WILLOW FODDER BLOCKS FOR GROWTH AND SUSTAINABLE MANAGEMENT OF INTERNAL PARASITES IN GRAZING LAMBS

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ABSTRACT

A rotational grazing experiment was conducted for 14 weeks in the summer/autumn of 2004/2005 on the lower eastern North Island, New Zealand, to compare the efficacy of grazing willow fodder blocks containing condensed tannins (CT), for sustainable control of internal parasites in 180 Suffolk x Romney weaned lambs. One third of the lambs grazed control perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture only, another third grazed pasture for 3 weeks followed by willow fodder blocks for 1 week (repeating the rotation; restricted access) and the last third of the lambs grazed on willow fodder blocks for the duration of the experiment (full access). All lambs were effectively treated with anthelmintics at the start of the experiment. Each group was divided into undrenched lambs and lambs regularly drenched every 4 weeks. Each of the six groups grazed separate areas at the same dry matter (DM) allowance, using rotational grazing with weekly breaks. Undrenched lambs would be trigger drenched if the faecal egg count (FEC) geometric mean of the group exceeded 1000 eggs/g wet faeces and/or liveweight gain (LWG) was reduced to zero and/or any one individual lamb exceeded 2500 eggs/g wet faeces, which never occurred.

Rectal faecal samples for FEC, larval counts (LC) and visual dag formation (Dag Score; DS) were assessed initially and at two week intervals throughout the experiment. All lambs were slaughtered at the end of the experiment, fatness (GR) and carcass weight (CW) measurements were recorded and representative samples of the abomasum, small intestine and large intestine were collected in the three undrenched treatments to determine total worm burdens.

Primary growth legume content in willow fodder blocks was similar to that of control pasture (20%), but willow fodder blocks secondary growth legume content (30%) was greater than in secondary growth control pasture (22%). Primary growth pre-grazing herbage mass (approximately 4800 kg DM/ha) and post-grazing herbage mass (approximately 3400 kg DM/ha) in willow fodder blocks (full and restricted access) was higher than that of control pasture (4400 and 3000 kg DM/ha respectively). Secondary growth pre and post-grazing herbage mass was similar in willow fodder blocks and control pasture (4200 and 3000 kg DM/ha respectively). Secondary growth mass of fodder trees (775 kg DM/ha) in the willow fodder block full access treatment was higher than primary growth (562 kg DM/ha). Pre-grazing herbage dead matter content was consistently higher in secondary growth (20-40%) than in primary growth (8-10%), for both control pasture and fodder blocks.

Condensed tannin concentration in willow fodder block herbage was 14.5 g/kg DM compared to the CT levels (6.2 g/kg DM) detected in control pasture diet selected. However, CT concentration in willow fodder block trees was particularly high (approximately 45.5 g/kg DM). *In vitro* OMD, DOMD and ME concentrations were higher for selected tree browse in willow fodder blocks (0.71; 0.65 g/kg DM; 10.6 MJ/kg DM respectively) when compared to herbage selected in either willow fodder blocks or control pasture (0.65; 0.60 g/kg DM; 9.7 MJ/kg DM respectively).

Regularly drenched lambs had significantly higher LWG and carcass weight gain (CWG) than undrenched lambs ($p < 0.05$) in all three groups. Lambs in willow fodder block full access had the highest LWG in drenched as well as undrenched lambs of 182 g/day and 154 g/day respectively.

Due to hot and dry summer conditions, growth rates of all treatments declined in the second half of the experiment as herbage nutritive value declined. Undrenched willow fodder block full access had the highest CWG amongst all undrenched treatments. Carcass weight gain reduction of undrenched lambs versus drenched lambs for the full access to willow fodder block group (12 g/day) was half of the reduction between control pasture groups (24 g/day).

Dag score increased with time until Day 70 of the experiment, with no differences between the six treatment groups. From Day 70 until the end of the experiment, dag scores of lambs grazing willow fodder block full access were consistently lower than lambs grazing willow fodder block restricted access or control pasture and were lower for drenched than for undrenched lambs. Drenched groups maintained low FECs throughout the experiment, whereas FECs of undrenched groups progressively increased with time. Both DS and LWG were similar for drenched lambs grazing control pasture and undrenched lambs grazing willow fodder block full access.

The parasites established in greatest numbers in undrenched lambs grazing control pasture were *Teladorsagia trifurcata*, *Nematodirus spathiger*, *Trichostrongylus vitrinus*, *Trichostrongylus colubriformis* followed by *Trichostrongylus axei* and *Teladorsagia circumcincta.* At slaughter, undrenched lambs grazing on willow fodder block full access had significantly lower *Nematodirus spathiger*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* worm burdens when compared to undrenched lambs grazing control pasture ($p < 0.05$), but greater burdens of *Haemonchus contortus* ($p = 0.0299$).

Undrenched lambs grazing willow fodder block with restricted access had significantly lower *Teladorsagia circumcincta*, *Teladorsagia trifurcata*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* worm burdens than undrenched lambs grazing control pasture ($p < 0.05$).

It was concluded that parasitism restricted lamb growth in all three undrenched grazing systems, showing a progressive increase in FEC over time. However, the reduction in carcass weight gain was greatest for undrenched control lambs and least for undrenched lambs with full access to willow fodder blocks. Grazing undrenched lambs on restricted and full access willow fodder blocks showed lower burdens of some parasites at slaughter compared to undrenched lambs grazing control pasture, which could be due firstly to an increased CT present in both willow and in fodder block herbage and their possible effects in increasing protein absorption. Secondly, CT could have interrupted parasite life cycles and/or, thirdly, decreased L_3 larval consumption could have occurred due to taller plant morphology of the trees, hence reducing the reinfection rate. There seemed to be no direct effect on killing established parasites, as if that had happened, there should have been a decrease in FEC in the first half of the experiment, before any effects of reinfection took place.

CT-containing forages could be used in conjunction with live weight gain monitoring and/or body condition score for the control of gastrointestinal nematodes, but it still needs further evaluation and a close collaboration of researchers and farmers.

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ABBREVIATIONS USED

Analysis of variance	ANOVA
Carcass weight	CW
Carcass weight gain	CWG
Condensed tannins	CT
Crude protein	CP
Dag score	DS
Degrees Celsius	°C
Digestible organic matter in dry matter	DOMD
Dry matter	DM
Eggs per gram	epg
Essential amino acids	EAA
Faecal egg count	FEC
Faecal egg counts	FECs
Figure	Fig.
First larval stage	L_1
Fourth larval stage	L_4
Gastrointestinal	GI
General linear model	GLM
Grams	g
Hectare	ha
Kilogram	kg
Larval culture	LC
Live weight	LW

Liveweight gain	LWG
Metabolisable energy	ME
MegaJoules	MJ
Meter	m
Millimetres	mm
Non ammonia nitrogen	NAN
Number	n
Organic matter digestibility	OMD
Post parturient rise	PPR
Second larval stage	L_2
Standard error	S.E.
Statistical Analysis system	SAS
Tannin protein complexes	TPC
Third larval stage	L_3
Tonne	t
Total nitrogen	N
Voluntary feed intake	VFI
Wet weight	W/W

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CHAPTER 1

LITERATURE REVIEW

1. Introduction

Sheep production in New Zealand is based on year round grazing of perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pastures. This is a low cost system of efficient meat production. New Zealand pastures have an organic matter digestibility (OMD) of approximately 80% in spring and 60% in summer, a metabolisable energy (ME) content of 8.8-11.7 MegaJoules (MJ)/kilogram (kg) dry matter (DM) and a total nitrogen (N) content of 25-35 g/kgDM (crude protein 155-220 g/kgDM; Waghorn and Barry, 1987). Pasture based systems have good nutritive values when the forages are maintained in the vegetative state, but have problems with internal nematode parasites that are currently controlled by oral application of anthelmintics, a process referred to in Australia and New Zealand as anthelmintic drenching (hence referred to as drenching). Drenching is usually administered to lambs at weaning (12 weeks of age) and then at monthly intervals until 6 weeks before slaughter.

In recent years, some nematode parasites have increased their ability to tolerate usual doses of anthelmintic drugs, a process described as anthelmintic resistance (Waller, 1985). Drench resistance is increasing and is a major problem in the New Zealand sheep industry (Waller, 1985; Leathwick, 2004). Alternative nematode control strategies are currently being explored to reduce drench resistance, including alternative anthelmintic controls and low chemical input options.

Condensed tannins (CT) present in some forages have the characteristic of reducing infective L_3 larvae motility, interrupting egg hatching and thus possibly acting as a

natural alternative control method for sheep parasites (Molan *et al.*, 1999). Furthermore, some studies have shown that lambs grazed on CT-containing forage legumes have lower nematode worm burdens, have a greater tolerance to internal parasitism and have higher growth rates than lambs grazing non CT-containing legume forages (Niezen *et al.*, 1998b; Ramírez-Restrepo *et al.*, 2005).

