Can mating ewes on *Lotus corniculatus* be used to reduce lamb mortality between birth and weaning?

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Chapter 1

Abstract

A grazing experiment was conducted over 74 days from late summer to early autumn in 2004 at Massey University's Riverside dryland farm, in the Wairarapa Region, New Zealand. The experiment compared the effects of grazing ewes on *Lotus corniculatus* (birdsfoot trefoil; cv. Grassland Goldie) versus perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture for 10 weeks during pre-mating and mating time, on reproductive performance of the animals and mortality of lambs from birth to weaning.

Shorn mixed age Romney ewes with an initial mean live weight of 56.7 Kg and mean body condition score of 2.9 were rotationally grazed on *L. corniculatus* (*n* = 300) or pasture (*n* = 300) at a herbage allowance of 2 Kg DM/ewe/day. At the end of the *L. corniculaus* feeding, animals from both treatment groups were joined and grazed on pasture until weaning.

Total condensed tannin (CT) concentration in the diet selected was 22g/Kg DM for *L. corniculatus,* with only trace amounts in pasture. Digestible organic matter in dry matter (DOMD; 0.66 vs 0.64) and metabolizable energy (ME) concentration (10.75 vs 10.42 MJ/ Kg DM) were slightly, but significantly, higher in *L. corniculatus* than in pasture. There was no difference in the nitrogen (N) content (g/Kg DM) neither in the

in vitro organic matter digestibility (OMD) between both diets, whilst the concentration of neutral detergent fiber (NDF) was lower in *L. corniculatus* than in pasture.

Liveweight gain (g/day) during the experimental feeding period was lower in the *L. corniculatus* group (94 g/day) than in the control pasture group (130 g/day), whilst increments in body condition score (BCS) during the same period were similar in both groups, with a mean total increment of 0.11 units. There were no significant differences in the reproductive rate of ewes (lambs born/100 ewes mated); however, there was a tendency in the lotus group to have a higher reproductive rate at scanning and lambing when data was corrected to similar liveweight gain during mating. There was no difference in fecundity rate (proportion of singles, twins and triplets) at lambing between treatment groups; however, there was a tendency for the lotus group to have a higher proportion of triplet lambs when data was corrected to similar liveweight gain during mating. The mean conception rate was 96.5% with no difference between treatment groups. Ewes in the lotus group lambed earlier than their counterparts in the control pasture group, with the difference being significant in twin and triplet-bearing ewes ($P<0.05$); differences in mean lambing dates were 0, 2 and 6 days for single, twin and triplet-bearing ewes respectively. Birth weight of female triplet lambs born to ewes mated on *L. corniculatus* was higher than for their counterparts born to ewes mated on control pasture ($P<0.05$); there was no other nutritional effect on either birth weight or weaning weight. Lamb mortality was directly proportional to birth rank regardless of nutritional treatment group, with mortality being of 14.7%, 26.7%, and 45.6% for singles, twins and triplet lambs respectively. Ewes' wool production was similar for both treatment groups.

Control pasture had an unusual high nutritional quality for a dryland area during autumn, which was due to an unusual wet autumn. The good nutritional status of ewes in the control pasture group was reflected in the high reproductive parameters measured in this experiment, leaving little room for differences when compared to the parameters observed in the lotus group. The co-variance adjustment to equal liveweight gain during mating suggests that in a normal dry autumn, mating on *L. corniculatus* would have increased the number of triplet pregnancies. It was concluded that the high lamb mortality rate in the lotus group was influenced mainly by the combination of three factors: a heavy storm during the third lambing week, a trend to more of triplet lambs in

this group, and a higher proportion of triplet bearing ewes lambing just before or during the storm in this group than in the control pasture group. It is suggested that the effect of *L. corniculatus* feeding during mating and early pregnancy on triplet pregnancies may be due to the increased EAA absorption from the action of the condensed tannins.

Factors which may have influenced the results of the present experiment are discussed, and suggestions are made concerning the design of future field experiment of this type.

Literature Review

1.1 Introduction

In countries like New Zealand, where the annual sheep production system is based on grazing of temperate forages, quality of pastures is one of the most important management issues to maximize production efficiency. When choosing forages to be planted on a farm, is important to consider, apart from their nutritional qualities, some other characteristics such as growth pattern, nutritional needs, soil requirements, production under grazing and adaptation to adverse weather conditions (e.g. drought conditions).

Combinations of temperate grasses and legumes in the sward are very common in the grazing systems of New Zealand. The advantages of using legumes are well known, including their effect on nitrogen fixation to the soil, their high nutritive value and their high palatability (Sheldrick, et al., 1995; Frame, et al., 1998). In New Zealand the most common legume used on farms is white clover, which is normally combined in the sward with perennial rye-grass (Waghorn and Clark, 2004). However, there are other forage legumes like *Lotus corniculatus,* that can be used on farms under specific circumstances.

Lotus corniculatus is a temperate forage legume that has proved to have some advantages when used in dry areas, like the East Coast in the North Island of New Zealand, due to its better resistance to drought conditions when compared to other legumes such as white and red clover (Ramirez-Restrepo, et al., 2004; Ramirez-Restrepo, et al., 2005a). In addition, its condensed tannin (CT) concentration represents an extra nutritional value, increasing the amount of 'bypass' protein in ruminants, which normally results in the improvement of productive and reproductive parameters of the animals (Waghorn, et al., 1987; Min, et al., 2003).

Ovulation rate and lambing percentage are two of the reproductive parameters that are normally enhanced when using *L. corniculatus* as grazing forage compared to common temperate grasses and legumes (Ramirez-Restrepo and Barry, 2005). Although the mechanisms by which these effects are achieved are not well understood yet, there are some hypotheses, which suggest that it is directly related to the nutritional status of the animals. There are some theories proposing that the improved supply of essential amino acids from the action of CT in *L. corniculatus* is important in the physiological processes involved in fetal development during pregnancy, and in post-natal survival (Barry, et al., 2004).

1.2 Sheep production systems in New Zealand

1.2.1 The annual cycle

The sheep production in New Zealand is a complex process that demands several skills from farmers in order to cover for the needs of both the animals and the pasture during the whole producing cycle, with the aim to have an efficient production at a low costs. This production process involves several activities all around the year. This section gives an overview of the annual cycle of commercial sheep production in New Zealand, with main activities being described.

Mating time. Lamb production is the priority of many sheep farms in New Zealand. That is why mating time is so important because management of the animals at this part of the production cycle has a huge influence on the total number of lambs produced at the end of lambing and at weaning. In order to achieve a good reproductive performance in the ewes during mating, the period of time between weaning and mating (summer time) becomes so critical because during this time the ewes have to gain weight and body condition (BCS) to reach a condition score around 3 (within a range from 1 to 5), which is related to a high ovulation rate during the coming season (Allen and Lamming, 1961; Abecia, et al., 1997). To hold a body condition score of 3 during summer, ewes need to consume 1 – 1.3 Kg DM per day of average good quality forage (10 to 12 MJ/Kg DM). During the mating time the ewes must maintain a high live weight and body condition score in order to reach high ovulation rates, which in turns generally increases the conception rate. During mating time the rams are with the ewes for about 35-42 days in ewe:ram ratios that can be 100:1; therefore just healthy and active rams are used with large testicular size to keep the sperm numbers high even when the animals serve many ewes per day (Geenty, 1997).

Pregnancy. Pregnancy develops during mid or late autumn and winter, and is commonly divided into early pregnancy (until day 50 of pregnancy), mid pregnancy (between days 50 and 100), and late pregnancy (from day 100 to lambing). Is in early pregnancy when the embryo implants into the uterus and the placenta begins to develop. Stress is one of the causes of embryonic losses; therefore, management of the animals at this time is minimal, avoiding activities such as shearing, and keeping to a minimum others like yarding and handling. Undernutrition is another important cause of embryonic death; therefore pregnant ewes must be kept well fed during the whole gestation but mainly at early and late pregnancy (Geenty, 1997; Jainudeen and Hafez, 2000b).

Feeding management of the animals during the last two thirds of pregnancy must be similar to management in early pregnancy maintaining the animals well fed and keeping the stressing factors at minimum. It is during mid pregnancy that ultrasound scanning is practiced in some commercial farms between days 60 and 90 of pregnancy. Ultrasound scanning is a reliable method to determine the number of fetuses carried by ewes, which allows farmers to identify empty ewes and those with single or multiple pregnancies for future management of each kind of animal; single-bearing ewes are also separated from multiple-bearing ewes, so that the animals can receive the best nutrition during late pregnancy and after lambing according to the number of lambs bearing and rearing (Geenty, 1997). Other activities to be carried out during mid pregnancy are to get all the non-productive animals off the farm, in order to allocate a higher amount of feed to productive animals; to start planning for lambing (get equipment and lambing paddocks ready); to carry out a feed budget to ensure proper feeding of the animals during lambing, and build up feed reserves for lambing (Dalton and Orr, 2004). During late pregnancy sheep farmers normally divide their flocks into groups by lambing date to control feeding, at this time they must be prepared for ewes that lamb before expected date, and have to check pasture growth and consider use of supplements or strategic use of nitrogen to provide feed for lactating ewes (Dalton and Orr, 2004).

Lambing. Lambing occurs during late winter or early spring depending on the mating dates, and the animals are allocated preferably in flat or gently sloping areas to facilitate the physical and visual contact between lambs and their mothers (Geenty, 1997). As in late pregnancy, good nutrition of the ewes at this point of the production cycle becomes so important to guarantee high milk production levels that enhance survival rate of the lambs. During lambing just minor sheperding activities are done such as assisting difficult births, lifting cast ewes, fostering mis-mothered lambs, and reducing constipation in lambs with stuck-down tails (Geenty, 1997; Dalton and Orr, 2004). However, the ranges at which these activities are practiced differ among farms, some of them covering the full range, and some others without any kind of intervention. After lambing ewes and lambs remain together for some months until weaning time in late spring (Geenty, 1997).

After lambing. After lambing lactating ewes are kept on the best feed to maintain lamb growth, regular assessments of feed on the farm must be done at this time to ensure proper feeding of both ewes and lambs at this time and after weaning; nitrogen can be used in some parts of the farm to increase pasture production for the following months (Dalton and Orr, 2004). Energy requirements of ewes are at a maximum during lactation (Table 1.1), emphasizing the need for high quality forages over this time. Lambs are normally reared by their dams for 12 weeks and then they are weaned; some weeks before weaning lambs can be docked (Geenty, 1997).

Table 1.1. Daily ME and DM intake requirements for ewes at different physiological stages.

Live weight (Kg)	**DM intake per day (Kg)**	**ME requirements per day (MJ)**
Maintenance		
50	1.0	8.4
60	1.1	9.2
70	1.2	10
Last month gestation (singles)		
50	1.6	14.2
60	1.7	15.6
70	1.8	16
Last month gestation (twins)		
50	1.7	17
60	1.8	18
70	1.9	18.5
First 6-8 weeks lactation (singles)	2.1	20.5
50	2.3	22.6
60	2.5	24.7
70		
First 6-8 weeks lactation (twins)		
50	2.4	23.5
60	2.6	25.5
70	2.8	27.6

(Pond, et al., 1995).

1.2.2 Grazing systems

The way that animals graze in a farm can be organized in different ways, which are commonly known as 'grazing methods' or 'grazing systems'. All these grazing systems have advantages and disadvantages; however, the main objective of all of them is to control and administer plant and animal resources, always attempting to satisfy the needs of both of them in order to maintain a high overall efficiency in the production system (Hodgson, 1990). An efficient grazing system matches animal feed requirements to the amount of pasture produced at all seasons of the year, always taking into account seasonal changes in pasture production. The choice of a grazing system depends on the physical characteristics and specific needs of the farm, and also on the farmer's preference. There is not a perfect grazing system since they vary widely in their adaptation to different vegetation types, precipitation zones, terrain, and soil type as well as in their management and investment requirements (Vallentine, 2000); therefore, they must be tailored to fit the needs of a specific case.

Continuous grazing. The main characteristic of this system is that the same area of pasture is grazed continuously by the livestock for long periods of time (Holmes, et al., 2002). It does not mean that there is a continuous defoliation of individual tillers or plants, in fact, some studies have shown that defoliation may vary from 5 days to as long as 3 to 4 weeks, although in this kind of grazing systems the access to particular areas of the sward is not controlled as in the case of rotational grazing (Hodgson, 1990).

Rotational system. This kind of system involves a regular sequence of grazing and rest over a series of paddocks, which means that the livestock are moved on to a fresh area of pasture at regular intervals and after each grazing there is a period of time when the area is not grazed, which gives the herbage time to recover (Hodgson, 1990; Holmes, et al., 2002). Rotational systems are so practical and useful to control grazing and conservation of pasture. In addition, they represent such an efficient way to use pasture, decreasing pasture wastage, ensuring that the animals receive fresh forage every day and that the pasture gets time to recover (Holmes, et al., 2002).

Rotational grazing is managed by the combined use of permanent and temporary electric fences in paddocks. The electric fences are useful to divide paddocks in grazing-blocks in order to achieve additional control over the grazing rotation. Back fences behind the animals can be used when necessary to avoid the animals grazing the pasture regrowth in the paddocks or blocks formerly grazed. The animals can stay in the same grazing-block from few days to one week (Vallentine, 2000).

Strip grazing. This grazing system is a high-intensity variety of the rotational system, and is based on confining grazing animals to an area under high stocking density and short grazing periods (half of a day to three days) (Vallentine, 2000). In general it is suggested that the animals stay in the same area no longer than three days to avoid newly grown tillers to be eaten off immediately after their emerge from the ground (Holmes, et al., 2002).

Deferred grazing. In this system, grazing is deferred in specific paddocks (not in systematic rotation with other paddocks) from the breaking of plant dormancy until after seedset or equivalent stage of vegetative reproduction, and then, the residual standing crop is grazed (Vallentine, 2000). Deferment grazing can be a one-time event or applied annually to the grazing units, and can be systematically rotated among the respective units in the system (Vallentine, 2000).

Deferred grazing can be useful to improve herbage density in specific areas by increasing seed production, enhancing seedling establishment, and protecting plants susceptible to trampling damage and defoliation in early spring (Vallentine, 2000). In addition, grazing after seed production is suggested for trampling the seed into the soil (Vallentine, 2000). Deferred grazing normally does not cause improvements in the productive performance of the animals because the herbage is grazed after reaching its reproductive stage, which decreases its nutritional value; however, this system represents a cheap method to conserve forage to feed the animals during the feed-shortage season, and to increase the herbage density in the paddocks through natural reseeding.

1.2.3 Grazing forages

The swards for grazing animals range from the most simple ones containing just one grass variety in extreme cases, to highly complex permanent pastures, which frequently contain several pasture species in some cases even more than ten (Hodgson, 1990). Botanical composition of long-established swards depends on the combined effect of factors such as type of soil, climate and management (Hodgson, 1990).

Pasture management and forage genotype are two of the most important factors influencing pasture production in the sward; however, in many circumstances grazing management (including fertilizer inputs) are likely to have a greater influence on herbage production than does plant genotype. Also, within limits, a high population of leafy tillers and a good ground cover are more important in determining the performance of the sward than is the genetic make-up of the plants of which it is composed (Hodgson, 1990).

