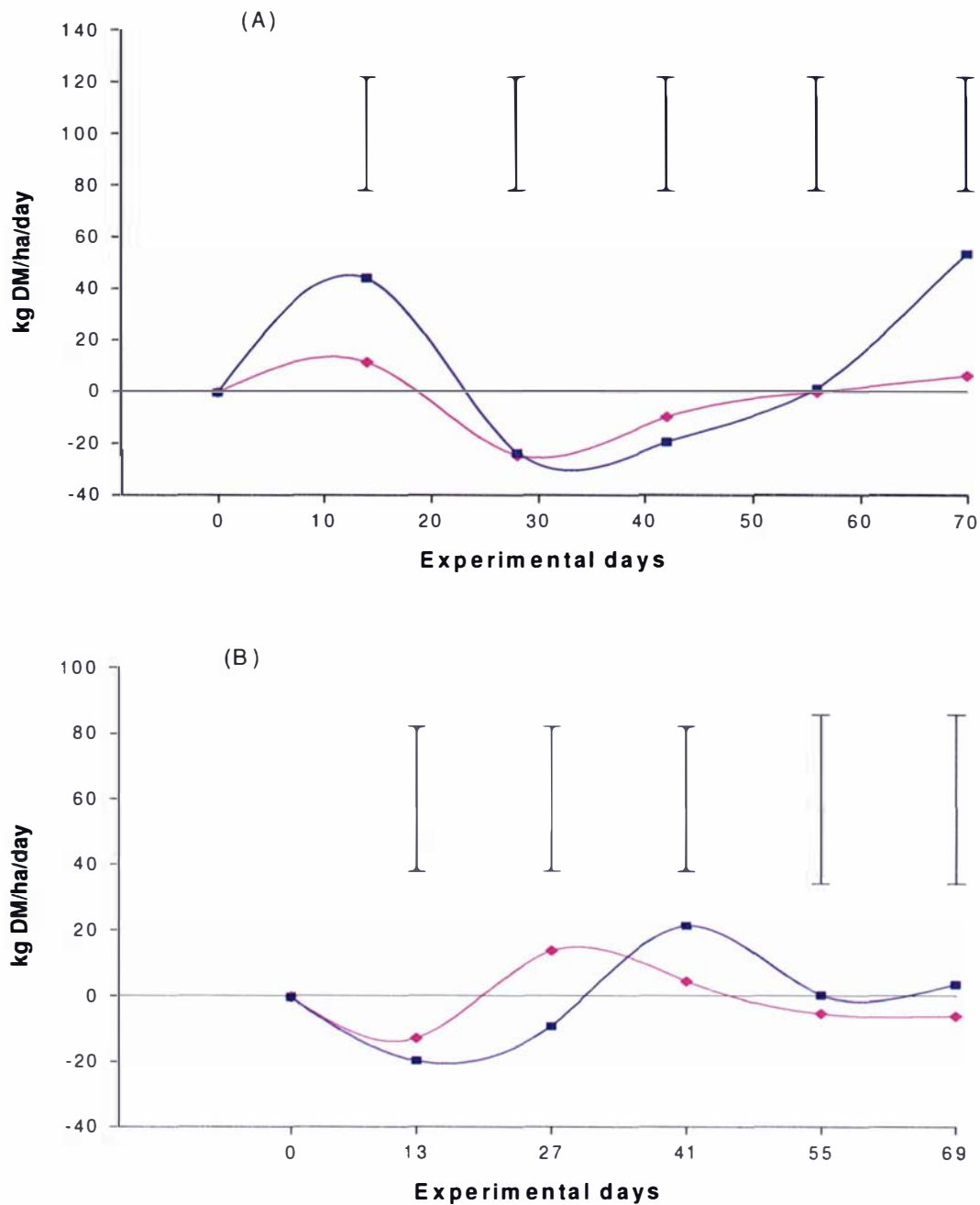


Figure 5.3. Comparative un-grazed net herbage accumulation rate (kg DM/ha/day) for summer/autumn of (■) perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture and (◆) *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie). Measured from (A) 16/02/01 to 27/04/01 and (B) 5/02/02 and 15/04/02 in a commercial dryland pastoral farming system in the Wairarapa on the East Coast of the lower North Island, New Zealand. Vertical bars (I) represent pooled standard error for clearer interpretation of trends.

5.3.5. Estimation of yields

Monthly herbage mass could be predicted in *L. corniculatus* from plate meter readings units (Table 5.3) and sward surface height (Table 5.4), with the linear regression calibration equations accounting for 71-89% and 67-91% of the variability (r^2) in the data set, respectively. Slopes of the *L. corniculatus* regressions were consistently different ($P < 0.0001$) from those of pasture, with the *L. corniculatus* regressions having a lower slope with plate meter readings units during late summer/autumn, mid and late winter, and early spring, whilst slopes in mid winter and early spring from sward surface height measurements were higher than those for pasture..

Additionally, slopes for the pooled calibration regressions to estimate changes in seasonal herbage mass measured to ground level from plate meter readings units were significantly lower ($P < 0.0001$) in *L. corniculatus* than in pasture over the summer/autumn period, whilst the regressions for sward surface height measurements had consistently lower slopes ($P < 0.0001$) in *L. corniculatus* than in pasture throughout the year (Table 5.5, Fig. 5.4 and 5.5).

Table 5.3. Comparative monthly regression parameters to estimate herbage mass (Y; kg DM/ha) at ground level from plate meter readings (X) for perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture or *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie).

Month	Pasture					Lotus					Significance ^a
	<i>n</i>	β_1	se	r^2	CV	<i>n</i>	β_1	se	r^2	CV	
January	358	160.76	3.12	0.88	37.7	355	165.00	3.75	0.85	42.6	0.0001
February	337	193.45	3.04	0.92	31.0	359	145.36	3.83	0.80	51.0	0.0001
March	395	218.63	3.72	0.90	36.3	401	184.79	4.32	0.82	49.2	0.0001
April	314	203.58	4.00	0.89	36.5	315	199.14	7.20	0.71	62.2	0.0001
May	152	141.76	6.55	0.76	55.0	143	161.23	7.62	0.76	53.7	0.0001
June	112	221.57	7.68	0.88	37.5	112	217.88	9.48	0.83	44.8	0.0001
July	88	203.69	8.38	0.87	43.3	93	171.24	7.07	0.86	39.5	0.0001
August	160	155.62	6.75	0.77	63.4	156	151.78	6.01	0.80	49.1	0.0001
September	184	127.12	5.71	0.73	60.1	191	150.37	3.79	0.89	37.6	0.0001
October	235	136.46	3.88	0.84	50.3	244	143.44	3.58	0.87	41.3	0.0001
November	306	137.87	3.07	0.87	40.7	314	162.71	4.08	0.84	45.4	0.0001
December	410	153.30	3.58	0.82	47.4	428	160.09	3.55	0.83	44.6	0.0001

Observations for analysis (*n*).

Slope (β_1),

Coefficient of determination (r^2).

Coefficient of variation (CV).

^a Significance of difference between slopes (β_1) of pasture and *L. corniculatus*.

Table 5.4. Comparative slopes (β_1) required to formulate a calibration regression ($Y = \beta_1 x$) between herbage mass (kg DM/ha) from ground level and sward stick height (sward height; cm; X) for perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture or *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie).

Month	Pasture					Lotus					Significance ^a
	<i>n</i>	β_1	se	r^2	CV	<i>n</i>	β_1	se	r^2	CV	
January	298	208.60	5.02	0.85	41.7	281	168.08	4.19	0.85	41.8	0.0001
February	328	212.60	4.96	0.85	43.7	304	139.19	4.57	0.75	56.7	0.0001
March	395	268.62	5.68	0.85	43.9	367	181.01	4.93	0.79	53.5	0.0001
April	314	249.12	6.38	0.83	45.9	315	189.44	6.86	0.71	62.3	0.0001
May	152	148.78	8.77	0.66	65.3	143	134.08	7.94	0.67	63.1	0.0001
June	112	271.44	10.55	0.86	41.4	112	275.02	11.05	0.85	41.9	0.0001
July	88	282.28	18.93	0.72	64.1	93	194.04	13.39	0.70	59.2	0.0001
August	160	137.20	10.51	0.52	91.7	156	162.06	6.89	0.78	52.0	0.0001
September	184	162.19	7.07	0.74	58.8	191	149.58	3.73	0.91	34.0	0.0001
October	222	169.84	6.01	0.78	59.0	177	164.13	4.98	0.86	42.3	0.0001
November	228	199.94	5.87	0.84	45.3	237	189.15	4.97	0.86	41.6	0.0001
December	294	191.42	6.93	0.72	56.3	262	181.33	4.99	0.83	43.3	0.0001

Observations for analysis (*n*).

Slope (β_1).

Coefficient of determination (r^2).

Coefficient of variation (CV).

^a Significance of difference between slopes (β_1) of pasture and *L. corniculatus*.

Table 5.5. Comparative seasonal slopes (β_1) required for the prediction of herbage mass ($Y = \beta_1 x$; kg DM/ha) at ground level from plate meter readings and sward stick height (sward height; cm) for perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture or *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie).

	Pasture					Lotus					Significance ^a
	<i>n</i>	β_1	se	r^2	CV	<i>n</i>	β_1	se	r^2	CV	
<i>Plate meter</i>											
Early spring ¹	344	134.22	4.33	0.74	63.0	347	150.66	3.10	0.87	41.7	0.0001
Late spring ²	541	137.55	2.37	0.86	44.2	558	153.82	2.79	0.85	44.4	0.0001
Summer ³	1105	162.25	2.09	0.85	42.7	1143	157.88	2.15	0.82	46.0	0.0001
Autumn ⁴	709	211.65	2.73	0.89	36.5	716	189.09	3.76	0.78	54.6	0.0001
Winter ⁵	352	170.20	4.68	0.79	51.3	348	177.49	4.89	0.79	49.4	0.0001
<i>Sward stick</i>											
Early spring	344	154.31	5.90	0.67	71.0	347	151.80	3.00	0.88	40.2	0.0001
Late spring	450	188.77	4.25	0.81	51.3	414	178.43	3.60	0.86	42.7	0.0001
Summer	920	203.45	3.33	0.80	48.6	847	163.11	2.70	0.81	47.8	0.0001
Autumn	709	259.62	4.25	0.83	44.9	682	184.22	4.04	0.75	57.5	0.0001
Winter	352	188.42	7.01	0.67	64.3	348	166.77	6.28	0.67	62.1	0.0001

¹ Calculated from August/September; ² October/November; ³ December/February; ⁴ March/April; ⁵ May/July.
Observations for analysis (*n*).

Slope (β_1), Coefficient of determination (r^2). Coefficient of variation (CV).