In this chapter, willow trees and their nutritive and feeding values will be reviewed, including their CT and other secondary compound contents. Particular attention will be given to evaluate the potential use of full or restricted grazing of willow fodder blocks for sustainable control of internal parasites in lambs, whilst also stimulating lamb growth rates. The use of diets containing CT as an antiparasitic control method could enable sheep producers to decrease chemical residues in lamb meat, accomplishing national and international trading market targets.

2. Sheep production and grazing systems in New Zealand

2.1 *Sheep production*

The agricultural industry is very important to New Zealand's economy, contributing 17% of the GDP (MAF, 2003). Pastoral farming covers approximately 13.5 million hectares from a total of 27.1 million hectares. Sheep numbers have decreased in recent years, from 69 million in 1980 to 40 million in 2003, whilst the dairy cattle population has increased from 3 to 5 million and deer numbers have increased from 100 thousand to 1.6 million over the same time period (MAF, 2003). One of the main reasons for the decrease in the sheep population is the elimination of all livestock subsidies by the New Zealand Government in the mid 1980's (Annual Review of the NZ Sheep and Beef Industry/NZ Meat and Wool Boards' Economic Service 1985 – 2003).

Table 1: Changes in the productivity of the New Zealand sheep industry from 1980 to 2003.

Year	**1980**	**1990**	**1995**	**2000**	**2003**
Lambing (%)	99.2	100.4	104.3	116.1	123.7
Wool production/ewe (kg)	5.54	5.28	5.51	5.72	5.65
Lambs slaughtered/annum (000-head)	28,692	25,149	26,684	26,050	25,978
Lamb carcass weight (kg)	13.61	13.71	14.83	16.61	16.86

Source: Annual Review of the NZ Sheep and Beef Industry/NZ Meat and Wool Boards' Economic Service (1985 – 2003).

Over the past 24 years, lambing percentage has increased from 99 to 123 lambs from every 100 ewes, wool production per ewe has stayed relatively stationary and average lamb carcass weight has increased by 3 kg (Table 1). Whilst there has been a 30% decrease in the sheep population, there has been a 52% increase in lamb meat produced per ewe. Sheep production and management is based on an annual cycle, with the breeding season in March/April, lambing in August/September, and weaning in November/December (Matthews *et al.*, 1999).

2.2 *Sheep grazing systems*

Pasture is the main source of most of the nutrients consumed by New Zealand grazing livestock; being a mixture of perennial ryegrass and white clover (80:20 ratio). Legumes (mainly white clover) are used for nitrogen fixation, but if used in excess can cause bloat problems in cattle. Ryegrass, because of its rapid establishment, persistence and steady growth through the year (including winter) is widely used. Several different grazing systems are available for utilising pastures by grazing livestock. These systems are designed to integrate a combination of animal, soil, and environmental components, with diverse systems designed to achieve specific results or goals of a producer (Clark and Kanneganti, 1998). A definition of these systems is as follows.

Continuous stocking system: Herbage intake and animal performance increase at a progressively declining rate while the animals continuously graze the same area for an undetermined length of time. Animals may stay in the same paddock throughout the season. The benefit of this system is that there is a lower input in the cost of fences, water and human labour (Clark and Kanneganti, 1998). If the surface

height of the sward declines below 6-7 cm, herbage intake and sheep performance tend to decline (Matthews *et al*, 1999).

Paddock grazing system: This is similar to the rotational stocking system, but differs in that the animals are wintered without an electric fence. Instead of dividing the area into different blocks for consecutive days, animals are left in the paddock for a specific period of time that is determined by herbage mass and stocking rates (Hodgson, 1990; Thomson *et al.*, 1993).

Rotational stocking system: Pasture is divided into smaller areas called breaks (often using temporary electric fences), where animals are moved after a determined length of time that depends on the growth, development and defoliation of the sward. The animals then progressively graze each individual break and then return to the first one, so completing the rotation. Second and subsequent rotations then follow. Another name for this method is block grazing, because animals are designated to an area for a fixed amount of time (i.e. 3-7 days) and then they are transferred to the next block (Hodgson, 1990; Thomson *et al.*, 1993; Clark and Kanneganti, 1998).

Strip grazing system: This technique is a modification of the rotational stocking system, in which animals are allocated a new area (strip) each day (i.e. 24 hours). Generally there is a fence in the front of the animal but there is no back fence (Thomson *et al.*, 1993; Clark and Kanneganti, 1998).

Leader follower grazing system: In this system, there are generally two or more groups of animals with different feed requirements. The group with the higher nutritional requirement grazes the paddock first, and then the next group grazes that same paddock as soon as the first group is moved into another area (Clark and Kanneganti, 1998).

On: Off grazing system: Animals are left in a determined area for a short period of time (i.e. 4 hours) and then they are taken out of the paddock and returned to the main grazing area for the rest of the day. The same sequence occurs the next day (Thomson *et al.*, 1993).

2.3 *Recommended pasture allowances*

In sheep production systems, lactating ewes and their lambs usually take top priority. Live weight gains of around 200 g/day for twin-born lambs and over 300 g/day for single-born lambs can be achieved during lactation with pasture allowances of about 6 kg DM/ewe/day. The New Zealand Sheep Industry target for lamb growth rates is 400 g/day (The New Zealand Sheep Council, 2000). Recommended post grazing swards and residual pasture mass for sheep grazing New Zealand pastures are shown in Table 2.

Table 2: Recommended post-grazing values for sward height with continuous stocking and post grazing residual herbage mass under rotational grazing for New Zealand sheep.

Animal class and performance	Post-grazing sward surface height (cm)	Post-grazing herbage mass (kg DM/ha)	Energy value of herbage consumed (MJ ME/kg DM)
Ewe plus twins, Early Lactation	7-8	1400-1600	11-12
Dry ewe	3-4	800-1000	10.5-11.5
Lambing season (spring pastures)	4-5	1200	11-12

Adapted from (Rattray and Jagusch, 1987; Matthews *et al*, 1999)

3. Willow trees on New Zealand farms

3.1 *Origin*

Willow (*Salix* spp.) trees, exotic to New Zealand, have been cultivated for more than 160 years in New Zealand for soil conservation, shade and shelter for animal welfare and as supplementary feed for livestock (Van Kraayenoord *et al.*, 1995; Wilkinson, 1999). Since 1969, willow breeding and selection programmes have been implemented in New Zealand with the help of the former National Plant Materials Centre at Aokautere near Palmerston North (Van Kraayenoord *et al.*, 1995; Bulloch and Wilkinson, 1993). However, since 1994 the Crown Research Institute, HortResearch, has taken over these functions with the objective to create clones and hybrids between *Salix matsudana* and various *Salix alba* willow more suitable for soil conservation and river protection, such as the clones Tangoio, Hiwinui, Wairakei, Makara, Adair, and Moutere (Van Kraayenoord *et al.*, 1995).

3.2 *Uses*

3.2.1 *Soil conservation*

Willows are one the principal trees for soil erosion control along with several other species of hardwood trees, such as *Acacia*, *Pinus radiata* and *Eucalyptus*. In New Zealand, trees planted on hill country have been shown to reduce mass movement of soil by 50 to 80% (Hicks, 1995). Eyles & Newsome (1992) estimated that soil conservation measures need to be taken in 33 and 25% of the North and South Islands, respectively.

Pasture productivity is reduced by 0.5% per year on erosion prone land without trees. The use of willow for conservation improves soil health by binding the soil with their fibrous root system. Willows are also beneficial in low-lying wet areas as they pump out the water from deep (transpiration) in the soil (Kemp *et al.*, 2001).

3.2.2 *Shade and shelter*

Willow trees can be planted at a low density (100-500 stems/ha) on established pastures or they can be planted in lines to provide shade and shelter (Gregory, 1995). Trees strategically planted for shade and shelter can help to alleviate the adverse effects of inclement weather conditions on livestock by reducing discomfort and distress attributable to heat, wind, rain or cold and reducing suffering and stress associated with events that lead up to death from hypothermia and hyperthermia (Flanagan, 1995; Gregory, 1995).

Protection from extreme weather conditions has been shown to increase pasture and animal production and reduce animal suffering (Gregory, 1995). Lynch and Donelly (1980) and Alexander *et al.* (1980) showed that providing shelter can improve growth and ovulation rates in cattle and sheep and can effectively reduce lamb mortality and abortions induced by hypothermia (Table 3). Furthermore, Arnold and Morgan (1975) found that 52% of all lambs born on rainy days died, accounting for 41% of total lamb losses.

Table 3: Beneficial effects of animals sheltered from cold climatic conditions on animal production. Adapted from Gregory (1995).

Lamb mortality	Lamb mortality during the first 48 hours decreased from 20% to 7% when a 5-8m high *Cupressus macrocarpa* shelterbelt was available in windy conditions (Egan *et al.*, 1972). Mortality decreased from 17 to 9% in single-born and from 51 to 36% in multiple-born lambs when phalaris grass shelters were provided (Alexander *et al.*, 1980).
Lamb growth rate	Growth rate to 21 days of age in lambs from sheltered paddocks was 7% greater than for unsheltered lambs (Alexander and Lynch, 1976).