When a sward is going to be sown with new forages, first is important to make an informed choice among the plant material available, taking into account primarily the productive potential (under the local circumstances) and the nutritive value of the plant species and cultivars (Hodgson, 1990). In the case of extreme climates, it is important to consider tolerance to winter cold or summer drought and resistance to pests and diseases, which are of equal importance as the former characteristics (Hodgson, 1990).

The pasture mixture in the sward commonly includes some legumes. White clover (*Trifolium repens*) is the most common legume available, which is flexible enough to consider for general-purpose grazed swards. The other major legumes, red clover (*T. pratense*) and lucerne (*Medicago sativa*) have their place as special-purpose crops, principally in dry areas (under specific management requirements). In the same way, *Lotus spp*. (*L. corniculatus and L. pedunculatus*) are two forage legumes suitable for soils of low fertility and dry areas (Hodgson, 1990). The presence of legumes in mixed swards is beneficial for the growth of the companion grasses due to the ability of legumes to fix atmospheric nitrogen; in addition, legumes in mixed swards normally improve the intake and the nutritive value of a grass diet (Hodgson, 1990).

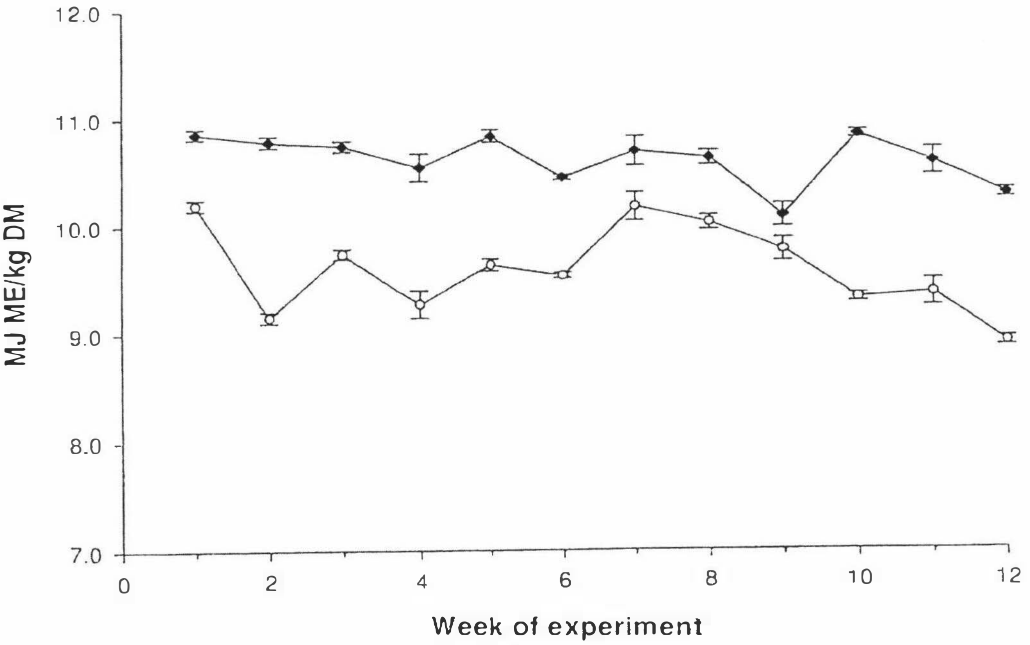
1.3 Lotus corniculatus

1.3.1 Nutritional characteristics

Lotus corniculatus, also commonly known as birdsfoot trefoil, is a temperate legume used as forage for grazing ruminants. The nutritive value of this legume is similar to that of Lucerne (*Medicago sativa*) (Sheldrick, et al., 1995; Frame, et al., 1998) and substantially higher than that of perennial ryegrass/white clover pasture when grown under New Zealand conditions (Table 1.2). Total herbage *in vitro* DM digestibility (IVDMD) of birdsfoot trefoil declines from the prebud stage onwards, because of the development of stems. In early summer the rate of decline is slower than in latter regrowths, falling from 68 to 66% over a 40-day period in the early season, compared with a decline from 67 to 58% latter (Frame, et al., 1998); stem IVDMD may decline at twice the rate of total herbage and the cell-wall content may increase during regrowths from about 30 to 45%, a change similar to that in red clover but lower than that in lucerne. Lignin content is consistently lower in birdsfoot trefoil than in other temperate legumes, such as white and red clovers and lucerne, but the *in vivo* digestion rates of organic matter (OM), hemicellulose and cellulose are similar to those in lucerne and sainfoin (Frame, et al., 1998). Drought normally increases the overall forage quality of birdsfoot trefoil, due to an increased leaf:stem ratio, delayed maturity and increased quality of each plant fraction (Frame, et al., 1998). Under summer grazing conditions in New Zealand, *L. corniculatus* was of higher ME value than perennial ryegrass/white clover pasture and declined less with time under very hot conditions (Figure 1.1).

The presence of condensed tannins (CT) in *L. corniculatus* is perhaps the most important nutritional quality of this legume. Condensed tannins are present in the foliar and stem tissues, and their capacity to bond with herbage protein plays an important role in protecting feed protein from ruminal degradation, and this allows more protein to reach the small intestine, increasing the absorption of essential amino acids, and thus improving productive and reproductive parameters in the animal (Waghorn, et al., 1987). In addition, CT from *L. corniculatus* may help to prevent bloat in ruminants by slowing down the rupture rate of herbage cell wall in rumen (Mangan, 1959), a rate that is slower than that for lucerne and clovers (Sheldrick, et al., 1995; Frame, et al., 1998).

Figure 1. 1. Metabolisable energy (ME) concentration (MJ/Kg DM) of diet selected by lambs grazing *Lotus corniculatus* (cv. Grasslands Goldie; ♦) and perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture (○) over the summer season of 2002-2003 in a New Zealand dry land pastoral system. (I = S.E.M.).



(Ramirez-Restrepo, et al., 2005b).

Table 1.2. Mean values of metabolizable energy concentration (ME; MJ/Kg DM), total nitrogen (N; g/Kg DM) and condensed tannin concentration (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* (birds foot trefoil; cv. Gasslands Goldide), or lucerne *(Medicago sativa)*[1].

			Pasture			*Lotus corniculatus*		
Reference	Animal species[2]	Season	ME	N	CT	ME	N	CT
Ramirez-Restrepo et al., (2004) Exp. 1[a]	S	Spring	9.8	29.7	1.4	10.6	30.3	24.1
Ramirez-Restrepo et al., (2004) Exp. 2[a]	S	Spring	10.2	26.2	0.5	10.7	33.5	26.7
Wang et al., (1996a)[b]	S	Spring/summer				10.8	35.5	44.5
Ramirez-Restrepo et al., (2005b); Regular-Drenched[a]	S	Summer	9.6	26.5	1.6	10.6	27.1	39.9
Ramirez-Restrepo et al., (2005b); Trigger-Drenched[a]	S	Summer	9.5	25.8	1.2	10.6	27.8	31.3
(Adu, et al., 1998)[b]	D	Summer	8.2	21.7	1.6	10.2	27.9	21.2
Wang et al., (1996b)[b]	S	Summer/autumn				10.8	31.4	34.0
Ramirez-Restrepo et al., (2005a); Exp 1[a]	S	Summer/autumn	8.3	18.4	1.8	10.9	28.4	18.4
Ramirez-Restrepo et al., (2005a); Exp 2[a]	S	Summer/autumn	9.1	25.5	1.3	10.4	26.0	28.6
Min et al., (1999)[b]	S	Late summer/autumn	10.9	46.0	4.1	11.7	42.2	12.8
Luque, et al., (2000)[b]	S	Late summer/autumn	8.7	22.8	1.3	10.2	26.0	23.7
Min et al., (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

[2] S = sheep; D = farmed deer

[1] (Wang, et al., 1996b) values for lucerne were 11.0 MJ ME/Kg DM, 42 g N/Kg DM and 0.3 g CT/Kg DM.

(Ramirez-Restrepo, et al., 2005a).

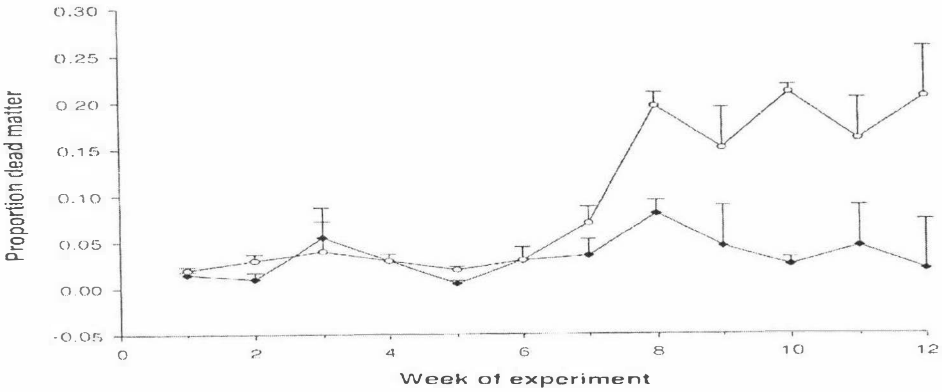
1.3.2 Lotus corniculatus as a grazing forage

Lotus corniculatus is a perennial forage with a deep taproot, that penetrates the soil almost as deeply as those of lucerne (usually 2 – 4 meters in depth), which make it drought resistant, but with a greater lateral spread, which leads to its better persistence than lucerne on undrained shallow soils (Sheldrick, et al., 1995; Frame, et al., 1998).

Lotus species in general have a slow establishment and poor competitive ability (McKersie, et al., 1981). However, in recent years, new improved cultivars have been released with much better seedling vigor and growth characteristics in addition to the experience accumulated both in establishing productive lotus pastures with minimum delay, and maintaining their persistence. As the seeds are small, a firm seed-bed is required with an optimum sowing depth of 10 – 15 mm under warm moist conditions (Charlton, 1984; Sheldrick, et al., 1995). For monocultures, *L. corniculatus* is generally sown directly in spring, when soil moisture is adequate, at seeds rates of 6-10 Kg/ha. It can be sown in autumn, but there has to be sufficient time for plants to develop winter-hardiness (Laskey and Wakefield, 1978). When sown in mixed cultivars, *L. corniculatus* has to be sown in simple mixtures with non-invasive grasses due to its low establishment rate. Some of the grasses that can be used as a companion grass for *L. corniculatus* are: timothy (*Phleum pretense*), smooth-stalked meadow-grass (*Poa pratensis*), brome grasses (*Bromus* spp.) or non-aggressive cocks foot (*Dactylis glomerata*) (Sheldrick, et al., 1995; Frame, et al., 1998). Therefore, grasses with an invasive and aggressive growth pattern such as perennial ryegrass (*Lolium perenne*) and bent grasses (*Agrostis* spp.), are not good companion grasses for *L. corniculatus* during establishment (Davies, 1969).

In general *L. corniculatus* is a drought resistant crop, well adapted to acid, infertile soils, however, it responds positively to less acid and fertile soils. This is shown in figure 1.2, where the lower dead matter content in *L. corniculatus* than perennial ryegrass-based pasture swards, over the New Zealand summer, indicates that *L. corniculatus* is more resistant to heat. It has been shown that two of the most important fertilizers to improve the *L. corniculatus* herbage and seed yields, and the winter-hardiness are phosphate (P) and potash (K) (Russelle, et al., 1991; Sheldrick, et al., 1995). Establishment of nitrogen fixation may be negatively affected at a soil pH lower than 6.2 (Beuselinck and Grant, 1995). Cutting height or residual grazing height is critical for re-growth rate; the root has few carbohydrate reserves, therefore, a rapid recovery after defoliation will depend on stem reserves and some residual leaf remaining (Sheldrick, et al., 1995).

Figure 1.2. Pregrazing dead matter in areas of *Lotus corniculatus* (cv. Grasslands Goldie; ♦) and perennial ryegrass/white clover pasture (○) grazed by lambs 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.



(Ramirez-Restrepo, et al., 2004; Ramirez-Restrepo, et al., 2005b).

Establishment of lotus swards may be complicated due to invasions of broad-leaved weeds. Such invasions can be minimized by good seed-bed preparation to ensure an initial weed-free environment, for example, incorporating some herbicides into the seed-bed prior to sowing to control invasive grasses and cereals. When weeds have already developed, cultural control can be done by cutting or grazing in some cases (for tall-growing annual weeds), or applying some herbicides for decumbent weed species (Frame, et al., 1998). It seems that among the different options to control weeds, herbicide use is the most successful judged by first harvest-year yield of the legume, while mowing is the one that reduces legume-plant population the most (Scholl and Brunk, 1962).

Pest and diseases do not represent a major problem in lotus grown for conserved forage or grazing. *L. corniculatus* is susceptible to the root-lesion nematode (*Pratylenchus* *penetrans*) and root-knot nematodes (*Meliodogyne* spp.), but due to its concentration of CT, lotus is generally resistant to insects (Briggs, 1991).

L. corniculatus is well accepted by livestock, and has proven to be a good grazing forage with adequate persistence on the sward over the years when grazed rotationally (Van Kauren and Davies, 1968; Van Kauren, et al., 1969). Since root carbohydrate reserves are lower in warmer climates, the controlled grazing system with rest periods become more necessary under warm than cold conditions (Nelson and Smith, 1968, 1969). Another controlled grazing system could be spring stockpiling for summer utilization, however is important to consider that the quality of herbage declines with increasing length of the stockpiling period (Collins, 1982).

Under intense grazing conditions, erect cultivars of *L. corniculatus* are less persistent than prostrate cultivars because of their higher exposure to be grazed (Van Kauren and Davies, 1968). The advantage of prostrate cultivars is that more leaves are left on the stems after grazing, which helps to get vigorous re-growth and plant persistence. However, erect cultivars are more productive than prostrate ones (Frame, et al., 1998). Table 1.3 shows data of annual dry matter production (t DM/ha) of *Lotus corniculatus* compared to perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) under New Zealand dryland conditions.

In mixed pastures, the acceptability of the companion grass by grazing livestock is another important factor influencing the persistence of *L. corniculatus* in the sward; grasses species with low acceptability may result in lotus being preferentially grazed, which may negatively affect its persistence. Whatever the case, severe grazing must be avoided, leaving stems of at least 10cm above the soil surface (Frame, et al., 1998).

Table 1.3. Annual and seasonal dry matter production (t DM/ha) of perennial ryegrass/white clover *(Lolium perenne/Trifolium repens*) pasture or *Lotus corniculatus* (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 corniculatus*	*P*
Annual production[1]			
2000-2001	7.03	8.46	
2001-2002	9.99	10.5	
2002-2003	7.06	5.30	
Average production:			
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.
NS = No significant.
(Ramirez-Restrepo, et al., 2004).