^a Significance of difference between slopes (β_1) of pasture and *L. corniculatus*.

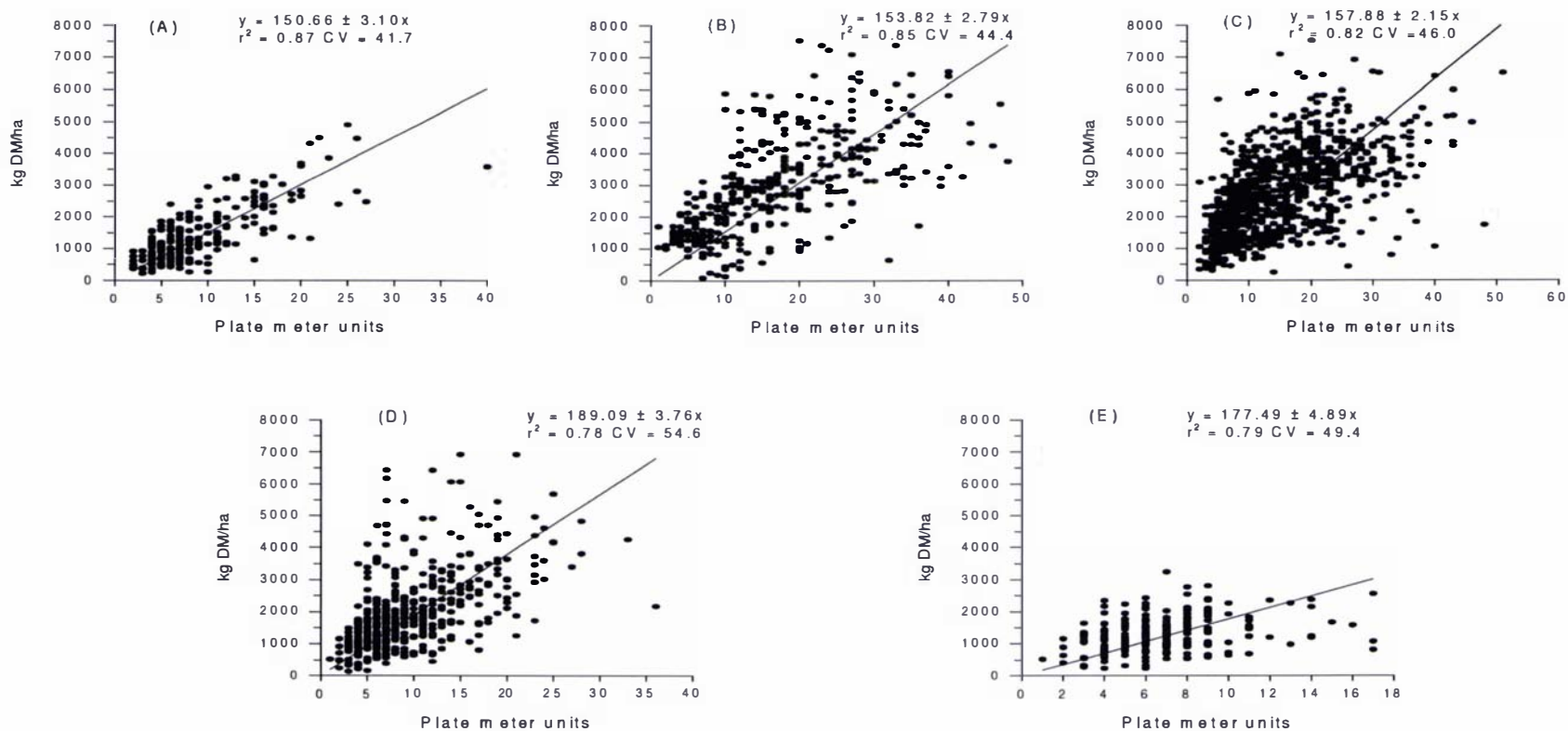


Figure 5.4. Calibration regressions to estimate herbage mass (kg DM/ha) as a function of plate meter reading (units) for *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) during (A) early spring, (B) late spring, (C) summer, (D) autumn and (E) winter.

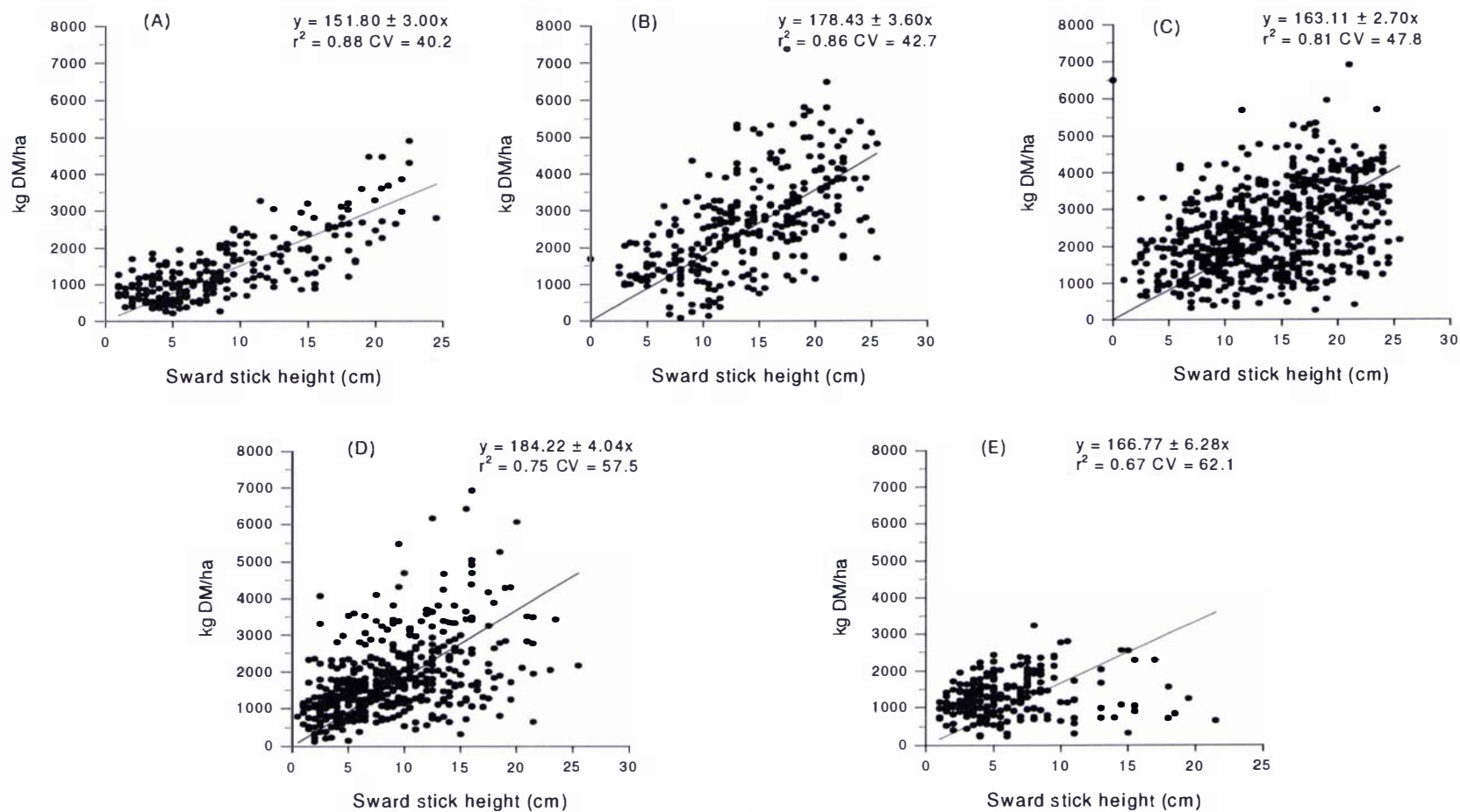


Figure 5.5. Comparative calibration regressions during early (A) and late (B) spring, summer (C), autumn (D) and winter (E) to estimate herbage mass (kg DM/ha) at ground level from sward stick height (cm) for *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie).

5.4 DISCUSSION

The objectives of this study were firstly to determine seasonal and annual growth patterns of herbage accumulation of semi-erect *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) in a commercial dryland pastoral system over a three-year period relative to perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*), under rotational grazing by sheep. Secondary objectives were to evaluate seasonal dynamics of net herbage accumulation rate in *L. corniculatus* and pasture and to develop calibration regressions to predict *L. corniculatus* herbage mass by the use of the rising plate meter or the sward stick. The most significant findings were that *L. corniculatus* growing in a moderately fertile and acidic soil in a dryland environment exhibited a similar level of seasonal and total DM production to that of pasture.

It is difficult to draw strong conclusions about the causes that limited the herbage mass accumulation of *L. corniculatus* in the third year under grazing management. However, these results might be explained as follows. The short lifespan of lateral branches and leaves being grazed by sheep resulted in slow regrowth which might have encouraged weed invasion (Barry *et al.*, 2003). Previously Van Keuren and Davis (1968), Van Keuren *et al.* (1969) and Chapman *et al.* (1990) reported reduced persistence of *L. corniculatus* under grazing management.

Turkington and Franko (1980) found that *L. corniculatus* is largely dependent upon carbohydrate production from the canopy photosynthetic area rather than from root reserves. This is in contrast to a larger tap-root legume, such as lucerne, but is

probably explainable, because whilst the regrowth of *L. corniculatus* depends largely on active upper axillary branching, lucerne tends to grow from both the crown (Nelson and Smith, 1968ab) and carbohydrate root reserves (Nelson and Smith, 1969). Alison and Hoveland (1989) showed yield reductions of 49% in cultivars of *L. corniculatus* after two years when the stubble height decreased from 10 to 3 cm during the spring with a 21-day harvest interval, whilst stand density of plants decreased by 48%. Defoliation height is often important to yield, especially during summer, when carbohydrates root reserves in *L. corniculatus* are at a low level, contrary to autumn when vegetative growth ceases (Smith, 1962; Smith and Nelson, 1967). Greub and Wedin (1971) reported that close defoliation to 3.8 cm removed leaf area, axillary bud sites and increased crown and root diseases. This is consistent with the observation by Van Keuren and Davis (1968) who indicated that *L. corniculatus* persists at a productive level better under rotational than under continuous grazing management. More recent studies (Ayala, 2001) strongly suggest that 20 to 30 day grazing intervals and defoliation heights of 6 cm to 10 cm during spring, and moderate cutting heights (≥ 6 cm) with rest periods during summer, were critical for persistence of *L. corniculatus*. Defoliation in late autumn and low cutting height (4 cm) or grazing in winter are detrimental to persistence.