3.2.3 *Feed for livestock*

In New Zealand, willow trees have been used as a source of alternative supplementary fodder for sheep and cattle, during summer/autumn, when there are feed shortages and droughts (Moore *et al.*, 2003). Willows provide palatable and nutritious foliage and primary growth that can be fed to sheep as a supplement during dry summers (McCabe and Barry, 1988).

There are three different methods of tree fodder supplementation. Firstly, willows can be mechanically trimmed, carried and fed as a supplement to stock fed in any situation. Secondly, they can be used in coppicing when required from trees planted for forage bank purposes and fed as supplements (Douglas *et al.*, 1996). Thirdly,

densely planted tree blocks can be grazed *in situ* by livestock as browse or fodder blocks, with the animal doing the harvesting (Pitta *et al.*, 2005a).

3.3 *Establishment, growth and management of willow fodder blocks*

One of the main reasons for the establishment of willows is that it is easy to propagate clones of known quality. Willow trees can be established from un-rooted stakes or poles, they have high evapotranspiration rates during the growing season (to dry out water-logged soils), they provide superior early growth rate when compared to other cool temperate tree species and they can offer shade and shelter for grazing stock (Wilkinson, 1999).

Establishment of fodder blocks can be achieved by vertically planting cuttings also called stakes, which are 1.0-1.2 m long, with a diameter of 15-25mm and 20-40mm, respectively (Van Kraayenoord *et al.,* 1986). However, Douglas *et al.* (2003) suggested that 2m poles would enhance yield per tree and per hectare because they have more growing points and more energy reserves for shoot growth. Willow cuttings can be either rooted or unrooted stems with both being very productive. However, unrooted cuttings are cheaper and easier to handle (Zsuffa, 1992). Research has shown that the total biomass production of Tangoio willow (2,700 stems/ha) can average 1.2 to 4.3 t DM/ha/year, with 25% being edible (leaves plus stem $\leq$ 5 mm diameter; Douglas *et al.*, 1996).

4. Willow Fodder

4.1 *Chemical composition and nutritive value of willow fodder*

Nutritive value, ease and low cost of establishment, are amongst the factors for willows being investigated as a supplementary feed in dry summer conditions (Oppong *et al.*, 2001). Moreover, McCabe and Barry (1988) found that the lignin and CT concentrations present in willow are greater than for most temperate forages. The nutritive value of edible fodder (leaves and fine stems $\leq$ 5 mm diameter) of shrub willow is adequate for the maintenance of sheep, goats, cattle and red deer and better than that of low quality summer pasture (McCabe and Barry, 1988; Kemp *et al.*, 2001; Moore *et al.*, 2003). Experimental data shows that the willow diet selected by sheep is superior to drought pasture with higher total N, OMD and ME than low quality drought pasture (Table 4; McWilliam *et al.*, 2005a).

Table 4: Chemical composition and nutritive value of the pasture diet selected by ewes grazing low quality drought pastures when supplemented with willow trimmings. Adapted from McWilliam *et al.* (2005a).

	Control drought pasture	Willow supplementation
Total nitrogen (g/kg DM)	15.9	24.7
Organic matter (g/kg DM)	915	913
Organic matter digestibility *in vitro*	0.49	0.70
Digestible organic matter in the dry matter *in vitro*	0.44	0.64
Metabolisable energy (MJ/kg DM)	7.2	10.4

Willow is also lower in neutral detergent fibre (NDF; 355 g/kg DM) compared with drought pasture (603 g/kg DM), and contains higher concentrations of secondary compounds such as CT, salicin and other phenolic glycosides (McWilliam *et al.*, 2005a).

Experimental data collected from grazing ewes, showed that drought pasture and herbage present in willow fodder blocks had low nutritive value, with a high NDF content (Table 5; Pitta *et al.*, 2005b). Nevertheless, selected browse in willow fodder blocks was superior in nutritive value, higher in digestibility and ME compared to control drought pastures (Table 5; Pitta *et al.*, 2005b). Pasture growing in willow fodder blocks had a higher concentration of CT than control drought pastures. However, tree fodder had the highest concentrations of secondary compounds, including CT (38.3 g/kg DM), flavenoid monomers (14.21 g/kg DM) and other phenolic glycosides (36.53 g/kg DM; Table 5; Pitta *et al.*, 2005b).

Table 5: Chemical composition, nutritive value and secondary compound content of pasture and willow diet selected (g/kg DM) by ewes grazing control drought pasture and willow fodder blocks. Adapted from Pitta *et al.*, 2005b.

	Control drought pasture	Willow fodder block	
		Herbage	Trees
Total nitrogen	24.2	20.0	13.6
Neutral detergent fibre	551	512	417
Organic matter	885	916	943
Organic matter digestibility *in vitro*	0.57	0.60	0.67
Digestible organic matter in the dry matter *in vitro*	0.50	0.59	0.61
Metabolisable energy (MJ/kg DM)	8.2	8.8	9.9
Condensed tannins	1.9	3.6	38.3
Catechin+epicatechin	0.11	0.14	2.85
Other flavenoid monomers	5.23	4.21	14.21
Other phenolic glycosides	4.44	5.13	36.53
Chlorogenic acid	0.24	0.26	1.09
Zearalenone (mg/kg)	0.13	0.16	0.31

4.2 *Edible fodder production*

If willows are to be used as an adequate edible biomass, it is important to maintain the initial harvest and frequency of harvesting when the regrowth period starts (Oppong *et al.*, 2002). For example, if trees are not harvested/grazed until late summer, the quality of the foliage declines and the quantity of edible forage outgrows what the animal can effectively reach to graze, whereas if they are harvested in late spring, larger quantities of edible forage are produced (Oppong *et al.*, 2002).

Total DM yield of willow changes depending on the cutting height, however, it doesn't significantly influence the edible forage yield or the regrowth of stumps (Oppong *et al.*, 2001). Edible forage production (leaves plus stem ≤ 5 mm diameter) from widely spaced willow trees ranges from 0.9-25.0 kg DM per tree depending on tree species and age (Oppong *et al.*, 1996; Kemp *et al.,* 2001), and 1-6 tonnes DM/ha from densely planted fodder blocks (Oppong *et al.* 1996; Hathaway, 1986).

Hathaway (1986) and McCabe and Barry (1988) identified cut forage of *S. kinuyanagi* and *S.matsudana* × *alba* (Tangoio) as a nutritionally acceptable supplementary livestock forage in summer and autumn. Even though green willow leaves have higher nutritive value than total edible forage, sheep readily browse the soft stems (Kemp *et al.*, 2001; Oppong *et al.*, 2001). Sheep should be encouraged to feed on green or just fallen leaves (OMD of 50%, ME 7-8 MJ/kg DM) because bark and senesced or dead leaves have a lower nutritive value (Kemp *et al.*, 2001.

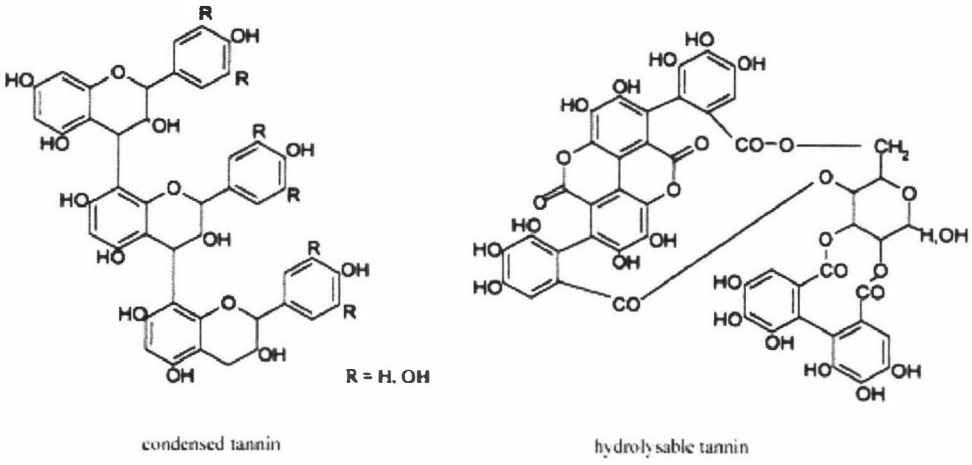
5. Plant secondary compounds present in willow fodder and its role in ruminant nutrition

5.1 *Condensed and Hydrolysable Tannins*

Polyphenolics are widely distributed in plants. Tannins are polyphenolic compounds classified into two groups (Haslam, 1981; McSweeney *et al.*, 2001); proanthocyanidins or condensed tannins (CT; polymers of flavanoids), and hydrolysable tannins, which are complex polyesters consisting of a carbohydrate core in which two or more hydroxyls are esterified with gallic and hexahydroxydiphenic acids (respectively, gallotannins, and ellagitannins; Nelson *et al.*, 1995; Silanikove *et al.*, 2001; McSweeney *et al.*, 2001).