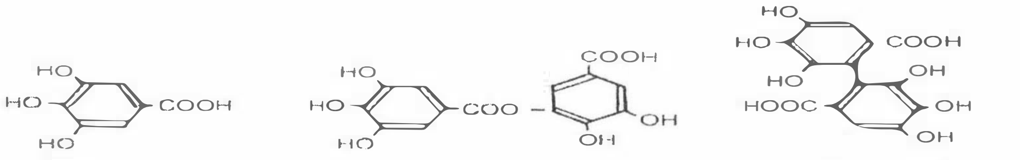
From information in Table 1.3 it can be seen that averaged over all three years, *L. corniculatus* produced similar amounts of DM to grass-based pastures, with a similar seasonal distribution. However, in the very dry autumn of 2000/2001, *L. corniculatus* produced better than grass-based pastures.

1.4 Condensed tannins

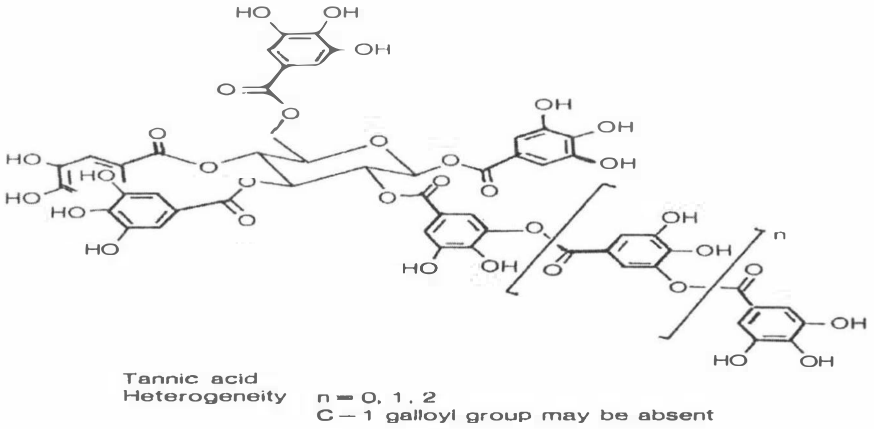
1.4.1 Chemical characteristics

Tannins are polyphenolic substances with various molecular weights and variable complexity that can have both adverse and beneficial effects for ruminants depending on their concentration, structure and molecular weight (Makkar, 2003). The term 'phenolic' is used to define substances that posses one or more hydroxyl (OH) substituents bonded onto an aromatic ring; compounds that have several phenolic substituents are referred to as polyphenols (Waterman and Mole, 1994). Tannins exist mainly in condensed (CT) and hydrolysable (HT) forms; the HT molecule contains a carbohydrate as a central core, which is normally D-glucose (Kumar and D'Mello, 1995). The carboxyl groups of these carbohydrates are esterified with phenolic groups such as gallic acid or ellagic acid (Figure 1.3), and unlike CT, their degradation products can be absorbed from the small intestine of animals, and can be potentially toxic to ruminants (Min, et al., 2001).

Figure 1.3. Constituents of hydrolysable tannins and the structure of tannic acid.



Gallic acid
m-Digallic acid
Hexahydroxydiphenic acid

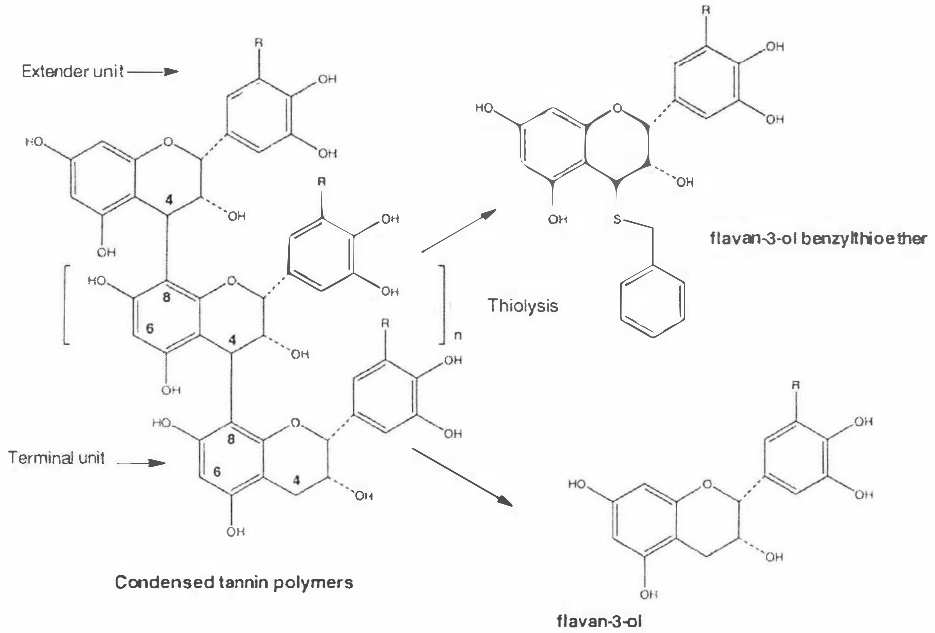


(Kumar and D'Mello, 1995).

Condensed tannins are widespread in the plant kingdom, common forages such as perennial ryegrass and white clovers normally contain trace amounts of these compounds but higher concentrations are commonly found in some forage legumes, trees and shrubs such as *Lotus corniculatus, L. pedunculatus,* sainfoin (*Onobrychis viciifolia*) and sericea lespedeza (*Lespedeza cuneata*) (Min, et al., 2001). Structurally CT are complexes of flavonoid (flavan-3-ol) units (oligomers and polymers) linked by carbon-carbon bonds, and normally occur in cell vacuoles (Waterman and Mole, 1994; Min, et al., 2003). When CT are heated with acid, the interflavan carbon-carbon bonds are ruptured and the flavan units are converted by oxidation to anthocyanidins; that is why CT are referred as proanthocyanidins (PA) (Haslam, 1989; Kumar and D'Mello, 1995).

Condensed tannins comprise two types of monomer units; procyanidins (PC), which are flavonoid units with hydrogen as radical, and prodelphinidins (PD) that have a hydroxyl group as radical; therefore, the degree of hydroxylation of CT and some of its chemical properties will depend on the relative proportion of each of these monomer units in the molecule (Meagher, et al., 2004). Regards to their stereochemistry, they may have a 2,3cis or 2,3-trans conformation; catechin (trans) or epicatechin (cis) for the PC-type, and gallocatechin (trans) or epigallocatechin (cis) for the PD-type (Figure 1.4). The degree of polymerization of CT may vary according to plant species, and the monomers can be linked by 4-8 carbon unions and more rarely by 4-6 in branched structures (Meagher, et al., 2004).

Figure 1.4. Thiolysis reaction of condensed tannins polymers with procyanidin (R=H) and/or prodelphinidin (R=OH) units. The trans-stereochemistry is associated with catechin and gallocatechin (not shown), while cis-stereochemistry is associated with epicatechin and epigallocatechin. All terminal units in the polymer were released as flavan-3-ols, and the extender unit as flavan-3-ol benzylthioethers.



(Meagher, et al., 2004).

Phenolic compounds have the capacity to form hydrogen bonds with other molecules through interactions between the acidic (positively charged) phenolic hydrogen and basic (negatively charged) centers in other molecules (Waterman and Mole, 1994). The reactivity of PA with molecules of biological significance has important nutritional and physiological consequences; their multiple phenolic hydroxyl groups lead to the formation of complexes with protein (Kumar and D'Mello, 1995), metal ions (Kumar and D'Mello, 1995; Foo, et al., 1997) and polysaccharides (Mueller-Harvey and McAllan, 1992; Kumar and D'Mello, 1995; Schofield, et al., 2001; Makkar, 2003). That is why the flavan-3-ol composition of CT (Prodelphinidin: Procyanidin ratio) in lotus species is so important for their protein-binding activity (Meagher, et al., 2004). Condensed tannins from *Lotus corniculatus* and *L. pedunculatus* are composed of both PC and PD units, although the proportion of PD in *L. corniculatus is* lower than in *L. pedunculatus* (Meagher, et al., 2004). An increase in the PD:PC ratio increases the ability of that CT to complex with proteins because of the larger number of hydroxyl groups in the PD units; that is why CT from *L. pedunculatus* are more effective at reducing the degradation of Rubisco (fraction-1-leaf protein) than CT from *L. corniculatus* (Aerts, et al., 1999b; Molan, et al., 2001; Min, et al., 2003).

Tannin-protein complexes are normally formed by hydrogen bonds between the phenolic groups of tannins and the ketoimide groups of the proteins, and by hydrophobic interactions between the aromatic ring structure of tannins and hydrophobic regions of proteins (Kumar and D'Mello, 1995). Some of the main factors that determine the relative affinities of protein for tannins are the size of the protein molecule and its amino acid composition; in general large proteins tend to bind tannin more tightly, although small proline-rich proteins are preferred. High proline content of proteins gives them an open structure that exposes accessible sites for hydrogen-bond formations with tannins. In addition, it seems that affinity of tannins for proteins increases proportionately to the molecular size of tannins, however when the molecular weight is too large tannins become insoluble and lose their affinity for proteins (Spencer, et al., 1988; Kumar and D'Mello, 1995; Meagher, et al., 2004). The protein-CT complex is stable in the pH range 4-7, and dissociates at pH values on either side of this range (Kumar and D'Mello, 1995).

In some studies the molecular weights of CT from Lotus species have been determined by gel permeation chromatography (GPC), and according to these studies, the molecular weight (MW) of these CT is in a range between 5300-5900 Da. When the phoroglucinol cleavage technique has been used, MW of CT has been reported to be 1900 Da for *L. corniculatus*, and 2200 Da for *L. pedunculatus* (Foo, et al., 1997; Meagher, et al., 2004). The identification of the type of subunits that make up a CT polymer, and estimation of the polymer length can be done by subjecting the CT to strong acid-catalyzed cleavage in the presence of phloroglucinol or benzyl mercaptan (Meagher, et al., 2004).

Condensed tannins can react with polyethylene glycol (PEG; MW 3, 500) forming strong chemical bonds, even stronger than those between CT and herbage proteins (Barry and Manley, 1986). Therefore, PEG can be used to inhibit the protein-binding effect of CT, and has been used in several studies to define the real effect of CT in animal nutrition.

1.4.2 Concentration in plants

In botanical terms vegetable tannins occur in one form or another in a substantial part of the Plant Kingdom mainly in higher plants, especially in families of dicotyledons such as Leguminosae (eg. *Acacia sp.*), Anacardiaceae (eg. sumach, quebracho), Combretaceae (eg. myrobalan), Rhizophoraceae (eg. grove), Myrtaceae (eg. eucalyptus), and Polygonaceae (eg. canaigre) (Haslam, 1989; Scalbert, 1991). Many of the plants containing tannins are located in tropical and sub-tropical areas; however plants rich in tannins also occur in temperate zones. Tannins may be found in almost any part of a plant: bark, wood, leaves, fruit and root (Table 1.4), they can accumulate in large amounts (often more than 10% of the dry matter) in almost every tissue, although they are often at higher concentrations in woody lignified tissues. They also occur in seeds and their production tends to be higher in plants under pathological conditions such as insect attack (Bernays, et al., 1989; Haslam, 1989; Scalbert, 1991).

Tannins in general are considered compounds that result from secondary metabolism in plants and they are thought to constitute one of the most important groups of higher plant chemical defenses against predators such as insects and herbivores, acting as quantitative-dosage dependent barriers (Bernays, et al., 1989; Haslam, 1989; Scalbert, 1991). In addition there are speculations about other possible functions of these polyphenolic compounds such as effects on plant apparency (eg. size and appearance) (Haslam, 1989), maintenance of basic metabolism when plants are under unfavorable conditions such as extreme nutrient unbalance (Bernays, et al., 1989), or protection of essential tissues such as wood against decay (Scalbert, 1991). The histological distribution of tannins indicates that the epidermis is the most common site for tannin deposition, which may be important in preventing penetration of saprophytic organisms such as fungi (Bernays, et al., 1989). Concentration of CT in plants depends on several factors such as plant species, plant tissue, developmental stage of plant tissue and environmental conditions (Table 1.5) (Bernays, et al., 1989).

Table 1.4. Plant materials that contain tannins.

Barks

Wattle (*Acacia* sp.), Mangrove (*Rhizophora* sp.), Oak (*Quercus* sp.), Spruce (*Picea* sp.), Hemlock (*Tsuga* sp.), Eucalyptus (*Eucalyptus* sp.), Avaram (*Cassia auriculata*), Babul (*Acacia Arabica*), Birch (*Betula* sp.), Willow (*Salix caprea*), Pine (*Pinus* sp.), Larch (*Larix* sp.), Alder (*Alnus* sp.).

Woods

Quebracho (*Schinopsis* sp.), Chesnut (*Castanea* sp.), Oak (*Quercus* sp.), Cutch (*Acacia catechu*), Wandoo (*Eucalyptus wandoo*), Urunday, Tizra.

Fruits and Fruitpods

Myrobalans (*Terminalia chebula*), Valonea (*Quercus aegilops*), Divi-divi (*Caesalpinia coriaria*), Algarobilla (*Caesalpinia brevifolia*), Tara (*Caesalpinia spinosa*), Teripods, Sant pods.

Leaves

Sumach, American Sumach (*Rhus* sp.), Gambier (*Uncaria gambier*), Dhawa or Country Sumach (*Anogeissus latifolia*), Badan (*Bergenia crassifolia*).

Roots

Docks (*Rumex* sp.), Canaigre (*Rumex hymenosepalus*), Siberian saxifrage (*Saxifragia crassifolia*), Garouille (*Quercus coccifera*), Sea lavender.

Plant Galls

Oak (*Quercus* sp.), Chinese (*Rhus semialata*), Tamarisk (*Tamarix articulate*), Pistacia (*Pistacia* sp.), Aleppo (*Quercus infectoria*).

(Haslam, 1989).

Table 1.5. The extractable and bound condensed tannin content of some legumes, grasses and herbs fed to ruminants in temperate grazing systems, measured by the butanol-HCl method.

	Condensed tannin (g/Kg DM)			
Forage	Extractable	Protein-bound	Fibre-bound	Total
Legumes				
Big trefoil (*Lotus*	61	14	1	77
pedunculatus)	36	9	2	47
Birdsfoot trefoil (*Lotus*				
corniculatus)	33	9	3	45
Sulla (*Hedysarum coronarium*)	29			
Sainfoin (*Onobrychis vicifolia*)	0.4	0.6	0.7	1.7
Red clover (*Trifolium pretense*)	0.0	0.5	0.0	0.5
Lucerne (*Medicago sativa*)				
Grasses	0.8	0.5	0.5	1.8
Perennial ryegrass (*Lolium perenne*)				
Herbs	1.4	2.6	0.2	4.2
Chicory (*Chicorium intybus*)	1.0	1.4	1.0	3.4
Sheeps burnet (*Sanguisorba minor*)				

(Barry and McNabb, 1999).