Thus, it might be assumed that under grazing management the rate of regrowth in *L. corniculatus* is a function of the residual leaf area (RLA), cutting or grazing height and the carbohydrate supply from the photosynthetic tissues or root reserves. However, it is possible that under year-round grazing conditions the desired RLA occurs before the planned defoliation height, which could potentially

slow the fodder crop regrowth. Consequently, it is necessary to understand the balance between herbage mass, defoliation height and RLA to develop an indicator for lax or intensive grazing that allows adequate grazing practices to reduce the risk of poor sward persistency. More intensive research is required on population dynamics under field conditions.

Based on the climatic variation reported (Table 5.1), it seems likely that failure of seed germination and early seedling establishment from the soil seed reserves to replace the original plants could have occurred, particularly during the wet conditions in year 2001-2002 due to a combination of factors such as low ambient temperatures and moisture stress during both early spring and autumn. *L. corniculatus* has poor seedling vigour (Seaney and Henson, 1970; Foulds, 1978; Beuselinck and Grant, 1995) so requires a long establishment period. Therefore, sub-optimal plant density could have contributed to a reduced *L. corniculatus* DM production during the third growing season. McGraw *et al.* (1986) found that plant population density (PPD) is critical in *L. corniculatus* as it requires a minimum PPD of 30 plants/m² for high DM production. Consequently, in interpreting the responses in this study it appears that under grazing management allowing a rest period at least every two years to allow natural reseeding and early seedling growth during summer/autumn could substantially reduce stand losses. Further agronomic research with emphasis on grazing management, prostrate and winter active cultivars, stem palatability, increased leaf/steam ratio and improved persistence reducing weed invasion of *L. corniculatus* is still required.

The negative growth rates in Figure 1 for some periods in late autumn and winter were likely due to low temperatures, resulting in *L. corniculatus* and pasture growth that was occurring more slowly than the rate of senescence, as a consequence presumably of reduced root activity in water and nutrient uptake (Sonneveld, 1962). The optimum temperature range for *L. corniculatus* and pasture is 18 to 25° C (Mitchell, 1956ab; Kunelius and Clark, 1970). Growth of *L. corniculatus* ceases below 9° C and that of perennial ryegrass ceases below 6° C (Mitchell, 1956ab; Kunelius and Clark, 1970).

The results of this study highlighted the importance of monitoring herbage mass for efficient forage management and to meet animal production targets over the entire year (Ramírez-Restrepo *et al.*, 2004abc). This is the first attempt to associate *L. corniculatus* herbage mass with plate meter readings and sward surface height. The relationship between herbage mass and both plate meter readings and sward stick height suggested that calibration regressions from both procedures are potential options for the estimation of *L. corniculatus* herbage mass in spring. However, both techniques are unlikely to be suitable for the semi-erect *L. corniculatus* (Birdsfoot trefoil cv. Grasslands Goldie) in summer, when the accuracy of these calibration equations, as measured by their coefficient of determination (r^2), or their coefficient of variation (CV; Fig. 4 & 5) is low (Piggot, 1986; Thomson, 1986). Also, the seasonal changes in the structure and morphology of *L. corniculatus* make it unlikely that a simple method for predicting herbage mass of *L. corniculatus* throughout its growing season can be developed.

The changes in the slopes and regression equations to predict herbage mass of *L. corniculatus* between spring and summer are most likely related to the production of erect flowering stems and the resulting changes in the morphological structure of the sward. Various authors (Earle and McGowan 1979; Thomson, 1983; Stockdale, 1984; Barthram, 1986; Thomson, 1986; L'Huiller and Thompson, 1988; Thomson *et al.*, 1997) have reported that climate, forage type, growth habit, plant morphology, botanical composition, plant density, DM content, soil surface and operator are components of variation that affect the accuracy of prediction equations for herbage mass. The RPM measures a combination of plant density and height (Hodgson, 1990), and SS the sward surface height (Barthram, 1986), with both methods developed to be applied on forages with high tiller populations and prostrate growth habit (Hodgson, 1990).

In summer the most reliable option is to estimate herbage mass of *L. corniculatus* by cutting samples to ground level with a description of the botanical composition of the sward. Additionally, the variability in the slopes of the calibration equations relative to pasture suggests that, in dryland conditions, further studies on the estimation methods of estimating pasture availability are required, since standard equations have not been calculated from data collected in dryland environments in New Zealand (Piggot, 1986; L'Huiller and Thompson, 1988; Hainsworth, 1999; Bishop-Hurley, 1999).

Records quoted by Radcliffe (1975) and Bologna *et al.* (1996) indicate that in a dryland environment, DM production of pasture and *L. corniculatus* ranges from 8.9 to 14.9 t DM/ha and from 7.5 to 13.1 t DM/ha respectively. The present study

provides direct evidence that *L. corniculatus* is as productive as perennial ryegrass/white clover pasture in the moderate P, acidic soil and dryland conditions of the Wairarapa and has the potential to support commercial sheep farming systems.

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CHAPTER 6.

ORGANIC MATTER DIGESTIBILITY OF CONDENSED TANNIN – CONTAINING *LOTUS CORNICULATUS* AND ITS PREDICTION *IN VITRO* USING CELLULASE/HEMICELLULASE ENZYMES

This chapter has been submitted to the *Animal Feed Science and Technology*

ABSTRACT

Three digestion experiments involving cryptorchid weaned lambs were conducted for 14 days over the spring, summer and autumn to determine the *in vivo* digestibility of dry matter (DMD), organic matter (OMD), digestible organic matter in the dry matter (DOMD) and estimated metabolisable energy (ME) concentration of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) at different stages of maturity. The experiments were carried out indoors at Massey University's Riverside farm, in the Wairarapa, North Island, New Zealand. *In vivo* digestibility samples were used as standards to investigate if the enzymatic *in vitro* system of Roughan and Holland (1977) could predict OMD and DOMD of condensed tannin (CT)-containing *L. corniculatus*.

Digestibility of *L. corniculatus* declined as it matured, but the rate of decline was much less than that which occurs for temperate grasses and for white clover. It was concluded that the *in vitro* enzymatic system of Roughan and Holland (1977) can be used to predict OMD and DOMD of *L. corniculatus*, provided a standard curve involving *in vivo* data generated with *L. corniculatus* is used. Using a standard curve with *in vivo* data from perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture led to bias which increased at lower OMD values. Reasons for the differences between *L. corniculatus* and pasture standard curves are discussed, including possible effects of CT.

Keywords: *Lotus corniculatus*; condensed tannins; perennial ryegrass/white clover pasture; *in vivo* digestibility; *in vitro* digestibility.

Abbreviations: **CT**, condensed tannins; **cv**, cultivar; **DM**, dry matter; **DOMD**, digestible organic matter in the dry matter (g)/100 g DM; **EAA**, essential amino acid; **kJ**; kilo joules; **ME**, metabolisable energy concentration; **MJ**, mega joules; **NDF**, neutral detergent fibre; **OM**, organic matter; **OMD** organic matter digestibility.

6.1 INTRODUCTION

Lotus corniculatus L. is a temperate forage legume plant for dryland farming that contains condensed tannins (CT; 30–40 g/ kg DM), which are known to improve the efficiency of protein digestion in ruminants (Waghorn *et al.*, 1987). In grazing trials, action of CT has increased animal production from *L. corniculatus* (Wang *et al.*, 1996ab; Min *et al.*, 1998, 1999, 2001; Ramírez-Restrepo *et al.*, 2002, 2004ab).

In grazing experiments there is a need to predict the organic matter digestibility (OMD) and digestible organic matter in the dry matter (DOMD) in the diet of animals grazing CT-containing forages, relative to that of sheep grazing non-CT forages, such as perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) (80: 20) pasture, which is the normal forage on New Zealand (NZ) farms. One such *in vitro* prediction technique is that of Roughan and Holland (1977), which involves neutral detergent extraction of ground herbage, followed by an incubation at 50° C with fungal cellulase (3.2.1.4; International Union of Biochemistry, 1984) and hemicellulase enzymes, and which was originally validated to predict the digestibility of dry matter (DMD) and OMD in non CT-containing temperate and tropical grasses, and temperate legumes.

Objectives of the present study was to measure *in vivo* OMD and DOMD in *L. corniculatus* as it matured and then to use diet samples from these *in vivo* studies as standards to investigate if the *in vitro* system of Roughan and Holland (1977) could be used to predict OMD and DOMD of CT-containing *L. corniculatus*.

6.2 MATERIALS AND METHODS

6.2.1. Forage and diets

Plots in a pure sward of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) (Ramírez-Restrepo *et al.*, 2003) were mechanically harvested at three distinct stages of growth during the flowering season in summer 2001 (very short vs. short; Experiment 1), vegetative growth in spring 2002 (short vs. long; Experiment 2) and mature stage in autumn 2003 (pods formed vs. pods containing seeds; Experiment 3). All feeds were harvested daily between 07.30 and 08.30 hours with a sickle bar mower at a height of approximately 5 cm above ground level, picked up and delivered to the animal house.

6.2.2. Animals and *in vivo* digestibility

Three *in vivo* digestibility experiments, involving 12 male cryptorchid lambs (6 per feed), were each conducted for fourteen days. The lambs were individually fed in metabolism cages, with the first half of each trial being to accustom lambs to their surroundings and to the new feed. Harnesses were then fitted for faeces collection. Fresh feed was offered daily at 09.30 and 16.30 hours. Water was available *ad libitum*. For the adjustment period feed was available *ad libitum* until

intake stabilised at the desired level of 1.3 kg DM/lamb/day. Feed offered, residues remaining and faeces produced were then measured over a 7 day collection period.

Live weight was recorded at the start and the end of each experiment, with the mean values (\pm SE) being 45.2 ± 1.07 kg (Experiment 1), 47.0 ± 1.12 kg (Experiment 2) and 41.8 ± 1.07 kg for Experiment 3. During the collection period, samples of feed offered and of the residue left by each sheep were taken daily for DM determination (drying for 16 h at 80° C) and for nutritive value analysis (stored at -20° C).

Faeces were collected at 11.00 hours from each animal for 7 days, weighed each day and pooled separately for each animal and stored at -20° C. At the end of each experiment, faeces were thawed for 3 days, mixed and triplicate samples taken for DM determination by drying for 72 hours at 80° C in a forced-air oven (Contherm; Thermotec 2000; New Zealand). Additional samples (300 g) from the mixed faeces of each animal were stored at -20° C for laboratory analysis.

6.2.3. Analyses

All samples of feed offered, feed refused and faeces were freeze-dried using a Cuddon 0610 freeze drier (W.G.G. Cuddon Ltd, Blenheim, New Zealand), and ground to pass through a 1-mm diameter sieve (Wiley mill, Swedesboro, USA) before laboratory analysis. Samples were analysed for DM, ash and gross energy (kJ/g DM). *In vivo* digestibilities of dry matter, organic matter and digestible organic

mater in the dry matter were calculated and metabolisable energy (ME) in MJ/kg DM was calculated as $16.3 \times \text{DOMD}$ (Ulyatt *et al.*, 1980).