Condensed and hydrolysable tannins have a large number of free phenolic groups that form strong hydrogen bonds, at multiple sites, with proteins and carbohydrates (Haslam, 1989). Tannins are found in approximately 80% of woody and 15% of herbaceous dicotyledenous species and can be present in high levels in some forages (Bryant *et al.*, 1992). Condensed tannins are the most common type of tannin found in plant vacuoles of gymnosperms and angiosperms of forage legumes, trees and shrubs, whereas hydrolysable tannins are found only in dicotyledons (Min *et al.*, 2003). Structurally, CT are complexes of oligomers that comprise ten to twelve polymerized flavan-3-ol-units, linked by carbon–carbon bonds, as seen in Figure 1 (Hagerman and Butler, 1989; Barry and McNabb, 1999; Min *et al.*, 2003).

Figure 1: Chemical structure of condensed and hydrolysable tannins. The hydrolysable tannin represented is the toxic compound (punicalagin) from *Terminalia oblongata* (Doig *et al.*, 1990).



In New Zealand, it was originally thought that the only CT-containing forages were sulla (*Hedysarium coronarium*), *Lotus* species, and sainfoin (*Onobrychis vicifolia*). Nevertheless, very low CT concentrations can be found in common grasses, legumes and herbs used in temperate grazing systems (Barry and McNabb, 1999).

5.2 *Beneficial and detrimental effects of condensed tannins*

Condensed tannins play a significant role in the nutrition of animals and cause both adverse and beneficial effects on the nutritional quality from herbaceous and shrub legumes and animal health and production (McLeod, 1974; Jones *et al.*, 2000). They protect plants from invasion of pathogenic microorganisms due to their antimicrobial properties and antifungal properties and also stimulate salivary flow in animals (Scalbert, 1991; Van Soest, 1994; Provenza, 1995; Silanikove *et al.*, 2001). The major

benefit of CT to ruminant livestock feeding is that they bind with dietary proteins during mastication and protect the protein from microbial degradation in the rumen, making it available for digestion and utilization in the abomasum and small intestine (Waghorn, 1990; Norton, 1999).

The ideal concentration of CT in temperate forage legumes, ranging from 20 to 45 g/kg DM, reduces rumen forage protein degradation, resulting in increased milk production, wool growth, ovulation rate, and lambing percentage (Jones *et al.*, 2000; Min *et al.*, 2003). Condensed tannin reduces bloat risk, improves bypass protein and essential amino acids (EAA) flow to the small intestine and can reduce internal parasite burdens (Jones *et al.*, 2000; Min *et al.*, 2003). Condensed tannin content of 50-100 g/kg DM in *Lotus pedunculatus* may act as a feeding deterrent causing negative effects on appetite (Barry, 1985), influence feed degradation in the rumen, affect digestibility of the whole diet, reduce the activity of rumen bacteria and impair the production of gut enzymes (Provenza, 1995; Barry, 1989; Jones *et al.*, 2000).

5.3 *Effect of condensed tannins on voluntary feed intake and upon nitrogen and carbohydrate digestion.*

The effects of CT on forage feeding value can be regarded as the sum of the effects on the digestive process, on the metabolism of absorbed nutrients and on voluntary feed intake (Kumar and Vaithiyanathan, 1990). In general, there is an inverse relationship between CT concentrations in browse sources and voluntary feed intake (VFI) by herbivores (Kumar and Vaithiyanathan, 1990). High concentrations of CT tend to reduce VFI and digestibility (Barry and McNabb, 1999) due to the unpalatability of CT-containing plants acting as a defence against consumption by herbivores (Barry

and Ducan, 1984; Waghorn *et al.*, 1994). High forage CT concentrations in *Lotus pedunculatus* (>55 g CT/kg DM) tend to reduce VFI and depress rates of body and wool growth of grazing ruminants (Barry, 1985). On the other hand, medium CT concentrations in sulla (45 g/kg DM) and in *Lotus corniculatus* (34 to 44 g/kg DM) had no effect upon VFI (Barry and McNabb, 1999).

Condensed tannin forms reversible complexes with proteins (tannin–protein complexes; TPC), reducing the wasteful degradation of protein in the rumen and improving the total availability of protein in forages. Thus the total supply of protein for absorption is increased, without significantly affecting the efficiency of microbial digestion (Barry *et al.*, 1986; Barry *et al.*, 2001; McSweeney *et al.*, 2001; Andrabi *et al.*, 2005).

Condensed tannin reacts with forage proteins by hydrophobic hydrogen bonding in a pH-reversible manner (Jones and Mangan, 1977). In the rumen, stable TPC are formed at pH 3.5–7.5 that dissociate post-ruminally in the abomasum (pH 2.5–3.5), releasing protein in response to the extremes of pH less than 3.5 (Min *et al.*, 2003). The low pH in the abomasum as well as the high pH in the small intestine can stimulate dissociation (Jones and Mangan, 1977; Barry and McNabb, 1999; Andrabi *et al.*, 2005). The reactivity of CT differs between species of plants that contain these compounds, with CT from some plants increasing the net absorption of EAA more than others (Barry and McNabb, 1999; Min *et al.*, 2003). During mastication of non CT-containing plants between 56 and 65% of the N concentration in the rumen is released, consequently large losses of N occur (25–35%) as ammonia is absorbed from the rumen (Min *et al.*, 2000). Condensed tannins bind with proteins, reducing

nitrogen availability to rumen microorganisms, reducing proteolysis and thus improving N retention by the animal (McSweeney *et al.*, 2001; Min *et al.*, 2003).

There is extensive absorption of NH_3 from the rumen when animals are fed low CT-containing forages such as perennial ryegrass (*Lolium perenne*), short-rotation ryegrass (*Lolium multiflorum* × *Lolium perenne*) and white clover (*Trifolium repens*), with duodenal non-ammonia nitrogen (NAN) flow is only about 0·75 of N intake (Barry and Manley, 1984). However, NAN flow in sheep fed *Lotus* spp. increased linearly with increasing CT concentration, equalling N intake at a concentration of approximately 40 g/kg DM (Fig. 2; Barry and McNabb, 1999).

Figure 2: Duodenal NAN flow (Barry and Manley, 1984)

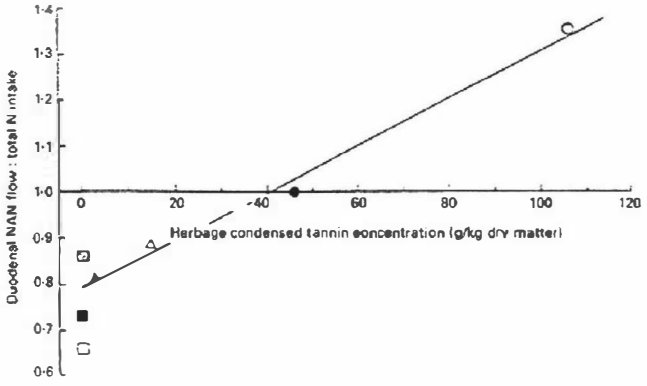


Fig. 2. Duodenal non-ammonia nitrogen (NAN) flow per unit total nitrogen intake as a function of herbage condensed tannin concentration in sheep fed on *Lotus* species. (○), High- and (●) low-tannin *Lotus pedunculatus*; (△), high- and (▲) low-tannin *Lotus corniculatus*. Results are compared with the non-tannin-containing herbages; (▨), short rotation ryegrass; (□), perennial ryegrass; (■), white clover. All results are for a nitrogen intake of 28 g/d and refer to fresh forages. From Barry & Manley (1984).

The ideal concentration of CT in temperate forage legumes generally ranges from 20–40 g/kg DM (Barry, 1985; Barry and McNabb, 1999). The high CT concentration in *Lotus pedunculatus* (95–106 g/kg DM) has been shown to reduce rumen digestion of readily fermentable carbohydrates (sugar, pectin) and hemicellulose (Barry and Manley, 1984; Barry and McNabb, 1999).

6. Internal parasites

6.1 *Gastrointestinal nematode classification*

Twenty-nine species of nematodes have been introduced with sheep into New Zealand. The gastrointestinal nematodes of major importance include *Haemonchus*, *Teladorsagia, Trichostrongylus, Nematodirus* and *Cooperia* (Vlassoff *et al.*, 2001) and are generally associated with production losses and clinical disease (Table 6; Brunsdon and Adam, 1975; Pomroy, 1997; Vlassoff *et al.*, 2001).