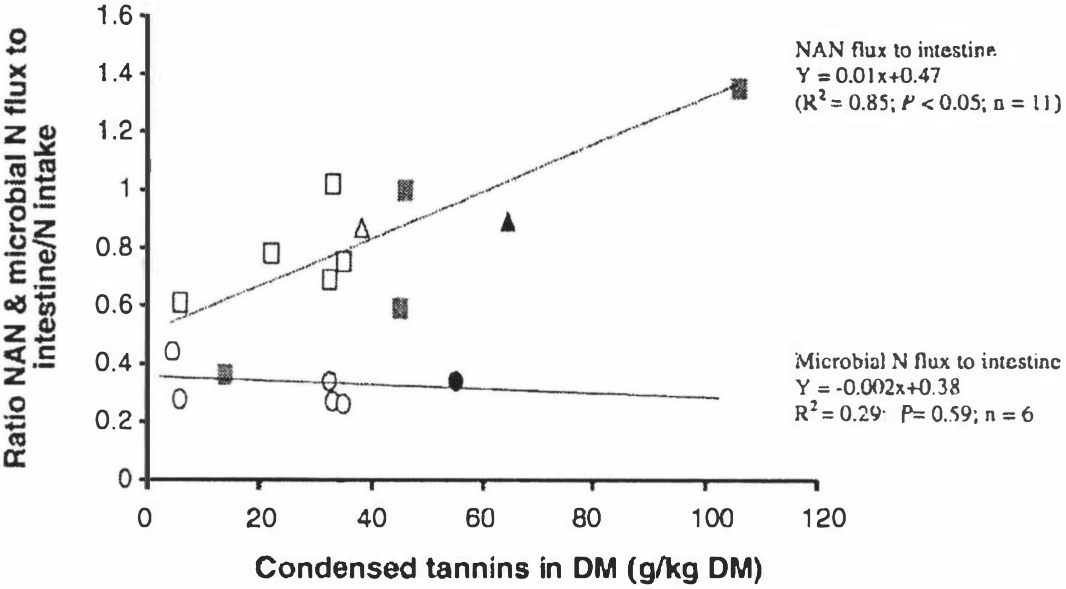
Lotus pedunculatus contains high concentrations of CT (80 – 110g/Kg DM) (Barry and Forss, 1983) mainly when it is grown under low fertility conditions, which may result in detrimental effects on animal productivity due to reduced voluntary feed intake (VFI) and reduced digestibility of forage (Barry and Duncan, 1984; Barry, 1985). *Lotus corniculatus* contains a lower concentration of these secondary compounds, however the quantity of CT in these species varies depending on the type of cultivar; the semi-erect cultivars of *L. corniculatu such as* Empire, Fargo and Winnar have a CT concentration within the rage of 1.3-8.4g /Kg DM, while the erect cultivars such as El Boyero, Franco, Ginestgrino, Granger, Leo, Lot, Maitland, Goldie and Sao Gabriel have a CT concentration between 16-39g /Kg DM (Lowther, et al., 1987). The CT concentrations in the erect cultivars of *L. corniculatus* are well below the VFI depressing concentration (Barry and Duncan, 1984) but are in the range where increased protein absorption occurs (John and Lancashire, 1981).

1.4.3 Effects on ruminal digestion

When ruminants are fed with common temperate fresh forages such as perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), which have just trace amounts of CT, about 75% of the feed protein is degraded by rumen microorganisms mainly to ammonia (NH_3) (Ulyatt and McRae, 1974) because this is the principal nitrogen product that rumen bacteria can use for their own metabolism. Not all the NH_3 produced in the rumen is utilized by ruminal bacteria, and when the animals are fed with fresh forages approximately 30% of the nitrogen eaten is absorbed as ammonia through the rumen wall to be eliminated as urea in the urine; therefore a total proportion of feed protein lost both in the rumen fermentation and the duodenal flow of NAN (non ammonia nitrogen) is about 65% of the total N eaten (Barry and McNabb, 1999; Min, et al., 2003).

Condensed tannins reduce both solubilization and degradation of forage protein by rumen microorganisms, specially the principal leaf protein, ribulose-bisphosphate carboxylase/oxygenase (Rubisco; fraction 1 leaf protein) (Barry and McNabb, 1999).That is why the duodenal non ammonia nitrogen flow (NAN), an index of the amount of feed protein leaving the rumen, increases when domestic ruminants are fed on forages containing CT like Lotus species, without significantly affecting microbial protein production (Figure 1.5) (Barry and McNabb, 1999).

Figure 1.5. The relationship between condensed tannin concentration in the dry matter of forage species (x), and (a) the ratio of non-amonia-nitrogen (NAN) flowing at the abomasum or duodenum (y: (□) *L.corniculatus*; (■) *L. pedunculatus*; (Δ) sainfoin; (▲) sulla), and (b) microbial N flow at the abomasum or duodenum per unit of N eaten by sheep (○) *L. corniculatus*; (●) *L. pedunculatus*).



(Min, et al., 2003).

Condensed tannin from *L. corniculatus* not only increases the NAN flow to the small intestine, but also increases the absorption of EAA from this site in sheep by 62% (Waghorn and Barry, 1987) which is contrary to the effect obtained with CT from *L. pedunculatus* that normally depresses digestion of EAA in the small intestine (Waghorn, et al., 1994) (Table 1.6). Therefore, although CT from both Lotus varieties decrease feed protein degradation in rumen and increase the amino acid flow into the small intestine, it seems that due to the differences in their chemical structure and concentration in plants, CT from *L. pedunculatus* attach to feed protein in a stronger way, decreasing the percentage of protein being released in the small intestine for digestion and absorption.

Table 1.6. Effect of condensed tannins (CT) upon the intake and absorption of essential amino acids (EAA) from the small intestine of sheep fed on fresh *L. corniculatus* and *L. pedunculatus* with or without an intraruminal infusion of polyethylene glycol (PEG) to inactivate the CT.

	L. corniculatus		*L. pedunculatus*	
	CT-acting group	CT-not acting group	CT-acting group	CT-not acting group
Rumen ammonia (mgN/l)	367	504	175	460
CT intake (g/day)	98.9	98.9	103.2	116.8
EAA[a]				
Abomasal flow (g/day)	84.7	55.5	121.1	105.6
Proportion intake	0.86	0.56	1.17	0.90
Apparent absorption from small intestine (g/day)	58.8	36.2	81.4	83.5
Proportion abomasal flow	0.67	0.67	0.66	0.79
Proportion intake	0.59	0.37	0.79	0.72

Each diet was fed containing respectively 22 and 55g CT/Kg DM.
[a] Excluding arginine.
(Waghorn and Barry, 1987; Waghorn, et al., 1994).

After mastication, a proportion of the CT gets associated with plant protein and another remains as free CT. In plants with high concentrations of CT such as *L. pedunculatus* (90g CT/Kg DM) aproximately 90% of CT get bound to plant protein; it seems that this concentration is the limit where the protein-binding system gets saturated, and increments in total CT concentration above 90g/Kg DM remain as “free tannins” (Barry and McNabb, 1999). It is suggested that high concentrations of free CT enhance the reaction of these compounds with bacterial enzymes and bacterial cell walls, which can interfere with the transport of nutrients into the cell and affect fermentation of other nutrients like carbohydrates. Free CT may also reduce fiber digestion by complexing with plant compounds such as lignocellulose, which makes more difficult its digestion, or by inhibiting cellulolytic microorganisms. The magnitude of those effects may vary with each type of CT (McSweeney, et al., 2001; Min, et al., 2003), and reduction of cellulolytic bacteria population not always results in impairments of ruminal microbial protein synthesis because the total population of fungi, protozoa and proteolytic bacteria may be not affected dramatically (McSweeney, et al., 2001). This explains why very high concentrations of CT from *L. pedunculatus* are associated with reduced rumen degradation of structural carbohydrates, especially hemicellulose (Barry and Manley, 1984).

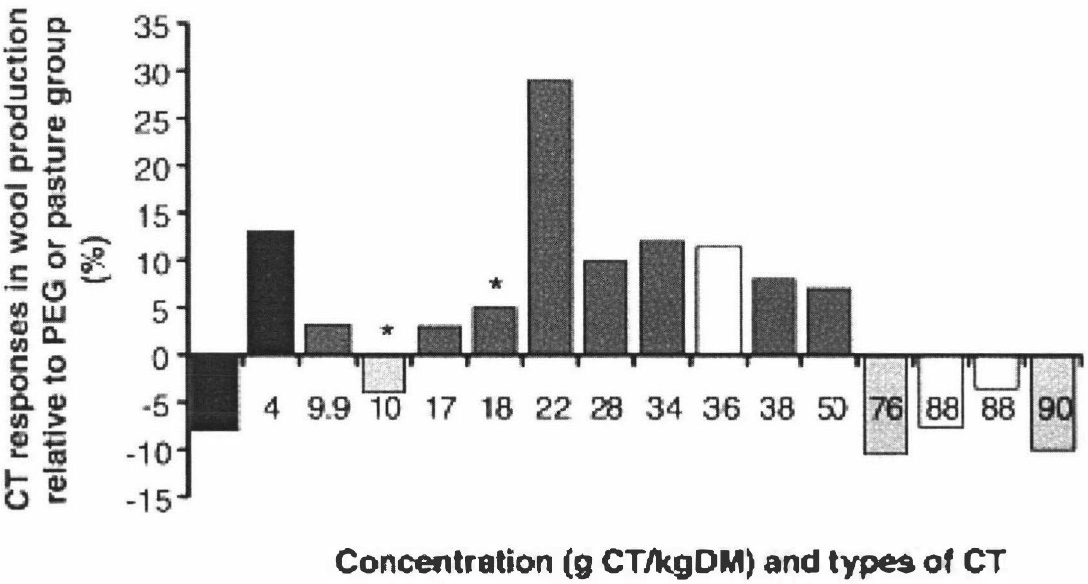
In general free CT may get bounded to some bacterial enzymes such as cellulases, pectinases, xylanases, peroxidase, laccases and glycosyltransferases, and inhibit their action (Scalbert, 1991). Another effect of tannins on bacteria may involve complexation of these compounds with metal ions, which would inhibit utilization of such ions by bacteria affecting directly the activity of metalloenzymes such as peroxidase and laccase (Scalbert, 1991). In addition, tannins may affect the metabolism of microorganisms; it has been found that bacteria such as *Pseudomona fluorescens, Escherichia coli* or *Cellvibrio fulcus* modify their morphology when grown in the presence of tannins; several mechanisms may induce such effects on bacteria metabolism, perhaps some of them affecting the integrity of their cellular membranes, and others affecting mitochondrial activity such as inhibiting oxidative phosphorylation and the electron transport system (Scalbert, 1991).

Besides the effects on forage protein, it seems that medium concentration of CT may have a positive effect on the efficiency of microbial protein synthesis; the decrease in the rate of digestion of feed by CT could help to synchronize the release of various nutrients and the speed at which microorganisms use them, increasing their partitioning into microbial protein synthesis rather than volatile fatty acid production (Makkar, 2003). Condensed tannins have the capacity to increase the molar proportion of propionate in the *in vitro* fermentation system, and decrease protozoal counts, and although results from studies *in vivo* are inconsistent, there is some evidence supporting these facts (Makkar, 2003). Therefore, in addition to the protein-sparing effect in ruminant, CT in the diet may lead to a reduction in methane production and nitrogen excretion to the environment; however, the concentration of tannins should be not too high to drastically decrease the true digestibility of the substrate.

1.4.4 Effects on animal production

Wool production. Wool production is a physiological process that demands high amounts of protein from the animals, mainly protein with high content of sulphur-containing amino acids (SAA) (Min, et al., 2003). Condensed tannins from *L. corniculatus* and *L. pedunculatus* reduce the degradation rate of SAA in the rumen; however, wool growth responses to CT depend on both the concentration and the type of tannin. In general CT from *L. corniculatus* with a concentration in the range 22-38 g/Kg DM has increased wool production in grazing sheep by about 10%, while concentrations above 50g/Kg DM from *L. pedunculatus* normally have negative effects (Figure 1.6) (Min, et al., 2003).

Figure 1.6. The effect of forage CT concentration on wool production of grazing sheep, calculated as the wool production response of CT-acting sheep relative to PEG-supplementation (CT-inactivated) or non-CT-containing pasture sheep; (■), temperate grass or temperate grass/white clover; (*), mixture of lotus species + lucerne or grass; (■), *Lotus corniculatus*; (▨), *Lotus pedunculatus*; (□), sulla.



(Min, et al., 2003).

An essential feature of action of CT under grazing is that it should reduce rumen ammonia concentration, as shown in Table 1.7. These effects of CT in *L. corniculatus* in reducing rumen ammonia concentration and increasing plasma EAA concentration explains how CT increases wool production and other aspects of animal production under grazing. Min et al., (1999), found that grazing sheep on *L. corniculatus* also reduced the urea concentration in blood, and increased the plasma concentration of most amino acids (52% EAA, 57% BCAA and 25% for methionine) (Table 1.8). In addition, clean fleece weight, wool production efficiency, staple length and mean fiber diameter were improved when the animals were fed on *L. corniculatus* apparently as a result of the effects of CT on plasma amino acids.

Table 1.7. Rumen concentration of ammonia and plasma concentration of urea in ewes grazing perennial ryegrass/white clover pasture and *L. corniculatus,* with or without twice-daily oral administration of polyethylene glycol (PEG; MW 3 500).

Group	Dietary treatment			S.E.M.	Contrast	
	Lotus + PEG	Lotus (CT-acting)	Pasture		Lotus vs. Lotus + PEG	Lotus vs pasture
Rumen ammonia (mg/l)						
Number of observations	10	10	10			
Maintenance fed (end summer)	130.6	119.7	336.8	13.22	NS	***
Maintenance fed (autumn)	318.2	255.8	334.3	17.02	**	**
Ad libitum feed (end summer)	268.9	168.1	268.5	13.44	***	***
Plasma urea (mM/l)						
Number of observations	20	20	20			
Maintenance feed						
Ovulatory cycles 1-3	7.13	6.59	8.80	0.153	**	***
Ad libitum feed	8.59	8.36	9.40	0.157	NS	***
Ovulatory cycles 1-3						

** ($P < 0.01$): level of statistical significance.
*** ($P < 0.0001$): level of statistical sifnificance.
(Min, et al., 2001).

Table 1.8. Plasma concentration of amino acids (μM) in ewes grazing perennial ryegrass/white clover pasture and *Lotus corniculatus* (cv. Grasslands Goldie) *ad libitum*, with and without twice-daily oral administration of polyethylene glycol (PEG;MW 3500).

	Pasture		Lotus		
Amino acids	PEG-sheep	CT-acting sheep	PEG-sheep	CT-acting sheep	S.E. (D.F. 69)
n	18	18	19	18	
EAA*					
Valine	138	126	161	199	5.2
Leucine	77	69	93	108	3.8
Iso-leucine	52	45	58	72	3.3
Tyrosine	46	41	41	49	2.8
Phenylalanine	30	30	30	38	2.7
Histidine	54	56	59	67	3.8
Tryptophan	106	105	177	247	6.4
Lysine	103	89	100	129	5.2
Arginine	34	51	71	68	5.7
Threonine	114	99	77	106	5.5
Methionine	12	12	12	15	1.8
Cysteine (*n =10)*	28	33	33	35	1.5
NEAA†					
Asparagine	17	27	21	25	3.2
Serine	85	85	62	86	4.7
Glutamate	188	186	192	254	6.4
Proline	61	68	69	96	5.3
Glycine	366	338	330	480	10.8
Alanine	139	116	120	149	5.5
BCAA‡	267	241	312	379	12.8
EAA*	786	742	894	1128	35.9
NEAA†	856	819	793	1091	35.8

* Essential amino acids (including BCAA and cysteine).
† Non-essential amino acids.
‡ Branched-chain amino acids (valine, leucine, and iso-leucine).
(Min, et al., 1999).