6.2.4. *In vitro* digestibility

In vitro digestibility of ground freeze dried herbage samples was performed using the technique of Roughan and Holland (1977), which has an initial solubilisation step with hot (90–96° C) neutral detergent solution, followed by centrifugation and washing the residue three times. Residues are then incubated with standardised fungal (*Trichoderma reesei*) cellulase and hemicellulase enzymes at 50° C for 5 hours, separated by centrifugation and incubated for a further 15 hours. The technique therefore measures the combined total solubilisation plus enzymatic cell wall degradation. The procedure is able to predict *in vivo* digestibilities of forages by comparing their *in vitro* digestibility values with the known *in vivo* values of the standards. To compare accuracy for predicting the digestibility of CT-containing forage samples, six separate repeat runs were performed using the *in vivo* *L. corniculatus* samples collected in this study as standards; *in vivo* pasture standards were also run at the same time, so that the *L. corniculatus* and pasture standard curves could be compared.

6.2.5. Statistical analyses

For herbage harvested at each stage of maturity, analysis of variance for daily intake, *in vivo* digestibility of DM, OMD, DOMD and ME concentration were performed using the MIXED procedure of SAS (2001). The linear model considered the fixed effects of diet, with final live weight of each lamb used as

covariate. Regressions for standard curves of *in vivo* on *in vitro* digestibility for all experiments were estimated using PROC REG (SAS, 2001). Comparisons of regression slopes and intercepts between *L. corniculatus* and pasture standard curves were performed using the PROC GLM (SAS, 2001).

6.3 RESULTS

Digestibility of *L. corniculatus* declined between the three experiments as the plant matured (Table 6.1); however the decline of *in vivo* digestibility of DM, OM and DOMD, and estimated ME concentration was not large. At each of the three stages of maturity, digestibility and estimated ME values between the two forms of *L. corniculatus* were not significantly different.

In vivo digestibility of *L. corniculatus* could be accurately predicted from its *in vitro* OMD (Table 6.2) and DOMD (Table 6.3), with the linear regressions accounting for 91-97 of the variability (r^2) and being repeatable over the six experiments. A similar level of precision was found for the pasture standard curve. Nevertheless, the slopes and intercepts of the *L. corniculatus* standard curves were consistently different ($P < 0.001$) from the pasture standard curves in all six experiments, with the *L. corniculatus* regressions having a lower slope but higher intercept (Fig. 6.1). This resulted in the two regression lines intersecting (Fig. 6.1); averaged over all the six experiments the mean *in vivo* values where the lines intersected was 0.72 for OMD and 0.67 units for DOMD.

Table 6.1. Chemical composition and least square mean values (\pm SE) of daily intake, *in vivo* digestibility for dry matter, organic matter, digestible organic matter in the dry matter and estimated metabolisable energy concentration (ME, MJ/ kg DM) of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) at different growth stages, determined with cryptorchid weaned lambs.

	Vegetative		Flowering		Very mature	
	Short lotus	Long lotus	Very short lotus	Short lotus	Lotus with pods formed	Lotus with pods containing seeds
<u>Chemical composition</u>						
Total N (g/kg DM)	35.1	34.6	27.2	23.3	25.1	16.9
Total CT (g/kg DM)	28.7	26.2	14.9	21.3	24.7	8.3
Bound CT (g/kg DM) ¹	8.3	7.9	8.1	8.7	8.4	7.0
(% total CT)	28.9	30.1	54.3	40.8	34.0	84.3
<u>Intake</u>						
Kg DM day ²	1.45 \pm 0.03	1.40 \pm 0.03	1.10 \pm 0.02	1.24 \pm 0.03	1.40 \pm 0.03	1.28 \pm 0.03
<u>Digestibility</u>						
Dry matter (proportion DM)	0.722 \pm 0.007	0.730 \pm 0.007	0.670 \pm 0.010	0.661 \pm 0.010	0.650 \pm 0.013	0.620 \pm 0.013
Organic matter (proportion OM)	0.736 \pm 0.006	0.742 \pm 0.007	0.687 \pm 0.010	0.676 \pm 0.010	0.661 \pm 0.013	0.626 \pm 0.013
Digestible organic matter (proportion DM)	0.673 \pm 0.006	0.683 \pm 0.007	0.640 \pm 0.009	0.634 \pm 0.009	0.622 \pm 0.012	0.590 \pm 0.012
<u>Metabolisable energy</u>						
MJ/ kg DM	11.0 \pm 0.10	11.1 \pm 0.11	10.4 \pm 0.16	10.3 \pm 0.16	10.1 \pm 0.20	9.6 \pm 0.20

¹ Bound CT = ((protein-bound + fibre-bound CT)/total CT). ² Adjusted to equal final lamb live weight.

Table 6.2. Standard curves for the prediction of *in vivo* organic matter digestibility (OMD; y) from *in vitro* organic matter digestibility (Roughan and Holland, 1977) (x) for perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture (80:20) or *Lotus corniculatus* L.

Experiment	Pasture curve	r^2	<i>Lotus</i> curve	r^2	Equivalence points ¹	
					y	x
1	$y = 0.704X + 0.177$	0.9655	$y = 0.466X + 0.374$	0.9710	0.7702	0.8438
2	$y = 0.689X + 0.229$	0.9725	$y = 0.361X + 0.458$	0.9423	0.7108	0.6995
3	$y = 0.774X + 0.134$	0.9363	$y = 0.389X + 0.411$	0.9469	0.6904	0.7186
4	$y = 0.666X + 0.241$	0.9183	$y = 0.442X + 0.409$	0.9159	0.7403	0.7499
5a	$y = 0.698X + 0.223$	0.9375	$y = 0.398X + 0.432$	0.9162	0.7100	0.6979
5b	$y = 0.674X + 0.237$	0.9508	$y = 0.381X + 0.446$	0.9512	0.7199	0.7175

¹ The y and x values where the two regression lines intersect.

Table 6.3. Standard curves for the prediction of *in vivo* digestible organic matter in dry matter (DOMD; *y*) from *in vitro* DOMD (Roughan and Holland, 1977) (*x*) for perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture (80:20) or *Lotus corniculatus* L.

Experiment	Pasture curve	r^2	<i>Lotus</i> curve	r^2	Equivalence points ¹	
					<i>y</i>	<i>x</i>
1	$y = 0.728X + 0.147$	0.9768	$y = 0.409X + 0.386$	0.9620	0.6922	0.7491
2	$y = 0.700X + 0.209$	0.9271	$y = 0.312X + 0.455$	0.9467	0.6527	0.6342
3	$y = 0.768X + 0.119$	0.9567	$y = 0.340X + 0.414$	0.9484	0.6487	0.6895
4	$y = 0.661X + 0.215$	0.9579	$y = 0.388X + 0.412$	0.9222	0.6912	0.7198
5a	$y = 0.694X + 0.199$	0.9658	$y = 0.346X + 0.433$	0.9134	0.6658	0.6727
5b	$y = 0.659X + 0.217$	0.9737	$y = 0.329X + 0.446$	0.9506	0.6739	0.6931

¹ The *y* and *x* values where the two regression lines intersect.

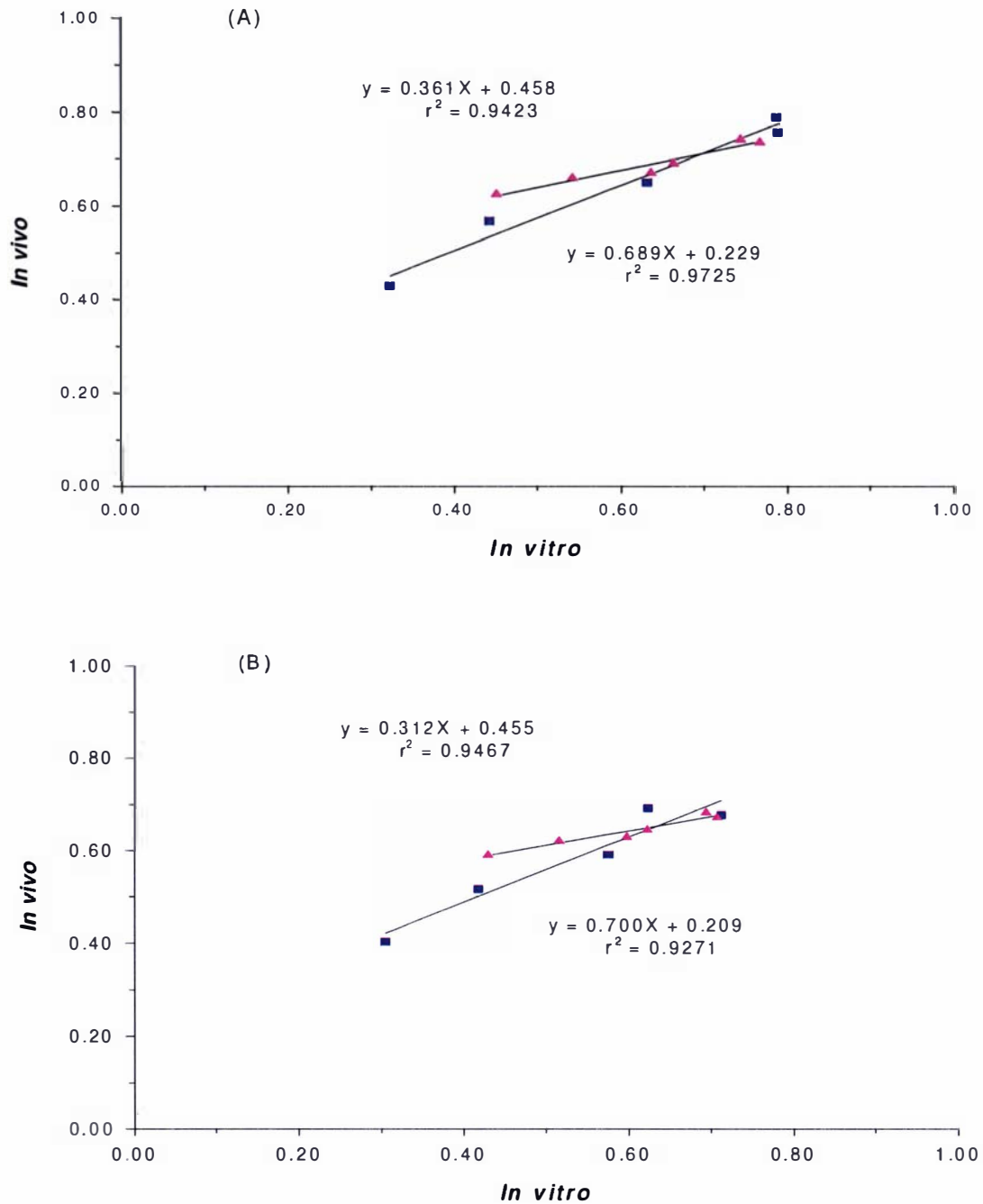


Figure 6.1. Relationships between *in vivo* and *in vitro* digestibility for (A) organic matter digestibility (OMD) and (B) digestible organic matter in dry matter (DOMD) for Experiment 2, using samples of the diet selected by sheep grazing (\blacktriangle) *Lotus corniculatus* L. (birdsfoot trefoil) and (\blacksquare) perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture.