Table 6: Important New Zealand nematode parasites of sheep (Adapted from Townsend, 1993; Charleston, 1982)

Major Importance	Secondary Importance
Abomasum	
Haemonchus contortus	*Ostertagia crimensis*
Teladorsagia circumcincta	*Teladorsagia pinnata*
Teladorsagia trifurcata	*Ostertagia ostertagi*
Trichostrongylus axei	
Small Intestine	
Cooperia curticei	*Bunostomum trigonocephalum*
Nematodirus filicollis	*Cooperia mcmasteri*
Nematodirus spathiger	*Cooperia oncophora*
Trichostrongylus colubriformis	*Cooperia punctata*
Trichostrongylus vitrinus	*Nematodirus abnormalis*
	Nematodirus helvetianus
	Strongyloides papillosus
Large Intestine	
	Chabertia ovina
	Trichuris ovis
	Oesophagostomum venulosum

6.2 *Epidemiological and seasonal pattern of larval development and survival in New Zealand*

6.2.1 *Developmental success in New Zealand's seasons*

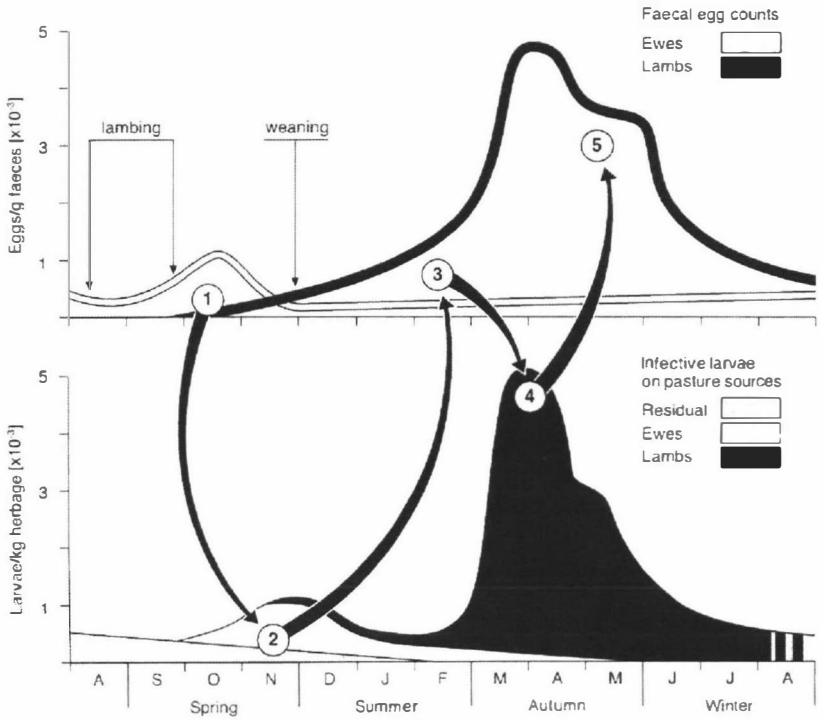
The prevalence and distribution of parasitic nematodes is governed by their ecological requirements for development and survival inside and outside the host (Vlassoff *et al.*, 2001). Due to the temperate climate and rainfall, free-living stages of nematodes generally have good conditions for developing on New Zealand pastures (Familton and McAnulty, 1994) This means that development can take place in all months of spring, summer and especially autumn (Vlassoff, 1982).

Nevertheless, the percentage of deposited eggs developing to 3rd stage larvae (developmental success) is very variable during the year (Vlassoff, 1982). Vlassoff (1982) described New Zealand's maximum developmental success on pastures to be around 20 - 25 % in late summer/autumn however; in most months, the developmental success is below 1 % (Vlassoff, 1982).

6.2.2 *Seasonal pattern of larval availability*

Vlassoff (1982) summarised the overall seasonal pattern of larval availability on New Zealand pastures as shown in Fig. 3. This excludes *Nematodirus*, which is principally a lamb parasite and is transmitted from the lambs in one season to those in the following season by eggs and larvae that over-winter each year (Vlassoff, 1973).

Figure 3: Representation of the seasonal interrelationship between pasture contamination by untreated ewes and lambs and the pattern of infective larvae availability on pasture (Vlassoff, 1982).



1. The post-parturient rise (PPR) in faecal egg count (FEC) of the breeding ewe is the main source of contamination contributing to the spring peak of larvae on pasture. This ensures that infective larvae at different stages will be available on the pasture to develop infection in the grazing flock (Georgi, 1985).

2. Pasture is re-contaminated in spring with infective larvae resulting in the first generation of worms that accumulate in the lambs in summer.

3. Eggs deposited by lambs during late summer early autumn produce a large autumn peak of infective larvae on pasture.

4. Larvae from the autumn peak produce the second generation of nematode worms in lambs that cause more pathogenic effects in autumn and winter (Georgi, 1985). A proportion of these larvae over winter as infective-stage larvae on pasture to provide a source of infection for ewes and lambs the following spring (Vlassoff, 1982).

5. L_3 stages on sheep pastures in New Zealand reaches the highest level in autumn (Vlassoff, 1982; Vlassoff *et al.*, 2001). However, due to low temperatures and excessively wet conditions most of the eggs deposited fail to develop.

Ewes can also be contributors to pasture larval contamination (West, 1982; Familton, 1991; Stafford *et al*, 1994). As the faecal output of ewes is considerable, even a low FEC will result in a high daily level of pasture contamination (Familton 1991; Vlassoff *et al.*, 2001). However, due to their higher feed intake (Jorgensen *et al*, 1998), adult ewes may remove a much larger proportion of infective larvae from pasture than lambs. Recent studies have also shown that ewes that have high levels of acquired immunity could reduce the viability of eggs to less than half (Jorgensen *et al.*, 1998). Consequently, Vlassoff *et al.* (2001) states that FEC alone is likely to overestimate the ewes' contribution to pasture contamination.

Leathwick *et al.* (1998) concluded that lambs that are not drenched provide the main source of nematode eggs, contributing to the greatest proportion of the larval population (Vlassoff *et al.*, 2001).

6.2.3 *Variation in larval number throughout New Zealand*

The general pattern of pasture contamination by nematode eggs and then larvae is considered to be similar throughout New Zealand as shown in Fig. 4. However, there is some variation in the pattern of different genera. Spring is dominated by *Nematodirus filicollis, Teladorsagia* spp. and small numbers of *Trichostrongylus* spp., whereas autumn is dominated by *Trichostrongylus* spp., followed by *Nematodirus filicollis* and *Nematodirus spathiger* (Familton and McAnulty, 1995). Vlassoff and McKenna (1994) found *Cooperia* spp. and *Trichostrongylus. axei* to be abundant in autumn and winter. *Haemonchus* spp. requires a higher range of temperatures for larval development and therefore species are predominantly found in the North Island, with a seasonal peak during January-May (Vlassoff 1982; Beckett, 1993; Vlassoff and McKenna, 1994).

6.3 *General Lifecycle*

The typical life cycle of nematodes infecting sheep comprises the egg, four larval stages and the adult stage (Charleston, 1982; Vlassoff, 1982). Most sheep strongylid nematodes share the same life cycle. Adult worms in the gastro-intestinal tract mate and females lay eggs that contain multi-celled embryos (morula), which pass out in the sheep's faeces. The first stage larva (L_1) develops in the egg, emerges, grows and moults to the second stage larva (L_2) and then to the non-feeding infective third larval

stage (L_3) which retains the cuticle of the L_2 and is more resistant to adverse conditions (Vlassoff, *et al.*, 2001). These subsequently migrate from faeces onto the herbage.

A suitable host is infected by ingesting the L_3 larvae. Before they reach their preferred site the CO_2 concentration, temperature and pH induce the L_3 larvae to ex-sheath. This is usually in the organ immediately prior to their preferred site. Exsheathment can take place in less than 10 minutes (Charleston, 1982; Georgi, 1985; Wharton, 1986). The fourth larval stage (L_4) and adult stage are parasitic stages that spend their entire lifetime within the alimentary tract of the host animal. They complete their development to mature adults in 15–21 days (Brunsdon and Adam, 1975; Vlassoff, 1982; Familton and McAnulty, 1997).

6.4 *Environmental effects*

Development of the free-living stages of gastrointestinal nematodes in and around the faecal pellets or dung pat can be influenced by intrinsic and environmental factors (Brunsdon and Vlassoff, 1982; Vlassoff, 1982; Familton and McAnulty, 1995).

6.4.1 *Temperature effect*

Required developmental conditions vary between nematode species but generally, optimum development occurs between 15–30°C. Below 10°C, development is slow, and most eggs fail to hatch (Leathwick *et al.*, 1999; Vlassoff *et al.*, 2001) whilst at higher temperatures (> 30°C), developmental success is limited as eggs are subjected to temperature stress (Vlassoff, 1982). However, it is important to mention that

common nematode hatching temperatures vary as seen in Table 7, although hatching times shown in the table should be considered only as guidelines.

Table 7: Upper and lower temperature limits for egg hatching in gastrointestinal New Zealand nematodes (Crofton, 1965; *Ahluwalia and Charleston, 1974).

Species	Minimum temperature for egg hatch, (°C)	Time to hatch at minimum temperature, (days)	Maximum temperature for Egg hatch, (°C)	Time to hatch at maximum temperature (hours)
Haemonchus contortus	9	7	36	13
Ostertagia circumcincta	4	7	34	17
Trichostrongylus axei	8 – 9	7	36	19
Cooperia curticei	16 (10*)	7	38	15
Chabertia ovina	6	7	36	17

Once the development to the L_3 stage is complete, they are more resistant to adverse conditions being able to survive around 2-3 months or even up to 12 months until the following summer (Vlassoff, 1982; Vlassoff *et al.*, 2001).

McKenna (1988) found that a decreasing number of L_3 stage larvae were recovered after culturing when faeces had been exposed to 4 °C for periods of time up to 12 days. *Cooperia* spp. was particularly susceptible after 1-3 days, whereas *Trichostrongylus* spp. and *Teladorsagia* spp. were affected after exposure for more than 12 days (McKenna, 1988).