Milk production. *L. corniculatus* may increase milk and protein production in dairy cattle by 30% and 10% respectively relative to cows grazing ryegrass, and 50% of these effects may be explained by the action of CT (Table 1.9) (Min, et al., 2003; Ramirez-Restrepo and Barry, 2005; Ramirez-Restrepo, et al., 2005b). Wang, et al., (1996a) studied the action of condensed tannins from *L. corniculatus* on milk production in ewes; they found that CT reduced the declining rate of milk yield, lactose, and protein concentration after lactation peak with total increases of 21%, 12% and 14%

respectively during the whole lactation with the main effects being observed during mid and late lactation.

Table 1.9. The effect of condensed tannins in *Lotus corniculatus* upon dry matter intake, milk yield, milk protein production and methane production in grazing dairy cows.

	Ryegrass		Lotus		SED
Chemical composition (g/Kg DM)	97		101		
Soluble carbohydrates	251		279		
Crude protein	411		284		
Neutral Detergent Fiber (NDF)	0		26		
Condensed tannins	0.77		0.81		
Organic matter (OM) digestibility					
	-PEG[1]	+PEG	-PEG	+PEG	
DM intake (Kg/cow/day)	14.9	14.9	17.4	17.1	0.46
Milk yield (Kg/cow/day)	18.5	19.0	24.4	22.1	0.70
Milk protein (g/Kg)	35.9	35.6	36.3	36.1	0.50
Methane production					
g CH_4/ Kg DMI	24.2	24.7	19.9	22.9	0.78
Proportion gross energy eaten	0.075	0.077	0.060	0.069	0.0027

[1] Polyethyleneglycol MW 3,350.
(Woodward, et al., 2004).

Meat production. In the two experiments carried out by Ramirez-Restrepo, et al., (2004), live weight gain (LWG) of lambs after 12 weeks of study was higher in the animals fed on *L. corniculatus* rather than ryegrass/white clover. Increments in LWG were about 37% in Experiment 1 and 52% in Experiment 2, and increasing weaning weight by 20% and 32% respectively. In the particular case of these two experiments, the positive effects of lotus on live weight of the animals perhaps were due to the higher nutritional value of lotus compared to pasture, the higher voluntary feed intake of the animals grazing lotus, and the action of condensed tannins increasing the amount of EAA absorbed from the small intestine.

Administration of PEG (MW 3,350) can be used to determine what proportion of LWG in sheep grazing *L. corniculatus* is due to actions of CT. A summary of some trials carried out in New Zealand during the 1990's (Table 1.10) shows that PEG supplementation has not consistent effects on LWG or carcass weight of lambs grazing *L. corniculatus*, but consistently reduced wool growth. This confirms that CT increase wool growth under grazing conditions, although there was no effect upon body growth under the conditions of these experiments. Therefore, it seems that the superior rates of body growth in lambs grazing *L. corniculatus* is due to the higher ME concentration and higher VFI in lotus than in grass-based pastures rather than to the its CT content.

Table 1.10. Voluntary feed intake (VFI), live weight gain (LWG), carcass gain and wool growth in lambs (Experiment 1) and dry ewes (experiment 2) grazing the forage legumes *Lotus corniculatus* (27-34 g CT/Kg DM) and lucerne (0.3 g. total CT/Kg DM) during summer.

	Lotus		Lucerne		
	CT acting	PEG supplemented	CT acting	PEG supplemented	SE
	Experiment 1 (1991/92; 27.9 Kg LW[1]; 4.5 Kg DM/lamb/day)[2]				
VFI (Kg/OM/day)	1.76	ND	1.65	ND	0.04
LWG (g/day)	228	ND	183	ND	8.2
Carcass weight (Kg)	20.4	ND	17.8	ND	0.82
Fleece weight (Kg)	2.78	ND	2.25	ND	0.091
	Experiment 2 (1994/95; 19.3 Kg LW; 5.3 Kg DM/lamb/day				
LWG (g/day)	271	250	ND	ND	8.0
Carcass weight (Kg)	21.1	19.8	ND	ND	0.57
Fleece weight (Kg)	1.75	1.78	ND	ND	0.067
	Experiment 3 (1992/93; 22.4 Kg LW; 2.5Kg DM/lamb/day)				
Rumen ammonia (mg N/1)	255	370	555	535	
VFI (Kg OM/day)	1.19	1.20	1.32	1.34	0.056
LWG (g/d)	203	188	185	178	5.8
Carcass gain (g/d)	79	75	68	63	2.9
Wool growth (g/d)	12.1	10.9	10.8	10.2	0.39
	Experiment 4 (1995/96; 54.0 Kg LW; 1.3 Kg DM/ewe/day)				
Rumen ammonia (mg N/1)	221	278	ND	ND	8.5
VFI (Kg OM/day)	1.23	1.20	ND	ND	0.051
LWG (g/d)	54	67	ND	ND	9.3
Wool growth (g/d)	13.2	11.1	ND	ND	0.66

ND = not determined
[1] Initial live weight
[2] Daily green forage allowance
(Barry, et al., 2001).

In contrast, very high concentrations of CT in *L. pedunculatus* (80-110g/ Kg DM) have been shown in PEG supplementation studies to depress rate of both body and wool growth (Barry, 1985).

Effects on animal reproduction. Reproductive performance is another important factor affecting farms' economics, therefore sheep farmers have to make constant efforts in order to increase the number of ewes getting pregnant during the mating season, and the number of lambs at weaning.

There are several factors affecting the reproductive performance of ewes, however nutritional level is one of the most important ones affecting the ovulation rate. The precise way by which nutrition makes such effect is not completely understood, but it has long been recognized that the amounts of energy and protein absorbed from the digestive tract have positive relationship with ovulation rate (Figure 1.7) (Smith, 1985).

Not all the amino acids have a positive relationship with ovulation rate. It seems that the strongest relationship is between ovulation rate and plasma concentrations of branched chain amino acids (BCAA; valine, leucine, isoleucine), and also phenylalanine and tyrosine (Table 1.11) (Waghorn, 1986; Smith, 1991). In fact it has been shown that intra-jugular infusions of some BCAA such as valine, leucine and isoleucine for 5 days before luteolysis can significantly increase the mean ovulation rate in ewes (Downing, et al., 1995).

Table 1.11. Concentration of plasma amino acids measured in wethers at 4 levels of intake and 2 levels of protein content in diet, and ovulation rates in ewes fed the same diets.

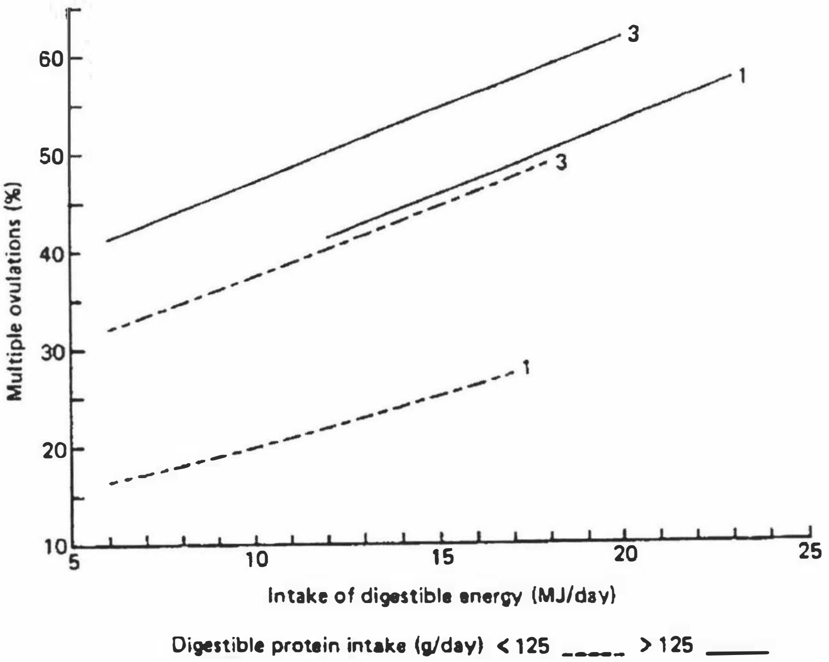
	Low protein				High protein			
	L1	L2	L3	L4	H1	H2	H3	H4
BCAA (nmol/ml)	555	643	989	887	906	1462	1959	1904
Phe + Tyr (nmol/ml)	160	244	486	358	302	437	638	604
Ovulation rate (%)	115	119	122	122	123	139	145	147

Intake levels (g DM/day): 1 = 444; 2 = 800; 3 = 1155; 4 = 1510.

Protein levels: Low = 12% (DM); high = 22% (DM)

(Waghorn, 1986).

Figure 1.7. Effect of intake of digestible energy (MJ/day) and digestible protein (g/day) on the percentage of ewes with multiple ovulations. Numbers 1 and 3 represent results from two different years.



(Smith, 1985).

Waghorn, et al., (1990) found that increasing the nitrogen intake of ewes from one estrous cycle to the next increases the ovulation rate significantly, with increases in plasma concentrations of EAA, mainly BCAA without changes in plasma concentrations of non essential amino acids (NEAA). Furthermore, it was found in the same study that when dietary changes resulted in increased ovulation in ewes, the increases in blood concentration of valine, leucine and isoleucine were at least twofold compared to those in ewes with no change in ovulation rate, and that the increases in plasma concentration of BCAA were double in ewes changing from two to three ovulations that of ewes changing from one to two.

The critical time for protein supplementation in order to achieve positive results in ovulation rate is during the luteal phase, approximately eight to five days prior to ovulation, with no increases in ovulation being obtained when supplementation is given immediately prior to ovulation (four days before) (Waghorn, et al., 1990; Smith, 1991). Therefore, it seems that the higher the amount of feed protein reaching the small intestine and being absorbed from there, the higher the reproductive rate and productive improvements that may be obtained in the animals, as long as the protein digested in the rumen supplies the metabolic requirements of ruminal bacteria. Several bypass-protein sources have proved to be useful in animal production systems; in the case of grazing animals, forages containing CT such as *L. corniculatus* have been shown to have the capacity for protecting feed protein from ruminal digestion and to improve reproductive performance in ewes (Table 1.12). Grazing ewes on *L. corniculatus* rather than pasture (rye grass/white clover) during mating time can increase lambing percentage (25%) due to increases in ovulation rate and fecundity; polyethylene glycol (PEG) supplementation has shown that the major reason for the effect of *L. corniculatus* on reproductive performance of ewes is due to its CT content, which may account for 50% of the reproductive improvements (Min, et al., 1999; Min, et al., 2001). Furthermore, feeding ewes on lotus increases the plasma BCAA and EAA by 57% and 52% respectively compared to plasma concentrations from animals fed on a mixture of rye grass and white clover (Min, et al., 1999; Min, et al., 2001).

Table 1.12. The effect of grazing ewes during mating on *L. corniculatus* or perennial ryegrass/white clover (pasture) and of supplementation with polyethylene glycol (PEG) on ovulation rate (OR).

Reference	n^a	LW[b]	Ovulation rate			Response to lotus (%)[c]
			Pasture	*L. corniculatus*		
			±PEG	+ PEG	- PEG	
Min et al., (1999) (second cycle)	200	54	1.33_a	156_b	1.76_c	32.3
Luque et al., (2000) (second cycle)	240	60	1.45_a	1.66_b	1.64_b	13.1
Min et al., (2002a) (third cycle)	225	51	1.48_a	1.66_b	1.80_c	21.6
Mean	222	55	1.42	1.62	1.73	22.3

[a] *n*: number of experimental animals.

[b] LW: mean liveweight.

[c] Calculated as: $\frac{\text{OR lotus (-PEG)} - \text{OR pasture (-PEG)}}{\text{OR pasture (-PEG)}} \times 100$

cycle = estrous cycle.

Means within the same row with different letters (a,b,c) are significantly different $P > 0.05$.

(Min, et al., 2003).

It has also been found that feeding ewes at mating with *L. corniculatus* under dry conditions may increase the ovulation rate in the ewes, the number of lambs born, and decrease the post-natal lamb mortality, which results in a higher number of lambs weaned (Table 13). Ramirez-Restrepo and Barry, (2005) concluded that ewes needed to graze *L. corniculatus* for 2-3 estrous cycles in order to maximize the increase in ovulation rate at mating.

Table 1.13. The effect of mating on *Lotus corniculatus* in 2001 on the reproductive performance (%ewes mated) of ewes on Massey University dryland farm in the Wairarapa. The period of grazing on lotus was 9 weeks.

	L. corniculatus	Pasture	Significance
Scanning (%)	179	170	*
Lambing (%)	175	159	*
Docking (%)	159	130	*
Weaning (%)	155	123	*
Birth weight (Kg)	5.5	5.5	NS
Weaning weight (kg)	32.1	32.3	NS
Fleece weight (Kg)	3.1	2.9	**
Lamb mortality: Lambing to weaning	11.4	22.6	*

NS: not significant.
* (P<0.05)
** (P<0.01)
(Ramirez-Restrepo and Barry, 2005).

1.5 Conclusions

Sheep production is one of the most important industries in New Zealand, and a high production of lambs each season is the main priority of many sheep farmers. The production process is based upon the grazing of forages throughout all seasons of the year and there are several factors that can influence the success of sheep producers in achieving the former goal; good nutrition of the animals at all stages of the productive process is one of the most important aspects to consider in order to increase, or to keep it in high levels. Therefore, management of forages at the farm and feed-budgeting are extremely important in order to promote good forage growth and nutritive value and to keep the animals well fed at all times.

Temperate forages are normally good quality forages with high protein and ME energy content. Although there are several forage species that can be used under grazing systems in temperate climates, it is common to find mixed swards combing grasses and forage legumes, which increases the nutritional quality of the total ration due to the higher nutritional quality of legumes compared to grasses, and enhance growth of the companion grass due to the ability of legumes to fix atmospheric nitrogen. White clover is the most popular forage legume among farmers in New Zealand, and is frequently combined with perennial ryegrass in a ratio of approximately 20:80.

Lotus corniculatus is another legume that is used as forage, with a nutritional value higher than that of the rye grass/white clover combination. Although it is difficult to grow this legume in combination with grasses, due to its low competitive growth ability, it represents a good option for farms in dry areas due to its resistance to drought conditions and easy adaptation to acid and infertile soils. In addition, the CT content of this legume increases its nutritional value further, increasing the proportion of amino acids being absorbed from the small intestine by reducing forage protein degradation in the rumen. This effect of CT in *L. corniculatus* enhances productive and reproductive performances of the animals in different ways; the increased amount of amino acids absorbed from the small intestine increases the concentration of total EAA and BCAA in blood, these being readily available for supporting productive and reproductive processes; reduction of protein break down in the rumen reduces the ammonia and plasma urea levels, which promotes an adequate oviduct-uterine microenvironment for viability of sperm and ova and represents a more efficient utilization of protein nitrogen.