6.4 DISCUSSION

The objectives of these experiments were firstly to measure effects on *in vivo* OMD and DOMD in *L. corniculatus* as the plant matured from the vegetative to the very mature stage. A secondary objective was to determine if the enzymic *in vitro* system of Roughan and Holland (1977) could be used to predict accurately OMD and DOMD of CT-containing *L. corniculatus*. The most significant findings were that as *L. corniculatus* matures, the decline in *in vivo* OMD and DOMD is less than would be expected for grass based-pastures (Waghorn and Barry, 1987) and that the enzymic system of Roughan and Holland (1977) can be used to predict *in vitro* digestibility of *L. corniculatus*, provided a standard curve of *in vivo* samples determined with animals fed *L. corniculatus* is used.

From Figure 6.1 it can be seen that the bias involved in predicting the OMD of *L. corniculatus* samples using a standard curve prepared with perennial ryegrass/white clover pasture (i.e. the normal for NZ) would depend if the sample OMD was above or below the point at which the *L. corniculatus* and pasture standard curves intersect (i.e. the equivalence point). For values of OMD above the equivalence point, using a pasture standard curve would overestimate the OMD of *L. corniculatus*, whereas below the equivalence point it would underestimate OMD.

Reasons for over-estimation are that the *in vitro* method of Roughan and Holland (1977) extracts soluble substances in the initial solubilisation step and therefore assumes them 100% digested, when they may be digested less than this *in vivo*.

An example is CT, that is not digested at all (Terrill *et al.*, 1994). However, this is unlikely to be a major source of error, as few *in vivo* OMD values for *L. corniculatus* (Table 6.1) were above the equivalence point.

Reasons for under-estimation are that the pasture-based standard curve is based upon forages that are predominantly perennial ryegrass and other grasses, with a low legume content and the Roughan and Holland (1977) method does not have a pepsin/trypsin digestion step to duplicate post-ruminal digestion. Relative to perennial ryegrass, less legume nitrogen (N) is digested in the rumen and more N is digested in the small intestine (McRae and Ulyatt, 1974). With the action of CT, the legume effect on this is likely to be even greater for *L. corniculatus* (Waghorn *et al.*, 1987), with a greater proportion of the N digested in the small intestine. Because the *in vitro* method does not have a pepsin/trypsin step, it may underestimate the *in vivo* value of legumes and specially *L. corniculatus* when a pasture standard curve is used. This is likely to be a substantial source of error, as most *L. corniculatus in vivo* OMD values (Table 6.1) are below the equivalence point. The only sure way of avoiding either over-estimation or under-estimation is to use a standard curve constructed with *L. corniculatus*, when using *in vitro* results to predict the *in vivo* OMD of CT-containing *L. corniculatus*.

It is possible that the neutral detergent extraction may leave a residual component of CT in the residue (Merkel *et al.*, 1999). If this occurs, it is likely to inhibit the cellulase/hemicellulase enzymes in the Roughan and Holland (1977) method and may lower *in vitro* OMD figures. There is evidence of this in Figure 6.1, for values below the equivalence point, where *in vitro* OMD and DOMD (at a given *in vivo*

value) are lower for the *L. corniculatus* standard curve than for the pasture standard curve. This can easily be corrected by using a standard curve also constructed with *in vivo* values from sheep fed *L. corniculatus*. It may be that separate standard curves are needed for each CT-containing forage.

Near infrared reflectance spectroscopy (NIRS) is a technique that is gaining popularity for predicting nutritive value (Clark, 1985), including OMD (De Boever *et al.*, 1995). This technique does not involve either solubilisation or enzymic degradation and instead relies on empirical relationships between infrared absorption at two or more wavelengths (Norris, 1985) and OMD in ground dried samples (De Boever *et al.*, 1995). These relationships could be influenced by CT and other phenolic compounds that occur in CT-producing plants. Thus, where NIRS is being used to predict OMD, it would seem advisable to also predict OMD of CT-containing forages using standard curves prepared with the same species that are being investigated.

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CHAPTER 7.

GENERAL DISCUSSION

This chapter has been submitted to *Animal Feed Science and Technology*

7.1 INTRODUCTION

The productivity of New Zealand pastoral farming systems has been developed on the efficient grazing of grasses, forage legumes, herbs or combinations of them to meet nutritional demands of farm animals. Therefore, the introduction and evaluation of improved and specialised grazing cultivars in dryland environments to overcome soil fertility and severe moisture deficits during the summer season is becoming important to improve traditional farm management, animal performance, sustainability and profitability under dryland farming conditions.

Given the importance of the year round productivity in a commercial dryland sheep farming system based on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) dominant pasture, the initial objective of this study was to assess the alternative use of *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) under commercial dryland farming, in particular to capitalise on the effects of its condensed tannin (CT) content in increasing amino acid absorption and in increasing animal production (Min *et al.*, 2003). First priority was to examine if feeding *L. corniculatus* during mating for at least 6 weeks with lighter ewes could increase ovulation rate (OR), lambing and weaning percentage under commercial conditions. A second aim examined the effect of feeding *L. corniculatus* to lactating ewes and their suckling lambs during spring on wool production and lamb weaning weight, whilst reducing anthelmintic inputs, to increase the proportion of lambs that can be drafted early for slaughter, before summer-dry conditions occur. The third objective evaluated the use of *L. corniculatus* for increasing the growth of post-

weaned lambs over the summer, to improve selling weights in the market with reduced reliance on anthelmintic drenches to control nematode parasites. Net herbage accumulation rate throughout 3 consecutive years was also measured, to compare the productivity of *L. corniculatus* with that of conventional perennial ryegrass/white clover pasture under commercial dryland farming conditions.

7.2 SYNTHESIS OF RESULTS

7.2.1. Reproductive performance

Results have shown that grazing ewes on *L. corniculatus* rather than pasture before and over the mating period in summer/autumn increased OR by up to 16% and the number of lambs weaned was increased by up to 14% (Chapter 3, Experiment 2), whilst the number of lambs born and weaned in Experiment 1 were increased by 10 and 26% respectively (Chapter 3). The increase in reproductive efficiency was due to increases in OR and to increases in fecundity, with more multiple births and less single births for ewes that were mated on *L. corniculatus*, with lamb mortality also being reduced in Experiment 1 for ewes mated on *L. corniculatus*.

A comparison of the results of the present studies with results of similar experiments conducted by other authors is shown in Table 7.1. Two types of experimental design have been used; repeat mating of ewes over one or two oestrous cycles using vasectomised rams, followed by intact rams for the final cycle (Min *et al.*, 1999, 2001; Luque *et al.*, 2000) or grazing different groups of

ewes on *L. corniculatus* for different periods of time and then mating them at the same time (this study, Experiment 2). Regardless of the experimental design used, it the maximum OR was achieved after ewes have been grazing *L. corniculatus* for between two or three oestrous cycles.

Results gained in the present study show that there is a linear response in OR to days of feeding *L. corniculatus* prior to mating in late summer/autumn, with at least 6 weeks being required to maximise reproductive efficiency under commercial dryland farming conditions (Table 7.1). These effects of mating on *L. corniculatus* compared to perennial ryegrass/white clover pasture are probably due to higher digestibility, higher metabolisable energy (ME) concentration and to the reduced rumen protein degradability by the CT-containing effect in *L. corniculatus*, leading to greater essential amino acid (EAA) absorption from the small intestine (Waghorn *et al.*, 1987). As there were higher responses at lambing for ewes that were mated on *L. corniculatus* relative to pasture (Table 7.1), it is possible that mating on *L. corniculatus* may reduce embryonic losses in addition to increasing OR. Additionally, field observations (Chapter 3; Experiment 1 and 2) indicated that mating ewes on *L. corniculatus*, including the period of early embryonic development, may have reduced lamb mortality between birth and weaning.

Table 7.1 Effect of feeding ewes for different lengths of time on *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) before mating on reproductive efficiency measured as ovulation rate (OR; *corpora lutea (CL)*/ewes mated) and as lambing percentage (lambs born/100 ewes lambing), relative to ewes grazing perennial ryegrass/white clover pasture.

	Days of grazing <i>L. corniculatus</i> before ovulation	Pasture	<i>Lotus</i>	Response to <i>Lotus</i> feeding (%)	Reference
<i>Ovulation rate</i>					
First cycle	8	1.35	1.43	6	Min <i>et al.</i> (1999)
Second cycle	25	1.33	1.78	33	
First cycle	16	1.60	1.77	11	Luque <i>et al.</i> (2000)
Second cycle	32	1.45	1.64	13	
Third cycle	48	1.65	1.77	7	
First cycle	16	1.37	1.58	15	Min <i>et al.</i> (2001)
Second cycle	32	1.60	1.70	6	
Third cycle	47	1.48	1.79	21	
First cycle	10		1.82	5	Chapter 3; Exp 2
	21		1.89	9	
	42	1.73	2.00	16	
<i>Lambing</i>					
	NA	136	170	25	Min <i>et al.</i> (1999)
	NA	122	169	39	Min <i>et al.</i> (2001)
	NA	159	175	10	Chapter 3; Exp 1
	NA	162	172	6	Chapter 3; Exp 2
<i>Weaning</i>					
		123	155	26	Chapter 3; Exp 1
		123	140	14	Chapter 3; Exp 2

NA; no apply. Initial weight during the mating season in the range of 53 to 60 kg.

This indicates that the duration (weeks) of CT-acting *L. corniculatus* feeding during mating and early pregnancy may play an important role to achieve a balance among OR, the uterine environment for conception, implantation and foetal development, but also may have a long term on lamb survival between birth and weaning. Possible mechanisms are reduced rumen ammonia concentrations and reduced blood plasma ammonia and urea concentration (Min *et al.*, 2001) and changes in the oviduct and uterus. Negative effects of diets with high levels of rumen degradable protein on survival of gametes, zygotes, utero-oviductal microenvironment and foetal growth have been previously reported (Blanchard *et al.*, 1990; Findlay *et al.*, 1990; Elrod and Butler, 1993; Elrod *et al.*, 1993; McEvoy *et al.*, 1997; Fahey *et al.*, 1998; Butler, 2000; McEvoy, *et al.*, 2001).