6.4.2 *Moisture level effect*

Moisture is generally considered to be less important than temperature but most free-living stages are considered to be susceptible to desiccation (Levine and Andersen, 1973; Gibson and Everett, 1976). The normal moisture content in freshly deposited faeces from sheep is 60-70 % however, if the moisture level is extremely high, it

inhibits the development of eggs due to the reduced supply of available oxygen to the egg (Silverman and Campbell, 1959; Young *et al.*, 1980; Gruner and Suryahadi, 1993). On the other hand, desiccation tends to destroy unembryonated eggs rapidly (Shorb, 1944; Rose, 1963; Gibson and Everett, 1967; Wharton, 1982) and inhibit the development of the larval stages (Hsu and Levine, 1977; Wharton, 1982; Rossanigo and Gruner, 1994).

6.4.3 *Oxygen availability effect*

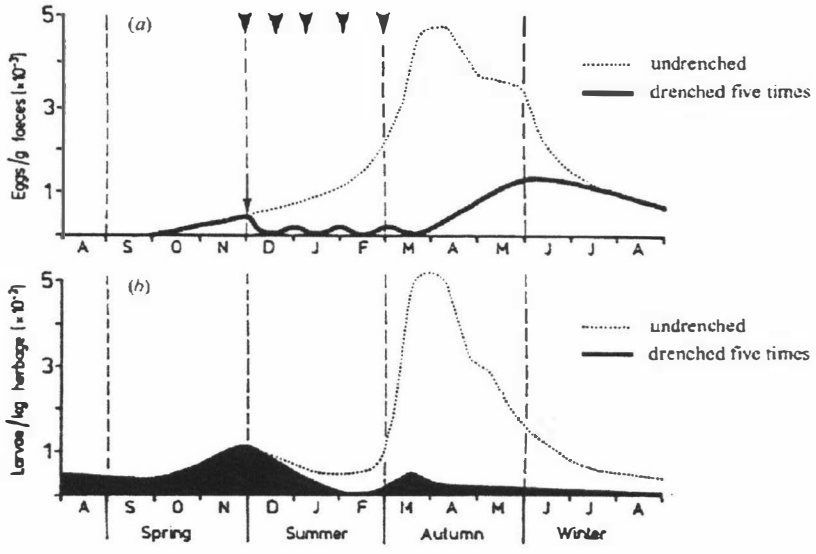
Oxygen is a very important requirement. A significantly higher rate of inhibited development has been shown in experiments with non-aerated egg suspensions as compared to aerated egg suspensions (Shorb, 1944; Silverman and Campbell, 1959). Conversely, in pelleted sheep faeces oxygen is not considered a problem for developing free-living stages on pasture (Christie, 1963).

6.5 *Anthelmintic control*

Traditionally, lambs were treated with anthelmintics when signs of parasitism were seen or expected and this involved treatment during late summer and autumn (Vlassoff *et al.*, 2001). However, higher production gains could be achieved with the implementation of a preventive approach to control parasitism in lambs (Vlassoff and Brunsdon 1981).

This preventive programme consisted of drenching 5 occasions at 28-day intervals, commencing at weaning in November-December (Fig. 4). Additional treatments were given as determined on the basis of FECs of the flock (Bisset *et al* 1996; Vlassoff *et al.*, 2001), although, Becket (1993) indicated that usually it was necessary to extend this to at least a 6 drench programme.

Figure 4: Seasonal faecal egg output (a) and pasture contamination with L_3 larvae (b) with undrenched and five times drenched ewes and lambs (Adapted from Brunsdon, 1981).



Despite the risk of developing anthelmintic resistance, farmers ended up drenching adult sheep at an average of 1–2 times and lambs almost 7 times each year (Brunsdon *et al* 1983; Familton 1991; Beckett 1993). Over time, this level of drench frequency has been considered to be an important contributing factor for the increase in genetically resistant worms in New Zealand, leading to treatment failure (Watson, 1994; Vickers *et al.*, 2001).

6.6 *Methods for detecting nematode parasites*

Faecal egg counts (FEC) are the most widely used *in vivo* technique for detecting nematode parasites. FEC levels help farmers to decide when or whether to drench sheep. With this technique the concept of 'trigger levels' evolved, on which drenching decisions were based.

Regardless of the decision, Cook (1997) and Vlassoff *et al.* (2001) suggested that FECs alone are not a suitable criterion to decide whether to drench or not, but FECs together with close liveweight gain monitoring and an understanding of the level of nutrition should help in the decision making. This is consistent with the view that FEC alone has inherent biological problems for the quantitative assessment of the intensity of the infection (Keymer and Hiorns, 1986; Cabaret *et al.*, 1998). A principle issue is that all strongylid eggs are similar in morphology (except *Nematodirus* spp.) and cannot be differentiated from each other (Keymer and Hiorns, 1986; Cabaret *et al.*, 1998).

Another downside of FECs is that the correlation with the actual number of worms in the host is not high (McKenna, 1981). FEC only measure eggs produced by mature worms and provides no estimate of larval numbers (Presidente, 1985; Taylor *et al.*, 2002).

Faeces can be cultured *in vitro* to allow eggs to develop to L_3 and these can be identified to genus. Infective larvae cultured from faeces are identified by morphological and morphometric keys after 10 days of incubation in laboratory conditions (Ministry of Agriculture, Fisheries and Food, 1986).

If animals are killed, the actual number of worms present can be counted. Post mortem worm counts are very expensive, but they do provide a more accurate estimation of the number of worms present in each animal (McKenna, 1981). However, Keymer and Hiorns (1986) suggested that even post mortem counts are not entirely precise because there are losses of parasites during the sieving and collection process and only an aliquot of these contents are actually examined.

Gardner and Craig (1961) proposed an estimation of parasite burdens based on a "point system" pathogenicity index, modified by Gordon (1973). Points are allocated to 4000 *Teladorsagia*, 500 *Haemonchus*, 6000 *Trichostrongylus*, 3000 *Nematodirus*, 200 *Chabertia*, 500 *Oesophagostomum*, 10000 *Cooperia*, 150 *Bunostomum* and 4000 immature worms (all species) were each is considered to represent one point. The assumption is that points are combined to estimate the pathogenicity of mixed infections, where figures of >2 points in young sheep and >4 points in adult sheep is considered to be economically important.

Studies showed there was a reasonable correlation (r=0.74) between mean FECs and worm burdens in sheep less than 12 months old when they are categorized into three FEC classes: "Low" being <500 epg, "Moderate" being 600–2000 epg, and "High" being >2000 epg and three worm burden classes: "Low" being <4000 worm count, "Moderate" being 4000-10000 worm count, and "High" being >10000 worm count (McKenna 1981; Vlassoff *et al.*, 2001). McKenna (1981) showed that for sheep beyond 12 months of age the correlation of strongyle egg counts (*Nematodirus* spp. excluded) and worm counts in these same classes is low (r = 0.23). It is usually considered that FECs and parasites with low fecundity such as *Teladorsagia circumcincta* have a poor correlation (Stear *et al.*, 1996).

6.7 *Dag formation*

There are various factors related to dag formation (accumulation of faeces in the wool surrounding the anus); (Leathwick and Atkinson, 1995), but the specific mechanism involved in the incidence of dags is yet unclear. Waghorn *et al.* (1999) considered factors such as parasitism, faecal moisture, changes in mineral absorption, genetic

variation, and endophyte toxins such as ergovaline or lolitrem B as all associated with dag formation (McEwan *et al.*, 1992; Scales *et al.*, 1995; Leathwick and Atkinson, 1995, 1998; Niezen *et al.*, 1995, 1998a,b; Ramírez-Restrepo *et al.*, 2002).

Dags represent losses in productivity due to the costs involved in removing them and the risk of flystrike. There are no statistics so far to be able to assess dag incidence or frequency in New Zealand (Leathwick and Atkinson, 1995). There is evidence of an unfavourable association between FEC and dagginess which suggests that the host response to parasite challenges may result in diarrhoea (Bisset and Morris, 1996). Niezen *et al.* (1995; 1998a) suggest that CT have the potential to control parasite infections and reduce dag formation.

7. Anthelmintic control

7.1 *Anthelmintic and drench resistance*

There are different classifications of broadspectrum anthelmintics. Class I drugs or benzimidazoles, include thiabendazole, mebendazole, albendazole, netobimine. Class II drugs or nicotinic agonists, include imidazothiazoles (levamisole), tetrahidropyrimidines (pyrantel and morantel). Class III drugs, or macrocyclic lactones, include the avermectins (ivermectin and doramectin) and milbemycins (moxidectin) (Waller, 1985; Merino *et al.*, 2002).

Nematode parasitism is one of the key limiting factors in sheep, goat and horse farming systems (Coles, 2002). However, in recent years, some nematode parasites have shown an increased ability to tolerate usual doses of anthelmintic drugs, a process described as anthelmintic resistance (Waller, 1985). This is a major worldwide problem that is increasing in New Zealand and around the world (Leathwick, 2004; Waller, 1985) and poses an indisputable menace to the long-term viability of the animal health industry (Hennessy, 1997; Waller, 1999). Several factors contribute to the development of anthelmintic resistance, which include the continuous use of the same anthelmintics and a high frequency of use (Waller, 1985).