The productive parameters that are enhanced by grazing sheep on *L. corniculatus* are: wool production, milk production, and liveweight gain. In the case of reproductive parameters, ovulation rate, fecundity, and lambing percentage are normally increased when mating ewes on *L. corniculatus* as grazing forage instead of rye grass/white clover pastur. In addition, recent studies have found that grazing ewes on *L. corniculatus* during pre-mating and mating time may have such a positive effect on survival of lambs, reducing lamb mortality from lambing to weaning; however, further studies with larger number of ewes are necessary to investigate this promising effect of *L. corniculatus* and the possible physiological mechanisms.

Chapter 2

Can mating ewes on *Lotus corniculatus* be used to reduce lamb mortality between birth and weaning?

2.1 Introduction

Some studies have reported that when the animals are feed on CT-containing forages such as *L. corniculatus,* productive parameters like wool production (Min, et al., 2003), lambs' growth rate (Ramirez-Restrepo, et al., 2004), and milk production (Wang, et al., 1996a) are normally increased.

In addition, it has been proved that CT from *L. corniculatus* can enhance reproductive parameters such as ovulation rate, conception rate and lambing percentage in ewes (Min, et al., 1999; Min, et al., 2001). For many years those effects have been attributed to the high nutritional value of *L. corniculatus* and the higher amount of both branched chain amino acids (BCAA) and essential amino acids (EAA) absorbed from the small intestine due to the effect of CT on herbage protein digestion (Barry and McNabb, 1999; Min, et al., 1999). However, the physiological mechanisms by which these effects on reproduction are achieved are not completely clear yet.

Ramirez-Restrepo, et al., (2005a) found that grazing ewes on *L. corniculatus* during mating time and early pregnancy, in addition to the already mentioned reproductive effects, may exert a positive effect on the survival rate of lambs from birth to weaning. There are several factors involved in lambs' survival after birth; however, it seems that birth weight is one of the most important ones. Therefore, it is possible that apart of direct effects on the nutritional status of ewes, CT may have an indirect effect on fetal development, with a supporting role in the production of some of the hormones involved in fetal growth during pregnancy.

The objective of this work is to determine if there is a real effect of mating ewes on *L. corniculatus* on improving survival rate of lambs from birth to weaning.

2.2 Material and methods

2.2.1 Experimental design

A grazing experiment was conducted at Massey University's Riverside Farm in the Wairarapa region in New Zealand. This area is on the Southern East Coast of the North Island and experiences regular dry summer conditions. The experiment was carried out to investigate if grazing ewes on *Lotus corniculatus* during breeding time and early pregnancy decreased the mortality rate of lambs from birth to weaning.

The experiment consisted of a grazing trial involving 600 mixed age Romney ewes, which was conducted for ten weeks; from the 1st of March 2004 (early autumn) to the 13th of May 2004 (late autumn). The experiment consisted of two groups each of 300 ewes, one of them grazing on perennial ryegrass/ white clover pasture (control group), and the other on *Lotus corniculatus* (lotus group).

The effect of the two different diets on the reproductive performance of the ewes was measured at mid-pregnancy (by ultrasound scanning), at lambing and at weaning time by analyzing the proportion of ewes pregnant, ewes lambing, and ewes having alive lambs respectively. In addition, the proportion of ewes carrying or having singles, twins and triplets was measured at each time. Wool production was determined by shearing the ewes at the beginning and end of the experiment.

2.2.2. Forage

The ewes grazed weekly breaks (seven days per break); the grazing areas were vegetative *Lotus corniculatus* (birdsfoot trefoil; cv Grasslands Goldie) and perennial ryegrass/white clover (*Lolium perenne/ Trifolium repens*). Measurement of the DM herbage mass before and after grazing, and botanical composition were determined for each weekly break by cutting random quadrants (8 x 0.180 m^2/break) to ground level. For determination of pre-grazing and post-grazing DM masses, 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 about 1.4 x 0.9 m were placed in each break immediately before sheep were introduced for grazing; after grazing each break the cages were removed and the forage was hand plucked corresponding to what the animals were eating (diet selected samples). These samples were pooled and stored at -20 °C until nutrient analysis.

Sheep grazing *Lotus corniculatus* in the sward.

2.2.3 Grazing Management

Ewes in the lotus group grazed rotationally on *Lotus corniculatus* for the ten weeks of the grazing trial; the control group in turn grazed rotationally perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) for the same period of time. Animals in both groups grazed weekly breaks built up with the use of temporary electric fences, offering them a feed allowance of approximately 2 Kg DM/ewe/day. The area of each weekly break was calculated as:

BA = 7 (days) x n (number of sheep) x FA / HM – 500

Where BA is break area (ha); FA is feed allowance (Kg DM /animal/day); HM is herbage mass (Kg DM/ha); and 500 is the amount of forage that remains in the paddock when the animals can not graze further because of the shortness of pasture. After the ten weeks of grazing trial, animals from both groups were combined together to graze perennial ryegrass/white clover swards as a single group.

2.2.4 Animals

Mean initial live weight of the animals was 56.7 Kg (S.E. 0.4), and mean initial body condition score (BCS) was 2.9 (S.E. 0.3) in a range from one to five. All ewes were weighed every two weeks, during the lotus feeding period, using electronic scales (Tru-test, Auckland, NZ), and BCS (Jefferies, 1961) was assessed every month during the same period. To advance the breeding season in the ewes from both treatments, vasectomized rams ran out with them in late summer from 18th February to 12th of March (ram effect) (Lindsay, et al., 1975; Al-Mauly N.Z.N., et al., 1991; Cushwa, et al., 1992). Afterwards, vasectomized rams were replaced by entire rams that ran out with the ewes for two estrous cycles (12th of March to 15th of April). The entire rams were harnessed carrying a crayon to mark the covered ewes; colors of the crayons were different in each cycle to differentiate the ewes covered during the first cycle from those covered during the second cycle or those repeating estrus. Approximate cover dates for each ewe were obtained by visual observations of the animals (twice per week during the two estrous cycles) and recorded. After the groups were joined, ewes were weighted and BCS assessed monthly up to weaning.

All the ewes were shorn before the starting of the experiment, and again at weaning time; fleeces were weighed at the final shearing to determine greasy fleece weight. Wool staple length (cm) from each animal was determined by measuring the length of ten randomly chosen unstretched mid side staples along a ruler.

During lambing (1st August to 15th of September), new born lambs were tagged every day, and their date of birth, weight and sex were determined and recorded. During the whole lambing period, tag numbers of dead lambs were recorded as well as the date of death. On the first of October all the lambs were weighed and tail-docked by the cauterization technique, which consist in cutting the tails pressuring them with a gas-heated iron that cut and cauterizes at the same time. The same day the lambs were tail-docked, male lambs were submitted to the cryptorchid technique by the application of a rubber ring to the neck of the scrotum with the appropriate applicator. The 15th of November all the lambs were weighed again and weaned from their dams.

Tagging and weighting new born lambs at lambing time.

2.2.5 Laboratory analyses

Forages. All samples of diet selected were stored at – 20 °C and then 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, New Jersey, USA) before laboratory analysis. Total nitrogen (N) was determined by the Dumas principle (Leco CNS 2000 Analyser, Model 602 600 200, USA). Neutral detergent fiber (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 fiber-bound CT fractions in forages were determined using a butanol-HCL colorimetric procedure (Terril, et al., 1992).

All CT concentrations were determined using CT extracted from *L. Pedunculatus* as a standard reference (Jackson, et al., 1996). *In vitro* organic matter digestibility (OMD) and digestible organic matter 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.

2.2.6 Statistical analyses

Pasture mass, dead matter content, total nitrogen (N), neutral detergent fiber (NDF) content, condensed tannins content, digestibility of the dry organic matter (DOMD), organic matter digestibility (OMD), and metabolizable energy content (ME), were analyzed by analysis of variance (ANOVA) using PROC GLM (SAS, 2001).

Live weight gain and body condition score (BCS) gain in ewes during the experimental period were analyzed by ANOVA using PROC MIXED.

Reproductive rate (proportion of conceptus or alive lambs per 100 ewes) was analyzed at four times: scanning, lambing, docking, and weaning using PROC MIXED;

reproductive rate data was corrected to equal live weight gain using live weight gain of ewes at the end of the nutritional period as a covariate.

Conception rate and fecundity rate at lambing were analyzed using PROC GENMOD with logit transformation assuming a binomial distribution; fecundity rate data was also corrected to similar live weight gain by analyzing the data with PROC MIXED using live weight gain at the end of the nutritional period as a covariate.

Total lamb mortality was analyzed per treatment and per birth rank (single, twin, triplet) using PROC GENMOD (SAS, 2001) with logit transformation assuming a binomial distribution. Lambing dates were analyzed by ANOVA using PROC GLM.

Birth weight and weaning weight of lambs were analyzed per birth rank and sex using PROC MIXED (SAS, 2001). Greasy fleece weight and staple length were analyzed by factorial design using PROC GLM (SAS, 2001).

2.3 Results

2.3.1 Forages

Perennial ryegrass-white clover pasture and *Lotus corniculatus* were in the vegetative state throughout the experiment. Both pre-grazing and post-grazing herbage mass were always higher for the lotus group than for pasture group (Table 2.1). The proportion of pre- and post-grazing dead matter content was also higher for the lotus group than for the control pasture (Table 2.1). However, pre-grazing dead matter content was low for both treatments.

There were some differences between treatments in the chemical composition of forage. Neutral detergent fiber (NDF) content was significantly lower ($P < 0.05$) in lotus than in control pasture (Table 2.2). The proportion of digestible organic matter in the dry matter (DOMD) and the metabolizable energy content (ME) were slightly, but significantly higher ($P < 0.05$) in *L. corniculatus* than in perennial ryegrass/white clover pasture (Table 2.2). Concentration of condensed tannins (CT) was 22 g/Kg DM in *L. corniculatus,* with control pasture containing just trace amounts of this secondary compound (Table 2.2). Total nitrogen content and organic matter digestibility (OMD) were similar in both forages; with mean values of 30.3 g/Kg DM and 71.63% respectively.

Table 2.1. Pre-grazing and post-grazing mass and dead matter content of forages grazed during the experiment (mean values with standard errors).

	Control pasture	*Lotus corniculatus*
n	10	9
Pasture mass (Kg DM/ha)		
Pre-grazing	2664.3 ± 234.1	3835.7 ± 246.8
Post-grazing	1278.8 ± 93.6	1613.6 ± 98.6
Dead matter content (%)		
Pre-grazing	12.26 ± 2.50	15.29 ± 2.63
Post-grazing	32.18 ± 4.0	49.83 ± 4.21

Table 2.2. Chemical composition of the diet selected of forages grazed during the experiment (g/Kg DM) (mean values with standard errors).

	Control pasture	*Lotus corniculatus*
n	10	9
Total nitrogen	29.9 ± 1.4	30.7 ± 1.5
NDF	397.8 ± 16.1	335.8 ± 17
CT	0.3 ± 0.10	22.4 ± 4.76
OMD	0.713 ± 0.82	0.720 ± 0.007
DOMD	$0.640 \pm 0.006_a$	$0.660 \pm 0.006_b$
ME (MJ/Kg DM)	$10.42 \pm 0.11_a$	$10.75 \pm 0.10_b$

NDF = neutral detergent fiber; CT = condensed tannins; DOMD = digestible organic matter in the dry matter; OMD; organic matter digestibility; ME = metabolizable energy.

Means within rows with different letters (a, b) differ significantly ($P < 0.05$).

2.3.2 Animals

Both groups of ewes gained weight during the 74 days of the nutritional period; however, animals in the lotus group gained significantly ($P < 0.05$) less weight than animals in the control group (Table 2.3). Both groups slightly increased in body condition score during the same period of time, without significant difference between treatments (Table 2.3). Live weight change pattern was similar in both groups through the whole experimental period (254 days); live weight remained almost constant after both groups were joined together at the end of the nutritional period until the beginning of lambing (days 74 – 149), with mean liveweights of 64.6 and 63.6 Kg for control and lotus ewes respectively, decreasing afterwards during the lambing and rearing period, and then starting to recover after day 237 (Figure 2.1). Body condition score (BCS) change pattern was also similar in the two groups, decreasing after the end of the nutritional period, with the lowest values being registered at docking time (day 210), with BCS of 2.02 and 1.87 units for control and lotus ewes respectively. After docking time, both groups started to increase their BCS to reach 2.5 units at the end of the experiment (weaning time) (Figure 2.2).

Table 2.3. Ewe live weight change (g/day) and body condition score (BCS) change during the 74-day of nutritional trial, and reproductive rate expressed as a percentage of the total number of ewes exposed to the ram (mean values with standard errors).

	Control pasture (300 ewes)	*Lotus corniculatus* (300 ewes)
Change in live-weight (g/day)	130 ± 2.8	94± 2.8
Change in BCS (units)	0.11 ± 0.03	0.12 ± 0.03
Reproductive rate		
Scanning	176 ± 3.7	175 ± 3.7
Lambing	160 ± 4.4	155 ± 4.4
Docking	123 ± 4.6	116 ± 4.6
Weaning	119 ± 4.6	112 ± 4.6
Reproductive rate corrected to equal live weight gain[1]		
Scanning	173 ± 3.7	181 ± 3.7
Lambing	157 ± 4.5	161 ± 4.5
Docking	118 ± 4.7	122 ± 4.7
Weaning	114 ± 4.7	118 ± 4.7

Means within rows did not differ significantly ($P > 0.05$).

[1] Using liveweight gain during mating as a co-variate.

Figure 2.1 Change in mean ewe live weight per treatment during the whole experimental period. The solid line indicates the nutritional period in which control and lotus ewes grazed pasture and *L. corniculatus* respectively. The broken line indicates period of lambing. (□) Lotus; (■) control pasture; (I) pooled S.E.M.