Further studies are required to investigate if feeding on the CT-containing legume *L. corniculatus* during mating and early pregnancy can reduce lamb losses from birth to weaning. These studies need to be done with the number of ewes/treatment group increased from 100 to 350. Additionally, areas of *L. corniculatus* used strategically into the farming system to allocate to ewes just mated on pasture could validate the hypothesis that feeding the CT-acting legume *L. corniculatus* in very early pregnancy might reduce both embryonic and post-natal lamb deaths.

7.2.2. Lactation and wool production

This study has shown that relative to conventional perennial ryegrass/white clover pasture, *L. corniculatus* can be used to increase pre-weaning lamb live weights, and both ewe and lamb wool production, whilst eliminating the need for prophylactic treatment in winter (pre-lambing), as judged by the lower levels of FEC and of dag formation (Chapter 2).

In a comparison with similar experiments conducted during lactation (Table 7.2), liveweight gain of lambs on *L. corniculatus* (mean 253 g/day) exceeded that on conventional perennial/white clover pasture (mean 198 g/day), with growth on lucerne (*Medicago sativa*) being intermediate (mean 237 g/day).

Wool production was ranked in the same order. Whilst these results are below the New Zealand Sheep Industry target of 400 g/day for lambs (The New Zealand Sheep Council, 2000), most of the lambs in these experiments were twins and the target growth of 400 g/day under grazing may be best achieved with single lambs.

Table 7.2 Comparative performance of non-parasitized (NP) and parasitized (P) lactating ewes and their lambs grazing *Lotus corniculatus* L., lucerne (*Medicago sativa*) or pasture (*Lolium perenne*/*Trifolium repens*).

	Plant fed						Reference
	<i>Lotus corniculatus</i>		Lucerne		Pasture		
	NP	P	NP	P	NP	P	
<i>Liveweight gain (g/day)</i>							
Ewes			- 89		35		^a Rattray <i>et al.</i> (1982)
					-199		^b McCall <i>et al.</i> (1986)
	251		59				Douglas <i>et al.</i> (1995)
	67						Wang <i>et al.</i> (1996a)
					75		Litherland <i>et al.</i> (1999)
		87				74	^c Experiment 1
		117				36	^c Experiment 2
Lambs				210		210	^a Rattray <i>et al.</i> (1982)
						213	^b McCall <i>et al.</i> (1986)
		275		263			Douglas <i>et al.</i> (1995)
		231					Wang <i>et al.</i> (1996a)
						217	Litherland <i>et al.</i> (1999)
		258				189	^c Experiment 1
		247				162	^c Experiment 2
<i>Wool growth (mg/100 cm² per day)</i>							
Ewes	133		123				Douglas <i>et al.</i> (1995)
	132						Wang <i>et al.</i> (1996a)
<i>Greasy fleece weight (kg)</i>							
Ewes			1.80		1.65		^a Rattray <i>et al.</i> (1982)
					1.65		^b McCall <i>et al.</i> (1986)
		4.22				4.07	^c Experiment 1
		1.69				1.43	^c Experiment 2
Lambs		1.17				0.98	^c Experiment 1
		1.17				0.81	^c Experiment 2

^a Mean values from two consecutive experimental years.

^b Mean values from a 2³ factorial experimental design.

^c Chapter 2.

Relative to sheep grazed on pasture, the high liveweight gain of lambs grazing CT-acting *L. corniculatus* during lactation in this study could be related to increased milk production and increased secretion rates of both lactose and protein from the action of CT during mid and late lactation (Wang *et al.*, 1996a), and also forage DM intake by lambs and the higher nutritive value of *L. corniculatus*. It is also possible that greater wool production in sheep grazing *L. corniculatus* was due to the increased availability and absorption of sulphur-containing amino acids from the small intestine (SAA; Reis, 1979), as CT in *L. corniculatus* reduced degradation of SAA in the rumen, increased irreversible loss (IRL) of cysteine from blood plasma and increased the flux of cysteine to body synthetic reactions (Wang *et al.*, 1994).

The grazing studies reported in this thesis during spring have shown reduced dag formation in both ewes and lambs grazing *L. corniculatus*. The absence of endophyte (*Neotyphodium lolii*) alkaloids, especially ergovaline in *L. corniculatus* compared to sheep grazing pasture, is possible one reason for the reduced faecal moisture content and reduced dag formation. Earlier research (Leathwick and Atkinson, 1995; Leathwick and Atkinson, 1998; Niezen *et al.*, 1998; Robertson *et al.*, 1995) have provided evidence that undrenched sheep grazing *L. corniculatus* had reduced incidence of dag formation compared with those sheep grazing high endophyte ryegrass cv. Nui or perennial ryegrass/white clover. In addition, enhanced tolerance to nematode parasites in sheep grazing *L. corniculatus*, due to increased EAA absorption, might reduce both anthelmintic treatment and production losses. The importance of protein supply in the diet (Donaldson *et al.*,

1997; Houdijk *et al.*, 2000) has been shown to be important in enabling ewes to better resist parasite burdens.

Therefore, this work shows that with increased efficiency of nutrient utilisation (higher estimated ME concentration; presence of CT) and increased efficiency of sheep production during the spring/summer season, the CT-acting *L. corniculatus* could have management opportunities for dryland farming systems associated with early lambing. Success with early lambing systems in dryland farming in New Zealand depends critically on two forage-related criteria; firstly ensuring sufficient high quality forage for flushing and mating in late summer/early autumn and secondly ensuring sufficient high quality feed for ewes to lamb on in late winter/early spring. It is shown in this thesis that *L. corniculatus* successfully meets the first of these criteria under dryland farming. However, there is a problem with the second criteria for the Wairarapa region of New Zealand, which is classified as a summer hot/winter cold climate, with insufficient early spring pasture growth to support large scale application of early lambing. For large scale application of early lambing to be feasible in the Wairarapa, additional supplementary feed is needed in late winter/early spring, such as silage or perhaps a winter active brassica crop such as turnips. Dryland areas further North in New Zealand, such as Hawke Bay and Poverty Bay, are classified as summer hot/winter warm climates and use of *L. corniculatus* for early lambing would fit well into farming systems in these areas. The same conclusion would probably apply in other dryland areas throughout the world, in that application of *L. corniculatus* for early lambing systems is probably best under summer hot/winter warm climates.

Application of *L. corniculatus* to early lambing systems under these conditions would bring benefits in less parasite problems, higher lamb selling live weights, low chemical residues in sheep products, and improved ewe live weights for the summer/autumn mating season. Therefore, further field studies are essential to evaluate the role of *L. corniculatus* for early lambing in order to capture premiums for chilled lamb and to increase the proportion of lambs drafted for sale before the onset of summer drought. Additionally, the performance of lambs that are conceived when their dams graze on *L. corniculatus* should be established on the legume during the spring/summer season.

7.2.3. Finishing of weaned lambs and sustainable control of parasitism

The current study has shown that over the late spring/summer, regular-drenched lambs grazing *L. corniculatus* produced the highest growth rates of approx 300 g/day over the 14 week period, which in spring were (350 g/day) close to the NZ Sheep Industry target of 400 g/day (The New Zealand Sheep Council, 2000). Trigger-drenched lambs grazing *L. corniculatus* produced lower mean liveweight gains of approx 230 g/day, but higher than that of either group grazing perennial ryegrass/white clover pasture (190–200 g/day). As carcass-weight gain of trigger-drenched lambs grazing *L. corniculatus* (99 g/day) was exactly half way between that of regular-drenched lambs grazing *L. corniculatus* (133 g/day) and pasture (66 g/day). The anthelmintic use can be restricted in weaned lambs grazing *L. corniculatus* but this will result in some loss of productivity (Chapter 4).

A summary of grazing trials involving CT-containing and non-CT-containing forages that evaluated parasite status and the performance of parasitized and non-parasitized weaned lambs is reported in Tables 7.3 and 7.4. The effect of withdrawing anthelmintic drench (i.e. non-parasitized – parasitized) depends upon the forage grazed. Withdrawing anthelmintic drench in lambs grazing pasture dramatically reduced LWG in two of the four experiments reported (50%) and similarly reduced LWG in three of the five experiments reported for lambs grazing lucerne (60%). In the two experiments reported for *L. corniculatus*, withdrawing anthelmintic drench dramatically reduced LWG in one of them. However, as shown in Chapter 4, anthelmintic drench could potentially be withdrawn in weaned lambs grazing *L. corniculatus* if the grazing period is 7 weeks or less. Withdrawing anthelmintic drenching had the least effect in reducing LWG for lambs grazing the CT-containing legume sulla (*Hedysarum coronarium*) or the herb chicory (*Chicorium intybus*). Worm burdens at slaughter were much lower in parasitized weaned lambs grazing sulla than for comparable parasitized weaned lambs grazing pasture, lucerne or *L. corniculatus* (Table 7.3).

Table 7.3. Comparative parasite status and liveweight gain of parasitized (P) and non-parasitized (NP) weaned lambs grazing perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture, lucerne (*Medicago sativa*) and condensed tannin (CT)-containing *Lotus corniculatus* L., sullia (*Hedysarum coronarium*), *Lotus pedunculatus* and chicory (*Chicorium intybus*).

	Pasture		Lucerne		<i>L. corniculatus</i>		Sulla		<i>L. pedunculatus</i>		Chicory	
	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
<i>Total GI worm burden</i>												
Niezen <i>et al.</i> (1995) ^c			19268				8016					
Niezen <i>et al.</i> (1998)	15806		18084		22990		13090		23665			
Chapter 4	9679				16812							
<i>LW gain (g/day)</i>												
Douglas <i>et al.</i> (1995) ^a				186		228						
Niezen <i>et al.</i> (1995) ^b			50	245			206	302				
Niezen <i>et al.</i> (1995) ^c			-39	184			129	200				
Scales <i>et al.</i> (1995) ^d	175	188	207	222							290	311
Scales <i>et al.</i> (1995) ^e	88	134	193	270							207	229
Niezen <i>et al.</i> (1998)	88	166	121	243	86	208	175	226	160	232		
Douglas <i>et al.</i> (1999)						215		227				
Chapter 4	187	200			228	298						

^a Experiment 2, ^b Experiment 1, ^c Experiment 2.

^d Experiment 1, ^e Experiment 2.

Table 7.4. Depression in carcass weight gain (g/day) caused by internal parasites (regularly drenched animals – parasitized animals) in lambs grazing five forages for 100 days at low and high allowances.