One important concept recommended to delay the development of anthelmintic resistance is to allow a proportion of organisms to escape exposure to anthelmintics whilst still achieving control.

This principle has been applied in entomology for crop protection from insects, so perhaps it is one of the possible solutions to control the rapid appearance of anthelmintic resistance worldwide (Coles, 2002). Therefore, alternative nematode control strategies are currently being explored to reduce internal parasite problems without selecting for resistance.

7.2 *Alternative nematode control strategies*

The development of resistant strains of nematodes to anthelmintics in addition to the worldwide growth of non-chemical farming of livestock (ecological, organic, green) (Hein *et al.*, 2001; Waller and Thamsborg, 2004), where the use of synthetic products is restricted, have increased the demand for alternatives to chemoprophylaxis (Athanasiadou *et al.*, 2000). Several strategies are currently being considered (Niezen *et al.*, 1996) such as grazing management and focusing more on an identified need to drench.

Another option is biological control through vaccination but to date this option has had no commercial success (Hein *et al.*, 2001; Waller and Thamsborg, 2004). This is due to the difficulty of producing antigenic fractions of parasite material in commercial quantities and the complexity of the host immune response to parasites, further complicated by the natural unresponsiveness that exists in the young animal (<6–9 months of age) and in the dam around parturition (Waller and Thamsborg, 2004).

An additional alternative is the use of nematophagous fungi that entrap the free-living larval stages found in faeces (Larsen *et al.*, 1994; Fernández *et al.*, 1999; Coop and Kyriazakis, 2001; Hein *et al.*, 2001). To date this has not become available in a commercial formulation.

Genetic selection for more resistant hosts (Hördegen *et al.*, 2003) is also considered to offer some hope for reducing reliance on anthelmintics.

Another alternative is the dietary supplementation with forages containing amounts of condensed tannins which may enhance the nutrition of the host and possibly have direct anthelmintic properties (Barry and McNabb, 1999; Sykes and Coop, 2001).

However, different plants contain very diverse CT structures, therefore it is not possible to predict the antiparasitic properties of different species just by their CT concentration (Coop and Kyriazakis, 2001; Sykes and Coop, 2001; Tzamaloukas *et al.*, 2005).

7.3 *Direct and indirect effect of condensed tannins*

Studies in New Zealand have shown that some plant species containing CT may reduce the degree of parasite infestation, provide sheep with the ability to withstand helminth infection and improve growth rates in sheep (Niezen *et al.*, 1993; Waghorn *et al.*, 1995; Waller, 1999). Two hypotheses have been developed; the first hypothesis proposes the direct effect of condensed tannins on parasite larvae, adult parasites and/or fecundity of the worms. However, mechanisms involved have not been determined (Athanasiadou *et al.*, 2000; Molan *et al.*, 2003). The second hypothesis suggests an indirect effect of condensed tannins with the improved protein nutrition that may enhance host immune response to gastrointestinal nematodes (Min *et al.*, 2004). Condensed tannins are known to affect the resilience and resistance of herbivorous hosts because they form complexes with dietary protein and consequently protect protein from rumen degradation until it dissociates in the acidic conditions of the abomasum and is again available for digestion (Barry and Manley, 1986).

This increases the availability of amino acids below the rumen (Coop and Kyriazakis, 2001) and protein availability to the host is increased (Barry and McNabb, 1999; Min *et al.*, 2000). Furthermore, it has been suggested that protein supplementation is effective in enhancing specific immune responses towards gastrointestinal parasites in ruminants (Athanasiadou *et al.*, 2000; Houdijk *et al.*, 2005) leading to reduced helminth survival, growth and/or fecundity (Waghorn *et al.*, 1995).

Nutrition influences the development of parasitism in three different ways. First, it improves the ability of the host to overcome parasitism (resistance) by limiting the establishment, growth rate, fecundity and/or persistence of a parasite population (Coop and Kyriazakis, 2001; Houdijk *et al.*, 2005). Second, it can affect the parasite population through the intake of alternative antiparasitic forages and thirdly, it can increase the ability of the host to cope with the adverse consequences of parasitism (Sykes and Coop, 2001; Coop and Kyriazakis, 2001).

It is useful to separate the possible nutrient requirements that are associated with the initial responses to parasite invasion in a naive animal from those that are mediated by changes in the supply of digested protein in animals who have acquired immunity (Coop and Kyriazakis, 2001; Sykes and Coop, 2001; Thi Mui *et al.*, 2005).

A partitioning framework suggests that responses to dietary supplementation in young animals is small at an early stage of infection, because the acquisition of immunity has a higher priority than body protein gain so as to fight any adverse parasitism before the animal reaches reproductive maturity as well as increase host resilience (Sykes and Coop, 2001; Coop and Kyriazakis, 2001).

In young sheep and goats, it has been shown that feeding additional undegradable protein is effective in enhancing specific immune responses as a first priority to allow the host to combat nematode infections by lowering worm fecundity and/or worm burdens as well as promote tissue growth (Coop and Kyriazakis, 2001; Sykes and Coop, 2001; Thi Mui *et al.*, 2005).

When parasitic gastrointestinal (GI) infections occur there is an endogenous loss of protein due to increased leakage of plasma protein, increased sloughing of epithelial cells and increased secretion of mucoproteins (Sykes and Coop, 2001; Coop and Kyriazakis, 2001). It is hypothesised that protein supplementation can replace lost protein and supply excess for growth as well as facilitate the immune response. If parasitised animals are supplemented with protein, eosinophils, globule leukocytes and mast cells increase in numbers in the GI mucosa (Sykes and Coop, 2001; Coop and Kyriazakis, 2001). Hence, host nutrition, will greatly influence functions such as controlling the parasite population (expression of immunity) (Coop and Kyriazakis, 2001; Molan *et al.*, 2003; Waller and Thamsborg, 2004; Thi Mui *et al.*, 2005).

Parasitism increases the demand for amino acids in the GI tract and this leads to a knock-on effect on protein metabolism in other tissues reducing the availability of absorbed amino acids for metabolism of peripheral tissues. Therefore making amino acids unavailable to peripheral tissues reducing the deposition of protein in the carcass and decreasing the rate of protein synthesis in muscle and wool (Sykes and Coop, 2001; Coop and Kyriazakis, 2001).

The effect of nematode parasites on animal metabolism has been widely recognised (Morris, 1988; Sykes and Coop, 2001). These include: metabolic disturbances such as impaired acid secretion in the abomasum, impaired protein and energy metabolism which reduces feed conversion efficiency (Bown *et al.*, 1991); reduction in VFI by up to 20% (Sykes and Coop, 1976); reduced retention and metabolic flows of calcium and phosphorus (Bown *et al.*, 1991); while being absorbed, amino acids are sequestered by the alimentary tract and are unavailable to peripheral body tissues; skeletal growth and liveweight gain (LWG) reductions of up to 50% (Sykes and Coop, 1976); impaired wool growth of up to 26% (Steel *et al.*, 1980); alterations of some structural characteristics of the wool (Steel and Symons, 1979); increased dags (faecal material accumulating around the anus of sheep) and flystrike (Waghorn *et al.*, 1999).

Furthermore, adult stages of nematodes in the gut can also cause leakage of plasma and extracellular fluids, thus increasing mucus production and reducing immunological competence (Sykes and Coop, 1976; Steel and Symons, 1979).

7.4 *Condensed tannins as gastrointestinal parasite control methods*

The term nutraceuticals refers to crops containing plant secondary metabolites (or nutricines), considered to be beneficial to health rather than because they can provide a direct contribution to the nutrition of animals. An example are CTs (Waller and Thamsborg, 2004). The main effect of dietary CTs in both sheep and goats seems to be reduction in the establishment, growth, persistence and worm fecundity of nematodes in the host (Molan *et al.*, 2003) in addition to the reduction in faecal egg counts and elimination of adult worms (Waller and Thamsborg, 2004).

Moreover, CTs may also interfere with parasite egg hatching and development to infective stage larvae (Thi Mui *et al.*, 2005). It is important though, to highlight that results shown *in vivo* do not necessarily relate to the beneficial effects of anthelmintic compounds found *in vitro.* Some interesting CT-containing crops that have been investigated for their antiparasitic activity are as follows.

7.4.1 *Quebracho (Schinopsis spp.)*

Quebracho is a South American evergreen hardwood tree belonging to the cashew family with a very hard tannin rich wood. Condensed tannins of quebracho extract have been shown to have a direct anthelmintic effect on an established *Trichostrongylus colubriformis* population, which is a small intestine parasite (Butter *et al.*, 2000), but not against the abomasal parasite *Teladorsagia circumcincta (*Athanasiadou *et al.*, 2000; Min *et al.*, 2004). In addition, it reduced egg excretion and fecundity of the female *Haemonchus contortus* worms in sheep and in goats (Paolini *et al.*, 2003).