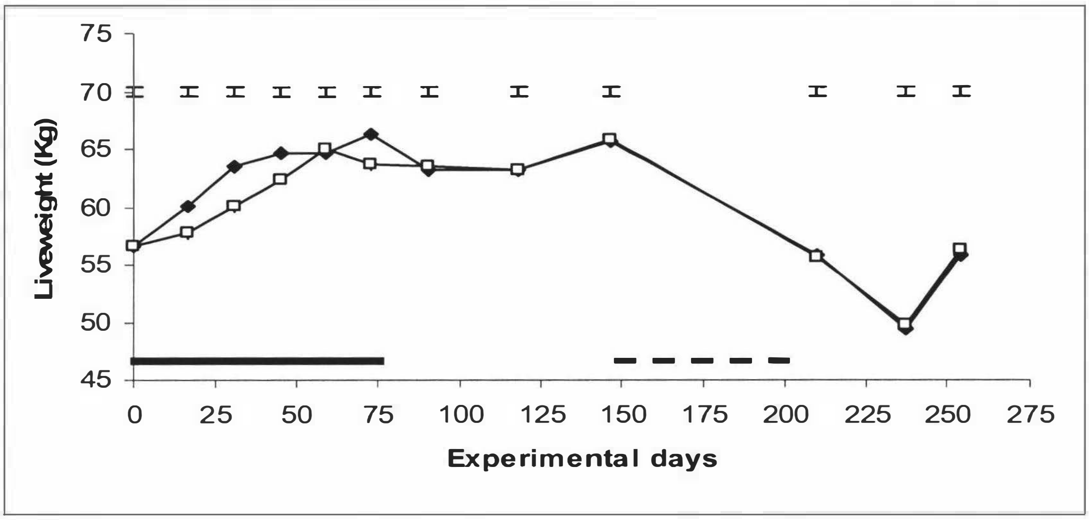
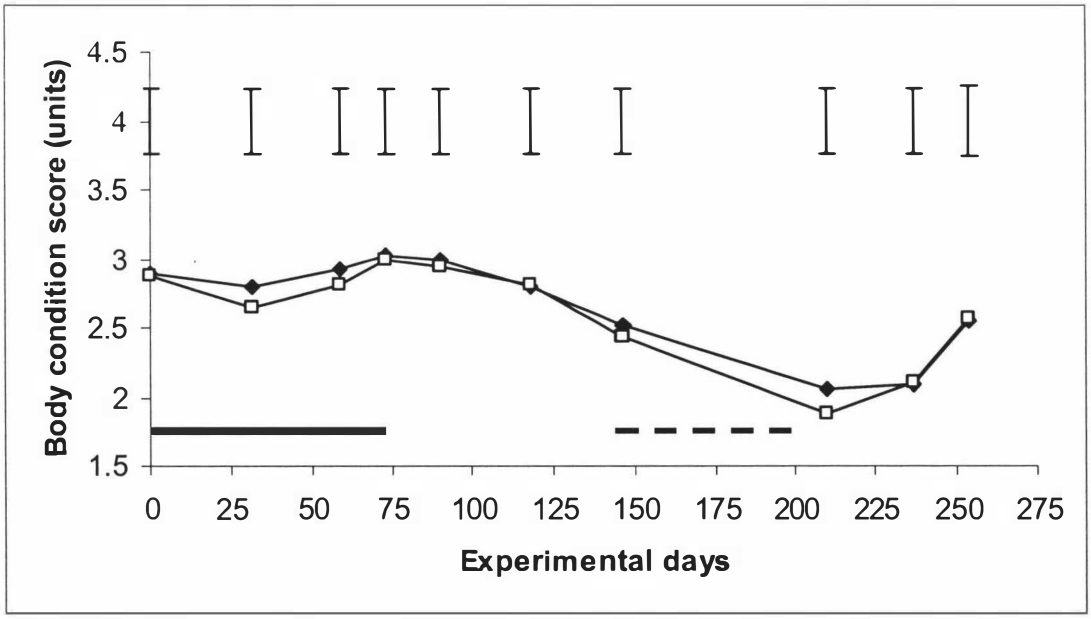


Figure 2.2. Change in mean ewe body condition score per treatment during the whole experimental period. The solid line indicates the nutritional period in which control and lotus ewes grazed pasture and *L. corniculatus* respectively. The broken line indicates period of lambing. (□) Lotus; (■) control pasture; (I) pooled S.E.M.



There were no significant differences in the reproductive rate (lambs per 100 ewes mated) of ewes between treatments at any of the periods analyzed (scanning, lambing, docking and weaning ($P > 0.05$; Table 2.3). When corrected to equal liveweight gain during mating, reproductive rate at scanning of ewes mated on lotus appeared to be slightly greater than that of ewes mated on control pasture, but the difference was still not statistically significant ($P > 0.05$). There were no significant differences in conception rate (ewes pregnant/100 ewes mated) between treatment groups, ($P > 0.05$; Table 2.4), with a mean conception rate of 96.5%. The proportion of ewes giving birth to single or triplet lambs was slightly higher in the lotus groups; however the difference was not significant ($P > 0.05$; Table 2.4). The differences were more apparent when the data were corrected to equal liveweight gain during mating, with ewes mated on lotus tending to have more triplet lambs and less singles and twins, but again this effect was not statistically significant ($P > 0.05$). Lotus treatment had a significant effect on mean lambing date, ($P < 0.05$) with ewes in this group lambing two days earlier than the ones in the control pasture group (Table 2.5). The largest advance in mean lambing date was in triplet-bearing ewes (6 days; $P < 0.05$), followed by twin-bearing ewes (1 day; $P < 0.05$).

Total lamb mortality and mortality per birth rank were not significantly different between treatment groups ($P < 0.05$). The only difference was observed among birth ranks (regardless of nutritional treatment); mortality increased proportionally to birth rank level; the mortality mean values for single, twin and triplet lambs were 14.7%, 26.7% and 45.6% respectively ($P < 0.0001$). Mean values for lamb mortality per treatment and birth rank are presented in Table 2.5.

There were no significant differences in birth and weaning weights of lambs between treatments, except for birth weight of triplet females, which was significantly ($P < 0.05$) higher in the lotus group than in the control pasture group (Table 2.6).

Table 2.4. Conception rate (at scanning), expressed as a percentage of the total number of ewes exposed to the ram, and fecundity as a percentage of lambing ewes (mean values with 95% confidence intervals).

	Control pasture (300 ewes)	*Lotus corniculatus* (300 ewes)
Conception rate	96.7	96.3
Range	93.9 – 98.2	93.5 – 97.9
Fecundity	(272 ewes)	(266 ewes)
Singles	29.7	32.8
Range	24.6 – 35.5	27.4 – 38.7
Twins	63.6	58.4
Range	57.6 – 69.1	52.3 – 64.2
Triplets	6.7	8.8
Range	4.3 – 10.4	6.9 – 12.9
Fecundity corrected to equal live weight gain[1]		
Fecundity	Control	Lotus
Singles	31.5	29.8
Range	24.6 – 35.5	27.4 – 38.7
Twins	62.4	60.7
Range	57.7 – 69.1	52.6 – 64.0
Triplets	6.0	9.4
Range	4.4 – 10.1	6.2 – 12.3

Means within rows do not differ significantly ($P > 0.05$).

[1] Using liveweight gain during mating as a co-variate.

Table 2.5. Total lamb mortality per treatment and per birth rank (%) from birth to weaning (mean values with 95% confidence intervals), and mean lambing date per treatment and birth rank (mean values with standard errors; days).

		Control pasture	*Lotus corniculatus*
		(481 lambs)	(468 lambs)
Total lamb mortality		27.2	27.3
	Range	22.1 – 33.0	22.3 – 33.0
		(81 lambs)	(88 lambs)
Singles		16.9	12.8
	Range	10.3 – 26.0	7.4 – 21.1
		(346 lambs)	(308 lambs)
Twins		25.7	27.7
	Range	21.4 – 30.6	23 - 33
		(54 lambs)	(72 lambs)
Triplets		42.6	48.6
	Range	30.2 – 56.0	37.1 – 60.1
Total mean lambing date		15 August $_{a}$	13 August $_{b}$
	SE	± 4.6	± 4.6
Singles		14 August $_{a}$	14 August $_{a}$
	SE	± 0.86	± 0.82
Twins		15 August $_{a}$	14 August $_{b}$
	SE	± 0.42	± 0.44
Triplets		16 August $_{a}$	10 August $_{b}$
	SE	± 0.72	± 0.40

Means within rows with different letters (a, b) differ significantly ($P < 0.05$).

Table 2.6. Lamb birth and weaning weights (Kg) per treatment, corrected for the small difference in the mean date of lambing (mean values with standard errors).

	Control pasture	*Lotus corniculatus*
Birth weight		
Single		
Male	5.75 ± 0.14	5.63 ± 0.12
Female	5.38 ± 0.11	5.36 ± 0.11
Twin		
Male	4.75 ± 0.06	4.72 ± 0.06
Female	4.52 ± 0.06	4.45 ± 0.06
Triplet		
Male	4.22 ± 0.17	3.90 ± 0.13
Female	$3.39 \pm 0.13_a$	$3.97 \pm 0.13_b$
Weaning weight		
Single		
Male	20.43 ± 0.56	19.97 ± 0.47
Female	19.24 ± 0.40	19.01 ± 0.43
Twin		
Male	16.80 ± 0.24	16.53 ± 0.26
Female	15.50 ± 0.23	15.47 ± 0.25
Triplet		
Male	15.18 ± 0.76	14.82 ± 0.66
Female	13.18 ± 0.63	13.87 ± 0.60

Means within rows with different letters (a, b) differ significantly ($P < 0.05$).

2.3.3 Wool production

Greasy fleece weight and staple length were not significantly different between ewes of both treatment groups (*P* > 0.05; Table 2.7). The only significant difference (P < 0.05) was observed among litter sizes (regardless of nutritional treatment), with mean greasy fleece weights of 3.07, 2.92 and 2.85 (Kg) and mean staple lengths of 125.27, 122.15 and 116.07 (mm) for single, twin and triplet bearing ewes respectively (*P* < 0.01; Table 2.7).

Table 2.7. Twelve-month wool production and staple length for each group (mean values with standard errors).

	Control pasture (261 ewes)	Lotus corniculatus (255 ewes)
Greasy fleece weight (Kg)		
Single bearing ewes	3.13 ± 0.06	3.02 ± 0.05
Twin bearing ewes	2.93 ± 0.03	2.91 ± 0.04
Triplet bearing ewes	2.79 ± 0.11	2.79 ± 0.11
Staple length (mm)		
Single bearing ewes	126.5 ± 1.96	124.04 ± 1.83
Twin bearing ewes	121.47 ± 1.29	122.83 ± 1.39
Triplet bearing ewes	119.68 ± 4.09	112.46 ± 3.59

2.4 Discussion

The main objectives of this study were to investigate if grazing ewes *on Lotus corniculatus* during mating time and early pregnancy had beneficial effects in terms of increasing their fecundity and reducing lamb mortality from birth to weaning. Unadjusted results from the present study (from data not adjusted to same LWG) show that mating ewes on *L. corniculatus* had no effect on either fecundity or lamb mortality under the conditions of this study. Possible reasons for these results will now be discussed.

Despite feed allowance being similar in both groups; live weight gain was lower in animals grazing lotus than control pasture when the opposite would be expected, based upon previous experiments (Table 2.8). According to these results, it seems that in the present experiment animals in the control group remained in a good nutritional status during the nutritional period, which led them to achieve a high reproductive performance in terms of ovulation rate, conception rate and lambing percentage (Coop, 1962; Knight, 1980; Smith, 1988).

Table 2.8. Liveweight gain (LWG) in ewes grazing either control pasture or *L. corniculatus* during mating in different studies in New Zealand.

Authors	Pasture Lwg (g/day)	*L. corniculatus* Lwg (g/day)
Min *et al*., (1999)	19	40
Luque *et al*., (2000)	-12	-25
Min *et al*., (2001)	43	22
Ramirez-Restrepo *et al*., (2005a)	-5	66
Ramirez-Restrepo *et al*., (2005a)	56	84
Present study	130	94

Table 2.9 shows the nutritional quality of the control pasture and *L. corniculatus* from the present experiment, in comparison with the quality of pasture and *L. corniculatus* from similar mating experiments carried out on the same farm in previous years. It can be seen that as OMD and ME in the control pasture increased, so did lambing percentage with the highest value (approximately 180%) obtained in the present experiment. As *L. corniculatus* OMD and ME was higher than that of pasture, this explains the increased lambing percentage in the work of Ramirez-Restrepo, et al., (2005a). As OMD and ME were high and similar in *L. corniculatus* and control pasture in the present experiment, the similar lambing percentage in the two groups suggests that both of them remained in a good nutritional status during the nutritional period, and that the genetic potential of the sheep for lambing percentage may had been attained, which means that responses to *L. corniculatus* in the present experiment could be limited by the genetics of the animals, with the highest genetic response being already achieved.

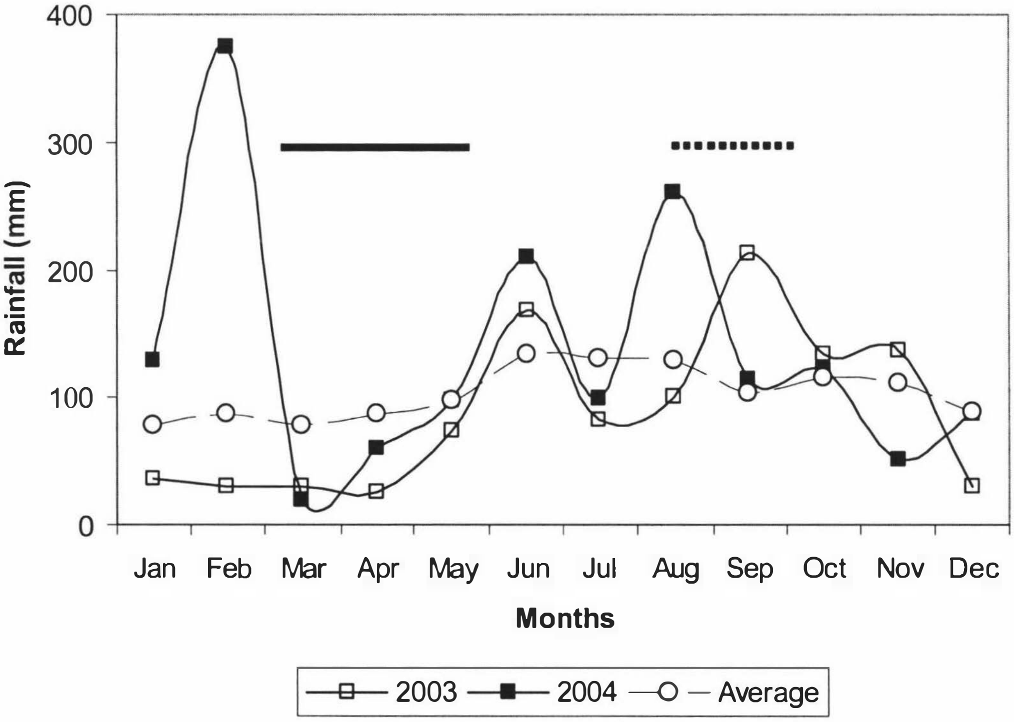
Table 2.9. Nutritive value of pasture and *Lotus corniculatus* during mating at Riverside farm, and ewe lambing percentage.

Author	Pasture			*Lotus corniculatus*		
	OMD	ME (MJ/Kg DM)	Lambing (%)[1]	OMD	ME (MJ/Kg DM)	Lambing (%)
	Drought autumn pasture					
(McWilliam, et al., 2004)	0.52	7.5	131			
(McWilliam, et al., 2005a)	0.53	7.5	144			
(McWilliam, et al., 2005b)	0.49	7.2	133			
	Normal autumn pasture					
Ramirez-Restrepo et al., (2005a)	0.54	8.3	159	0.72	10.9	175
Ramirez-Restrepo et al., (2005a)	0.61	9.1	162	0.68	10.4	176
	Wet autumn pasture					
Present study	0.71	10.4	182	0.72	10.8	182

[1] Lambs born/100 ewes lambing.