	Herbage allowance	
	Low (2 kg DM/lamb/day)	High (4 kg DM/lamb/day)
Forage		
<i>Lolium perenne</i> (perennial ryegrass cv. Nui)	32*	26*
<i>Dactylis glomerata</i> (cocksfoot cv. Wana)	24*	32*
<i>Festuca arundinacea</i> (tall fescue cv. Au Triumph)	26*	31*
<i>Medicago sativa</i> (lucerne cv. Otaio)	30*	50*
<i>Cichorium intybus</i> (chicory cv. Puna)	3	8

*Indicates effects significantly different from zero.

Adapted from Scales *et al.* (1995).

Experimental evidence (Scales *et al.*, 1995; Table 7.4) also indicates that carcass weight gain of weaned lambs grazing perennial ryegrass, cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*) and lucerne were all depressed by parasitism, whereas weaned lambs grazing chicory were unaffected by nematode parasites. Similar resilience to internal parasitism and increased animal productivity in autumn has been found in parasitized young farmed red deer grazing chicory compared to pasture (Hoskin *et al.*, 1999; 2003; Table 7.5) or fed sulla in indoor studies (Hoskin *et al.*, 2000). Thus of all the forages evaluated, sulla and chicory appear to have the most to offer for sustainable long term parasite control.

Table 7.5. Effect of grazing weaner red deer on perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture or chicory (*Cichorium intybus* cv. Grasslands Puna) with regular and trigger anthelmintic drench input upon animal productivity, clinical parasitism and nematodes worm counts from the lungs and gastrointestinal tract at slaughter. Grazing period was autumn 2002.

	Pasture		Chicory	
	Regular-drenched	Trigger-drenched	Regular-drenched	Trigger-drenched
Average autumn LWG (g/day)	134	60	208	175
Final live weight (kg)	62	57	66	64
Carcass weight (kg)	31	30	37	37
Dressing (%)	53	54	58	58
Clinical parasitism (%)	-	35	-	-
Lungworm (No)	1	643	0	311
GI nematodes (No)	0	2642	52	2240

Adapted from Hoskin *et al.* (2003).

Reasons why parasite problems are less in lambs grazing some forages than others include taller plant morphology and plant density (Knapp, 1964), giving less opportunity for infective larvae to migrate into the zone eaten by grazing ruminants and the presence of one or more secondary compounds that are known to have anti-parasite properties. A summary of these effects for the forages being discussed is presented in Table 7.6.

Reasons for sulla and chicory being so effective in parasite control, as well as promoting high animal growth rates, include tall plant morphology (Moss and Vlassoff, 1993), the presence of high concentrations of an astringent CT in sulla

(Terrill *et al.*, 1992; Douglas *et al.*, 1999) and the presence of low concentrations of two secondary compounds, CT and sesquiterpene lactones, in chicory that have anti-parasite properties (Molan *et al.*, 2000b; Schreurs *et al.*, 2002; Molan *et al.*, 2003). Reasons for grasses and lucerne being ineffective in parasite control include an absence of anti-parasite secondary compounds and a short grazing height in the case of grasses, leading to high larval intakes by grazing animals. *L. corniculatus* appears to fit between these two categories.

The beneficial effects may partly explained by differences in CT concentration, chemical structure and reactivity between these forages (Barry and McNabb, 1999). Therefore, the effects of grazing *L. corniculatus* in the present study on liveweight gain, worm burden and resilience of parasitized lambs may have been due to increased efficiency of protein digestion to overcome enteric loss of protein and reduced efficiency of food utilization (Sykes and Coop, 1976; Bown *et al.*, 1991). In previous metabolism studies with lambs Abbott *et al.* (1988) found a positive response of the immune system to protein intake. Immunology measurements therefore need to be made in future grazing experiments of this type involving CT-containing forages and management of internal parasites. The CT in sulla also increase EAA absorption in sheep (Bermingham *et al.*, 2001) and sulla CT are particularly effective in larval migration inhibition (Molan *et al.*, 2000a).

Table 7.6. Concentration in secondary compounds concentration in temperate forages species with pastoral value for New Zealand farming systems.

Forage	Total condensed tannin content (g/kg DM)	Other known plant secondary compounds	Morphology under grazing
Grasses			
<i>Lolium perenne</i> (perennial ryegrass)	1.8	Endophyte alkaloids 12 to 30 mg/kg DM	short
Legumes			
<i>Lotus corniculatus</i> (birdsfoot trefoil)	47	0	medium
<i>Lotus pedunculatus</i> (big trefoil)	77	0	medium
<i>Hedysarum coronarium</i> (sulla)			
Spring	84	0	tall
Autumn	51	0	tall
<i>Trifolium pretense</i> (red clover)	1.7	Iso-flavones 7 to 14 g/kg DM	tall
<i>Medicago sativa</i> (lucerne)	0.5		tall
Herbs			
<i>Chicorium intybus</i> (chicory)	4.2	Sesquiterpene lactones 3.6 g/kg DM	tall
<i>Sanguisorba minor</i> (sheeps burnet)	3.4	0	
<i>Plantago lanceolata</i> (plantain)	14	Iridoid glycosides ^{ab} ^a Catalpol 8 g/kg DM ^b Acubin 22 g/kg DM	medium

Short; recommended grazing height 6 cm for sheep and 10 cm for cattle under set stocking (Hodgson, 1990).

Tall; recommended grazing height approximately initial 30 cm down to 15 cm under a rotational grazing.

Medium; in between these heights under rotational grazing.

Adapted from Barry *et al.* (2002).

This trial (Chapter 4) highlights also the importance of alternative control of gastrointestinal nematode parasites to develop sustainable methods with reduced dependence on anthelmintic use. The most likely cause of this is the CT content of *L. corniculatus*. Recent *in vitro* studies (Molan *et al.*, 1999; 2000a) suggested that CT extracted from *L. corniculatus* have inhibitory activity against sheep *Trichostrongylus colubriformis* nematode parasites, and specifically reduced egg

hatching, the development of eggs to L₃ larvae and the motility of L₃ larvae, as demonstrated for CT extracted from *L. pedunculatus*, sulla and sainfoin (*Onobrychus viciifolia*) or chicory (Molan *et al.*, 2000b).

However, patterns of strongyloid nematodes parasites at slaughter in lambs grazing *L. corniculatus* suggest a decreased activity on parasite control from the abomasum to the rectum (Chapter 4). This may have been due to nematode species susceptibility to CT. Alternatively, changes in the CT molecule (Terrill *et al.*, 1994) after release protein at pH < 3.5 in the abomasum (Jones and Mangan, 1977) may explain the reduced effect on gastrointestinal nematode parasites from the abomasum to the large intestine. Moreover, it has been found that the *in vitro* parasite inhibitory activity of CT in chicory is reduced from rumen to abomasal fluid by 7% (Molan *et al.*, 2003). In contrast, sesquiterpene lactones extracted from chicory were not affected by the pH of either rumen or abomasal fluids to reduce parasite activity (Molan *et al.*, 2003).

Therefore, the greatest impact of the control of gastrointestinal parasites in grazing systems is not in terms of eliminating parasite infestation altogether, but rather the incorporation of new alternative temperate forages (Table 7.6) to reduce losses in production from parasites. The beneficial effects of developing more sustainable grazing systems with this germplasm relative to pasture-based livestock farm systems in dryland areas could be due to: concentration of plant secondary compounds, increased protein supply, high feeding value, plant structure, ability to grow in low or moderate fertility soils and seasonal forage production to meet deficit periods.

As *L. corniculatus* in the current study has also the potential to increase liveweight gains with traditional farming practices, further grazing trials will be needed to evaluate the effect of feeding *L. corniculatus* to hoggets during summer/autumn and its effect on mating and on reproductive performance. Further research is needed to assess the pastoral value of the herb chicory for year round productivity in sheep dryland commercial farming systems.

7.2.4. Agronomic value of Lotus corniculatus

It has been shown in the present study that relative to a perennial ryegrass/white clover pasture-based system, *L. corniculatus* increased sheep performance due to its ability to grow in acidic soils (pH 5.4), its similar annual dry matter (DM) production, but greater DM production during summer/autumn moisture deficits, its high feeding value, and both the moderate (18 to 40 g CT/kg) and beneficial concentration of condensed tannins (Chapter 5) to improve protein digestion. For these reasons *L. corniculatus* persists better under dryland farming than under high rainfall conditions and in terms of a systems approach seems better suited to integrating into dryland farming systems. Given these advantages, the farm management goals and objectives over late summer/autumn (Chapter 3), spring (Chapter 2) and late spring/summer (Chapter 4) were achieved.

However, there are in theory more opportunities to maximise animal performance, meeting feed demands for hogget growth rates to a suitable level for mating or additionally to contribute and test the hypothesis that year round lamb production is a viable option in New Zealand pastoral systems, even with emphasis in dryland

environments. Other advantages would depend on fertilizer inputs to increase the stock carrying capacity, dynamics of plant density and botanical composition

Although not measured in this study, making the assumption that *L. corniculatus* persists better under alternative management and that the legume has an adaptive capacity to respond to either grazing or harvesting techniques, the real value of *L. corniculatus* could be in the context of even more specialized production systems. Collins (1988) showed that, relative to pressed (mechanical fractioning that is critical factor for a forage harvesting system) lucerne and red clover (*Trifolium pratense*), pressed *L. corniculatus* was the highest in N concentration, the lowest in neutral detergent fibre (NDF), whilst its *in vitro* DM digestibility was least affected by pressing. Additionally, under hay-reseed management, natural reseeding may increase plant density by up to four-fold and compensate losses in DM production for the time that reseeding was permitted (Taylor *et al.*, 1973). Stands of *L. corniculatus* persisted after 12 years when seed production was allowed (Templeton *et al.*, 1967).

Therefore, these studies and the current findings (Chapter 5) suggest that after establishment, a recommended grazing programme and/or associated with adequate harvesting techniques, could achieve maintenance and a long lifespan of productive stands of *L. corniculatus*. This, in turn, should compensate for the long establishment period (Seaney and Henson, 1970; Foulds, 1978; Beuselinck and Grant, 1995), reduced persistence under grazing management (Van Keuren and Davis, 1968; Van Keuren *et al.*, 1969; Chapman *et al.*, 1990) and weed invasion (Douglas and Foote, 1993; Beuselinck and Grant, 1995). Persistence of *L.*

corniculatus may therefore be much longer if a component of the management system includes mechanical harvesting.

There are also encouraging indications of effective selection of cultivars to improve the competitive persistence of *L. corniculatus* during winter and reduce limitations under grazing conditions by selecting for a different plant morphology and a stable content of CT. As a result, in pastoral systems in Uruguay, the winter active cultivars, INIA San Gabriel and Draco, had 2.6 times greater levels of annual net herbage accumulation rate than winter dormant cultivars (Grasslands Goldie and Steadfast; Ayala, 2001). Similarly, a selection programme has been carried out in New Zealand to produce a "Grasslands creeping selection of *L. corniculatus*", containing moderate but stable concentrations of CT. This material is currently being evaluated in Canterbury as pure stands and with white clover and tall fescue as companion species. Seed is available for research in other projects in New Zealand (W. Rumball, personal communication).

Because of the effects of its CT in increasing EAA supply in sheep (Waghorn *et al.*, 1987), *L. corniculatus* is currently the best option available for grazing ewes during mating to increase reproductive performance (Chapter 3). However, whilst *L. corniculatus* does have some role in reducing parasite problems, the legume sulla and the herb chicory may be better forage options for this purpose (Chapters 2 and 4). However, rhizobial requirements, low persistence (2 years maximum), price and the lack of a commercial seed supply could be considered a barrier for both on farm trials and adoption of sulla as a legume alternative in agricultural activities (Rolston, 2003).

Chicory may have the potential to benefit dryland pastoral farming systems due to its moderate persistency (4 to 6 years), growth from spring to autumn, palatability and tolerance to *Sclerotinia* (root disease). Relative to grasses and legumes under rotational grazing, chicory is well adapted to summer/autumn moisture stress because of its deep taproot system (Stewart and Charlton, 2003). Similarly, compared to perennial ryegrass, its forage contains high concentrations of readily fermentable carbohydrates (sugars and pectin), metabolisable energy (ME) and higher digestibility of organic matter (OM) concentration (Barry, 1998). In addition, chicory also contains plant secondary compounds (Rees and Harborne, 1985) and high concentrations of minerals (Barry, 1998). A limitation is that it is a herb, and therefore does not fix nitrogen, but this can be overcome by sowing it with a companion legume.

This suggests that, as a pure stand or associated with prostrate species (clovers), chicory could have a role in mating and finishing systems, but there could be a possible restriction over lactation in spring due to degradation of plant secondary compounds in chicory (dihydrolactucin, hydroxyphenylacetic acid and tetrahydrolactucin; Barry, 1998), which causes taint in the milk of dairy cows.

Further research on farm is suggested to assess the feasibility of chicory to increase reproductive efficiency, weaning and post-weaning lamb live weights and wool production, as part of an integrated approach to nematode parasite control. This possibility needs to be investigated.

7.2.5. Chemical composition and nutritive value of *Lotus corniculatus*.

The *in vivo* digestibility of dry matter (DMD), OMD, digestible OM in the dry matter (DOMD) and estimated metabolisable energy (ME) concentration of *L. corniculatus* were similar between diets at the same stage of maturity, but declined as *L. corniculatus* matured from vegetative growth to very mature stage (Chapter 6), with the rate of decline being much less than occurs for temperate grass-based pastures.

Mean values of ME, N and CT concentration for *L. corniculatus* and pasture in a range of experiments are compared in Table 7.7. Overall mean values for all three were higher for *L. corniculatus*, with the ME value of *L. corniculatus* showing little variation between experiments. Pasture ME values showed more variation between experiments, with particularly low values during drought years. Therefore, the nutritional benefit of grazing *L. corniculatus* compared with grass-based pastures is likely to be maximal in drought years. Condensed tannin was consistently present in *L. corniculatus* in the range 13–45 g/kg DM (mean 27 g/kg DM), whereas pasture contained only traces of CT (0.5–4.1 g/kg DM). Total N concentration was similar for the two forages.

These data also demonstrated that increase in sheep productivity in the current studies are the result of high *in vivo* digestibility, high nutritive value and improved protein digestion due to the moderate concentration and beneficial effects of CT in *L. corniculatus*.

Table 7.7. Mean values of metabolisable energy concentration (ME; MJ/ kg DM), total nitrogen (N; g/kg DM) and condensed tannin concentrations (CT; g/kg DM) of the diet selected by sheep and deer grazing perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) dominant pasture, *Lotus corniculatus* L. (birdsfoot trefoil; cv. Grasslands Goldie) or lucerne (*Medicago sativa*).

Reference	Animal Species ¹	Season	Pasture			<i>L. corniculatus</i>			Lucerne		
			ME	N	CT	ME	N	CT	ME	N	CT
Chapter 2, Exp 1 ^a	S	Spring	9.8	29.7	1.4	10.6	30.3	24.1			
Chapter 2, Exp 2 ^a	S	Spring	10.2	26.2	0.5	10.7	33.5	26.7			
Wang <i>et al.</i> (1996a) ^b	S	Spring/summer				10.8	35.5	44.5			
Chapter 4, Regular-drenched ^a	S	Summer	9.6	26.5	1.6	10.6	27.1	39.9			
Chapter 4, Trigger-drenched ^a	S	Summer	9.5	25.8	1.2	10.6	27.8	31.3			
Adu <i>et al.</i> (1998) ^b	D	Summer	8.2	21.7	1.6	10.2	27.9	21.2			
Wang <i>et al.</i> (1996b) ^b	S	Summer/autumn				10.8	31.4	34.0	11.0	41.8	0.3
Chapter 3, Exp 1 ^a	S	Summer/autumn	8.3	18.4	1.8	10.9	28.4	18.4			
Chapter 3, Exp 2 ^a	S	Summer/autumn	9.1	25.5	1.3	10.4	26.0	28.6			
Min <i>et al.</i> (1999) ^b	S	Late summer/autumn	10.9	46.0	4.1	11.7	42.2	12.8			
Luque <i>et al.</i> (2000) ^b	S	Late summer/autumn	8.7	22.8	1.3	10.2	26.0	23.7			
Min <i>et al.</i> (2001) ^b	S	Late summer/autumn	9.8	37.9	2.2	11.2	44.8	17.6			
Mean			9.4	28.1	1.7	10.7	31.7	27.0			

^a ME = DOMD x 16.3

^b ME = OMD x OM x 16.3

¹ S = sheep; D = farmed deer.

7.3 IMPLICATION FOR PRODUCTION SYSTEMS; FUTURE TECHNOLOGY TRANSFER AND RESEARCH NEEDS

There is substantial evidence relative to perennial ryegrass/white clover pasture of the potential value of *L. corniculatus* in dryland farming systems to increase sheep productivity during mating (Chapter 3), lactation (Chapter 2) and finishing seasons (Chapter 4). These advantages are by producing similar annual DM production, adaptation to drought conditions during summer/autumn (Chapter 5), high nutritive value and high digestibility (Chapter 6). Additionally, as a component of dryland production systems, *L. corniculatus* could also be considered to improve hogget growth, prior to mating. However, whilst *L. corniculatus* is currently the best alternative forage available to dryland farmers for increasing reproductive performance, it seems that both chicory and sulla could be better options for controlling parasites in weaned lambs. Given the agronomic limitations of sulla, the best alternative to *L. corniculatus* for controlling parasites in weaned lambs is therefore chicory, probably sown in a mixture with a companion legume (red or white clover).

In relation to future work, translating the results of the present series of experiments into recommendations for commercial sheep farming systems is seen as the first priority (i.e. technology transfer). However, for applied scientists, consultants and farm decision makers to develop, promote and support adequate extension programmes, caution should be exercised in focusing on the impact of variable percentages of the farm that might be sown on *L. corniculatus*. Therefore,

the development of a decision support model should be considered to determine the point at which the seasonal and annual DM production of *L. corniculatus* at the whole farm level result in a marked change in existing farm systems.

Conceptually, the deterministic and biological Stockpol® modelling programme developed by the modelling group at Whatawhata Research Centre (AgResearch) in New Zealand (Marshall *et al.*, 1991) provides the opportunity to predict the effect of input of *L. corniculatus* (birdsfoot trefoil; cv. Grasslands Goldie) into the farming system in 'average', dry and wet years from both a physical and a financial perspective (N.M. Shadbolt, personal communication). Probabilities could then assumed for each of these conditions, based on historical climatic data, market prices and costs of production. This suggests that cumulative probability curves for a range of relevant outcomes for the farm business may be also determined by use of the complementary risk analysis programme @RISK®. Through this process, the influence of inputs of *L. corniculatus* on the viability of commercial dryland farming systems could be quantified and the inherent risks and their impact identified. The proportion of the dryland farm to sow in *L. corniculatus* to give the greatest financial return could be quantified. This information will assist commercial lamb producers in their decision on whether to adopt this new technology and, based on their attitude to risk, to what extent to adopt it. (N.M. Shadbolt, personal communication).

The second priority is research involving long term studies that investigate the efficient use *L. corniculatus* and other alternative forages at strategic times in the year round dryland sheep production system. As improved reproductive efficiency

was reached on *L. corniculatus* in terms of OR and lambing percentages (Chapter 3), a number of research opportunities may be identified. First, to keep *L. corniculatus* as a specialist forage to increase OR and to increase lamb growth in spring, and to use chicory to promote growth in weaned lambs in late spring/summer with reduced anthelmintic input. A secondary use of chicory could be as a pre-mating and mating crop. Investigating if mating ewes on specialist forages can reduce lamb mortality between birth and weaning is a key research area.

Use of chicory supported in the low ratio of structural : readily fermentable carbohydrates may increase microbial protein flow for absorption, reducing loss of N across the rumen (Barry *et al.*, 1998), contributing then to regulate the uterine environment for conception, implantation and foetus development (Chapter 3) as proposed for *L. corniculatus*. Thus, at mating weight, hoggets could be moved earlier onto *L. corniculatus*. Additionally, the post-mating option should bring two extra opportunities for the sheep commercial systems: (i) increased number of mixed aged ewes mated on the legume or (ii) reduced time (weeks) that ewes would require grazing *L. corniculatus* to decrease losses from conception to lambing with increased lamb viability to weaning. Additionally, a reasonable interpretation of the present findings is that chicory in a mixture with legumes following the same scheme noted above could lead to considerable production gains with reduced anthelmintic input.

It would appear, using the above mentioned assumptions, that chicory could have a particular role to complement systems based on *L. corniculatus* or pasture for

post-weaning growth, lactation or perhaps as a mating feed. Thus, grazing systems could be more sustainable for lamb sales or hogget replacements due to a higher proportion of chicory DM production during summer/autumn (Barry *et al.*, 1998), particularly under moisture stress. The anthelmintic effect of sesquiterpene lactones and CT in chicory (Molan *et al.*, 2000b; Schreurs *et al.*, 2002; Molan *et al.*, 2003) will play an important role to develop alternative approaches on parasite epidemiology research in pastoral dryland farming systems.

Finally, in addition to the results described from *L. corniculatus* in the present study, effects of feeding the legume upon animal productivity may be further discussed in terms of the use of winter active and creeping germplasm that may further enhance sustainable productivity in specialised dryland farming systems. Mechanical harvesting and effective conservation strategies of surpluses may also be evaluated; an example of the use of *L. corniculatus* as a component of total mixed rations (TMR) for dairy cows in New Zealand, as proposed by Woodward *et al.* (2004).

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