The good nutritional status of the animals in the control group was due to the high nutritional value of the control pasture. Former studies have shown that productive and reproductive parameters can be increased in sheep when the animals are provided with good quality forages, such as *L. corniculatus,* during autumn on dry-land farming areas (McWilliam, et al., 2004; Ramirez-Restrepo, et al., 2005a) when common pastures, such as ryegrass/white clover, normally decrease their nutritional value at this time due to the drought conditions. In the present experiment (2004), the weather conditions during the nutritional period (late summer-early autumn) were completely unusual for this dry-land farming area, with a very wet autumn, which allowed the control pasture to remain in a high nutritional value during the whole nutritional trial (Figure 2.3).

Figure 2.3. Rainfall per month (January to December) in three consecutive years at Riverside farm. The solid line indicates the nutritional period, and the broken line the lambing period in this experiment. Average line represents the average rainfall from 1989 to 2004.



The positive effects of the high quality control pasture, and the consequent higher liveweight gain on the reproductive performance of the animals in the control group is confirmed by the fact that when reproductive rate and fecundity rate data were adjusted to similar live weight gain, animals in the lotus group tended to perform slightly better than their counter parts in the control pasture group, mainly in terms of higher reproductive rate at scanning and a higher percentage of triplet lambs at lambing. However, even with reproductive data adjusted to similar liveweight gain the differences between treatment groups did not attain statistical significance ($P>0.05$).

Despite the fact that there was no significant difference in overall lamb mortality between both treatment groups, the rate of mortality in lambs was very high especially in triplet lambs, and these results were strongly influenced by a heavy storm that occurred during the third week of lambing. Although there was not a significant difference ($P > 0.05$) in the number of triplet lambs between treatment groups, there was a tendency to be higher in the *L. corniculatus* group, in addition, a higher number of lambs from the *L. corniculatus* group were born before and during the storm (with a high proportion of triplet lambs) in comparison with the control group (Figure 2.4). Therefore, it is possible that the bad weather at early stages of the lambing period obscured any effects of nutritional treatments on lambing mortality, having a more critical and negative effect on the lotus group due to the tendency in this group to have a higher proportion of triplets, and a higher proportion of lambs being born before and during the storm. (Figure 2.4 and 2.5).

Figure 2.4a. Lamb mortality per treatment during lambing weeks. The solid line at the top of the chart indicates the period of time during which storm occurred.

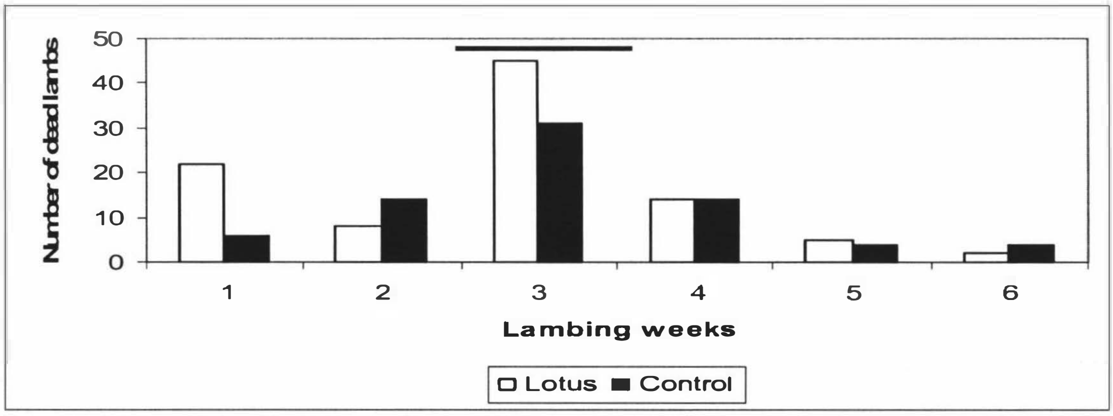


Figure 2.4b. Lambs born per week, in each treatment group, during lambing weeks. The solid line at the top of the chart indicates the period of time during which storm occurred.

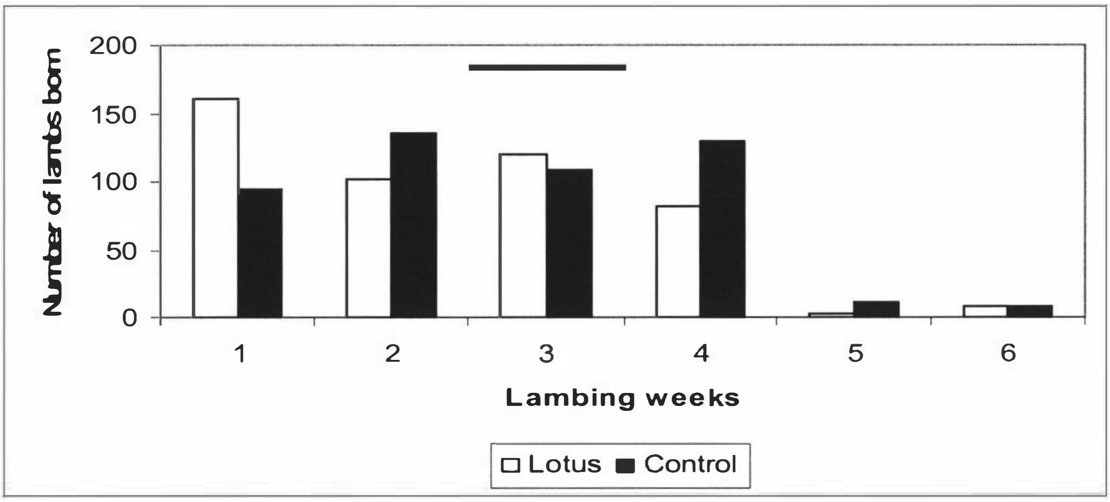


Figure 2.4c. Number of ewes lambing per week, in each treatment group, during lambing weeks. The solid line at the top of the chart indicates the period of time during which storm occurred.

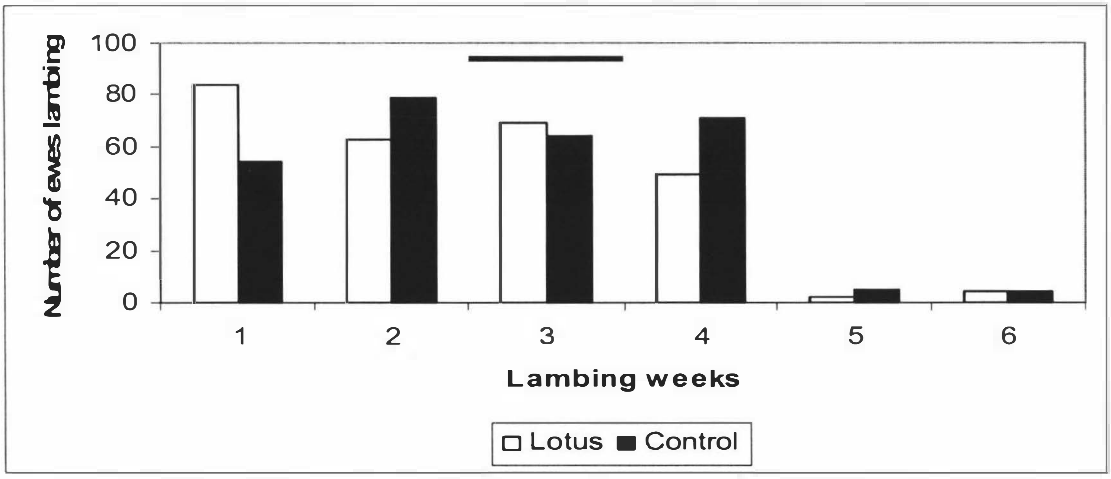


Figure 2.5a. Lamb mortality per birth rank during lambing weeks in the lotus group. The solid line at the top of the chart indicates the period of time during which storm occurred.

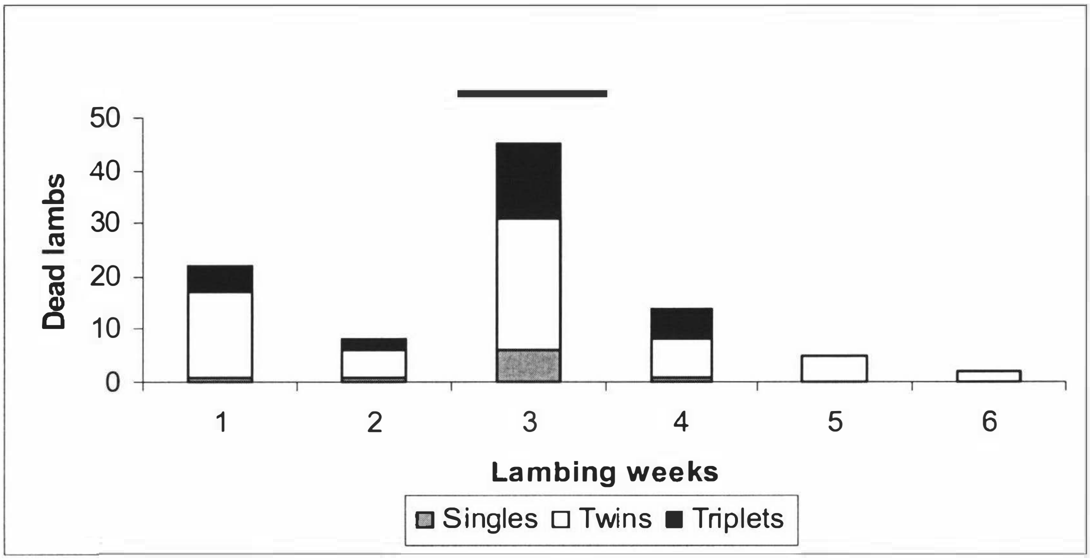
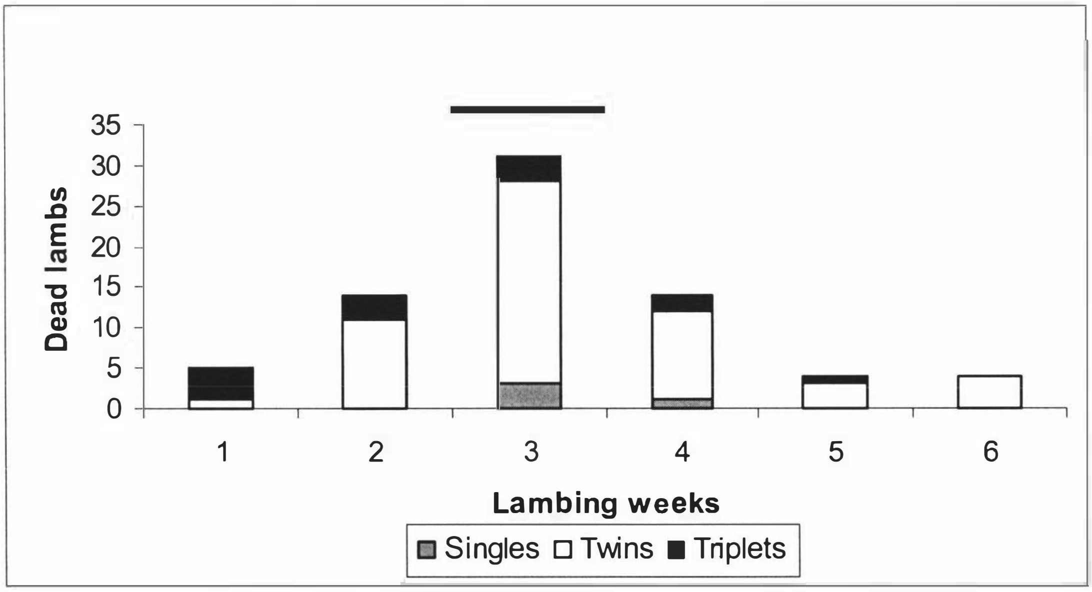


Figure 2.5b. Lamb mortality per birth rank during lambing weeks in the control pasture group. The solid line at the top of the chart indicates the period of time during which storm occurred.



Results from lamb mortality in this experiment show that triplet lambs are highly susceptible to the adverse weather conditions at and immediately following birth. Therefore, triplet bearing ewes may need special treatment at lambing. This aspect is being researched by On-farm Research Ltd, at Poukauwa Research Centre in the Hawkes Bay. Initial results show that the provision of more shelter during lambing reduced triplet lamb mortality (Thomson personal communication), as also found by other workers for single and twin-bearing ewes (Lynch, et al., 1980; Gregory, 1995). Another solution being researched at Poukauwa is putting triplet-bearing ewes in a big shed during lambing time, and then putting them out to pasture one week after lambing, with a view to reduce the mortality in triplet lambs. This research is on-going.

Whilst there was no increase in reproductive rate from mating on *L. corniculatus* in the present experiment, the adjusted data indicate that there would have been more triplet births to ewes mated on *L. corniculatus* if autumn conditions had been less favorable for the growth and nutritive value of grass-based pasture, as would be the case in a normal year. The six day earlier mean lambing date for triplet-bearing ewes mated on *L. corniculatus* and the increased birth weight of triplet females from the same group suggest that this treatment may have reduced gestation length by accelerating fetal development and growth; gestation length needs to be measured in future experiments of this type. As OMD and ME of the diet selected were similar for ewes mated on *L. corniculatus* or control pasture, it is suggested that the effect of mating on *L. corniculatus* upon triplet conceptions could be due to the increased essential amino acids absorption from the action of CT.

In addition, although a similar number of lambs was born in both groups, and lamb mortality in the *L. corniculatus* group was increased due to the heavy storm at lambing time, at the end there was a similar number of weaned lambs in both groups. This may means that in fact there was a higher survival of lambs in the *L. corniculatus* group, and that if that storm had affected both groups in the same way, perhaps there would have been a statistical difference in the survival rate of lambs between treatments, with a higher survival rate in the *L. corniculatus* group.

2.5 Conclusions

The main objectives of this study were to investigate if grazing ewes on *L. corniculatus* during mating time and early pregnancy had beneficial effects in terms of increasing their fecundity and reducing lamb mortality. *Lotus corniculatus* has proved to have several advantages, compared to common grasses, when used as grazing forage in dry areas improving the productive and reproductive parameters of ewes in parameters such as LWG, wool production, conception and fecundity rates. This is due to its resistance to drought conditions, remaining in a leafy status and keeping its high nutritional value even under these adverse conditions, while common grasses normally decrease their nutritional quality. However, according to the results from this study, it seems that under good weather conditions, with enough environmental moisture for common pastures to grow and remain with a high nutritional quality, ewes perform in a similar way weather they graze on *L. corniculatus* or common grasses such as rye grass/white clover.

Lamb mortality was high mainly in triplet lambs, which highlights the susceptibility of this kind of animals to adverse weather conditions. Lamb mortality is one of the most important economical problems in farms whose main activity is lamb production, therefore any action made in pro to reduce lamb mortality could lead to improve the economical profit of these farms. In the discussion two alternatives have been mentioned, both of them imply modifications to the common flock's management, and monetary investment to provide the extra shelter or the big shed, and because of this they may result no attractive for some farmers; however, it would be interesting to make a cost-effective analysis before making any decision.

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