Sheep trickle-infected with *Trichostrongylus colubriformis* larvae were offered a diet containing condensed tannins from quebracho extract at 8% total intake for 10 weeks, compared to sheep offered a quebracho-free diet, showing an immediate reduction on the FEC (Athanasiadou *et al.*, 2000).

7.4.2 *Sulla (Hedysarum coronarium)*

Sulla is a tanniferous legume with a two year lifetime (Niezen *et al.*, 1994; Niezen *et al.*, 2002). The presence of high concentrations of an astringent CT in sulla (Terrill *et al.*, 1992a) have demonstrated antiparasitic properties which are suggested to be either direct anthelmintic properties or indirect effects through nutrition or immunity

(Waller and Thamsborg, 2004; Molan *et al.*, 2002; Hoskin *et al.*, 2000). The CT in sulla increased essential aminoacid absorption in sheep (Bermingham *et al.*, 2001). It has been shown to reduce egg hatching and inhibit larval migration of *Trichostrongylus colubriformis* in *in vitro* experiments (Molan *et al.*, 2003).

By contrast, studies conducted by Tzamaloukas *et al.* (2005) showed no *in vivo* effect on decreasing adult worm burdens in sheep when compared to animals grazing control pastures of grass and clover for 2 weeks. Niezen *et al.* (1998b) conducted a 6-week experiment with sheep grazing sulla and showed an effective reduction in FECs when compared to sheep grazing control pastures, which suggests an indirect anthelmintic effect rather than a direct effect of sulla on parasites.

7.4.3 *Lotus (Lotus spp.)*

Previous metabolic studies with lambs fed *Lotus corniculatus*, showed lower faecal egg counts and higher faecal dry matter than lambs fed ryegrass (Ramírez-Restrepo *et al.*, 2004), which is not necessarily a direct anthelmintic effect. The difference in FEC in this experiment was probably due to an increase in the faecal output of sheep fed lotus because of a higher food intake and the lower digestibility of lotus compared to sheep grazing grass/clover pastures (Athanasiadou *et al.*, 2005). On the other hand, numbers of *Trichostrongylus colubriformis* nematodes were not affected when sheep grazed lotus (Niezen *et al.*, 1998a) contrary to studies conducted when lambs were fed quebracho (Athanasiadou *et al.*, 2000). Ramírez-Restrepo *et al.* (2005) *in vivo* experiment indicated that sheep fed with *Lotus corniculatus* had a reduced

establishment of *Haemonchus contortus*, *Teladorsagia* spp., *Nematodirus* spp. and *Cooperia* spp., in comparison to sheep fed ryegrass pasture.

7.4.4 *Willow (Salix alba)*

Currently, to the best of our knowledge, there is no published evidence of either direct or indirect effects of feeding willow, against gastrointestinal nematodes in sheep.

7.4.5 *Chicory (Cichorium intybus)*

Chicory is a legume member of the Compositae family. It contains sesquiterpene lactones as well as low concentrations of CT (secondary compounds) which have been shown to have some antiparasitic properties (Scales *et al.*, 1994; Marley *et al.*, 2003; Waller and Thamsborg, 2004). However it is not a tannin-rich legume.

There are several reasons for chicory being able to influence parasite numbers (Scales *et al.*, 1994; Moss and Vlassoff, 1993; Tzamaloukas *et al.*, 2005). As an upright plant it inhibits the vertical migration of larvae thus reducing the ingestion of L_3 by livestock when compared to grasses and white clover (Moss and Vlassoff 1993; Hein *et al.*, 2001; Ramírez-Retrepo and Barry, 2005).

A study conducted by Hoskin *et al.* (2003), showed that the withdrawal of anthelmintic drenching had no overall effect in LWG for farmed deer grazing the CT-containing herb chicory (Table 8). Young sheep fed chicory maintained high levels of productivity as well as low levels of parasites when compared to sheep and farmed deer grazing perennial ryegrass / white clover pastures (Hoskin *et al.*, 2003; Min and Hart, 2003; Table 8).

Table 8: Average autumn liveweight gain, carcass weight, dressing % and gastrointestinal nematode numbers of deer grazing either pasture (perennial ryegrass) or chicory with anthelmintic drench (four weekly) or trigger drenched. Adapted from Hoskin *et al.*, 2003.

	Pasture		Chicory	
	Drenched	Trigger	Drenched	Trigger
Average LWG (g/d)	134	60	208	175
Final live weight (kg)	62	57	66	64
Carcass weight (kg)	31	30	37	37
Dressing percentage	53	54	58	58
GI nematodes (No)	0	2642	52	2240

Recent studies support chicory as an effective controller of *Teladorsagia circumcincta* burdens (Marley *et al.*, 2003; Tzamaloukas *et al.*, 2005). Molan (2002; 2003) have demonstrated that chicory is effective against *in vitro* immature stages of nematodes such as *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. CT extracted from chicory had direct inhibitory effects on the motility of L_3 larvae of deer-origin gastrointestinal nematodes, immobilising larvae and preventing larval passage through 20 and 25 μm nylon mesh sieves (Molan *et al.*, 2003).

7.5 *Larval development*

Condensed tannins may affect developing larvae in several ways. Nematode larvae can ingest CT, which may bind to the intestinal mucosa and cause autolysis (Athanasiadou *et al.*, 2001). Another possibility is that tannins could bind to the free protein available in faeces for larval nutrition, hence reducing nutrient availability and cause larval starvation and death.

They may bind to the cuticle of larvae, which is high in glycoprotein and cause their death (Athanasiadou *et al.*, 2001) although the actual mechanism is unclear.

It is apparent that CT can affect larval motility under *in vitro* conditions. When measuring L_3 larvae migration through 20 µm sieves, inhibitory effects on the motility of the larvae caused by polymerised flavan-3-ols and flavan-3-ol gallates can be demonstrated (Molan *et al.*, 2003). Moreover, viability of infective larvae from abomasal species was affected under exposure to quebracho (Athanasiadou *et al.*, 2001). However, whether this effect occurs in nature is less certain.

7.6 *Establishment of worms*

It is difficult to differentiate effects on establishment from those against an existing adult burden. *In vivo* studies with young farmed deer have shown that there is a significant negative linear relationship between abomasal nematode burden and dietary CT concentration (Hoskin *et al.*, 2000). Research on the effects of chicory, quebracho (8% w/w) and sulla containing 35g CT /kg DM (Barry *et al.*, 2002) on total helminth parasites shows that these forages reduce the establishment of adult abomasal nematode parasites in sheep when compared to control groups (Marley *et al.*, 2003; Athanasiadou *et al.*, 2001). More specifically, research with *Lotus pedunculatus*, high in condensed tannins, shows a reduced establishment of *Teladorsagia circumcincta* worms (Niezen *et al.*, 1998b).

8. Conclusions

Willow (*Salix* spp.) trees, exotic to New Zealand, have been planted on farms for more than 160 years in New Zealand for soil conservation purposes, shade and shelter for animal welfare and recently as supplementary feed for livestock. In New Zealand, willow trees have been used as a source of alternative supplementary fodder for sheep and cattle, during summer/autumn drought, when there are feed shortages, providing palatable and nutritious foliage that can be fed to sheep as a supplement during dry summers. Farmers consider tree fodder as a useful or valuable feed, successfully integrating tree fodder into beef, sheep and deer farming systems.

There are three different methods of tree fodder supplementation. Firstly, mature willow trees can be mechanically trimmed carried and fed as a supplement to grazing livestock. Secondly, they can be used by coppicing trees densely planted for forage bank purposes, cutting and then feeding them as supplements to grazing animals. Thirdly, densely planted tree blocks can be grazed by livestock as browse or fodder blocks, with the animal doing the harvesting. The annual edible DM yield of coppiced fodder trees is between 1-4 t DM/ha per year for willow.

Condensed tannins (CT) play a significant role in the nutrition of ruminant animals and cause both adverse and beneficial effects on nutritional quality and animal health and production. They have antimicrobial and antifungal properties and also stimulate salivary flow in animals. However, the major benefit of CT to ruminant livestock feeding is that they bind with dietary proteins during mastication and protect the protein from microbial degradation in the rumen, making more protein available for

digestion and utilization in the abomasum and small intestine. The ideal concentration of CT in temperate forage legumes (such as *Lotus corniculatus*), ranges from 20 to 45 g/kg DM, reduces rumen forage protein degradation and increases EAA absorption from the small intestine, resulting in increased milk production, wool growth, ovulation rate, and lambing %. The effects of CT on forage digestion depends on the molecular weight, structure and reactivity of the CT. Higher concentrations of CT (i.e. 100g/Kg DM) depress VFI.

Traditionally, lambs were treated with anthelmintics when signs of parasitism were seen or expected and this involved treatment during summer and autumn. However, higher production gains could be achieved with the implementation of a preventive approach to control parasitism in grazing lambs, which consisted of drenching on 5 occasions at 28 day intervals commencing at weaning in November-December. Additional treatments were given as determined on the basis of FECs of the flock.

Despite the risk of developing anthelmintic resistance, farmers drenched adult sheep at an average of 1–2 times and lambs almost 6 times each year. However, this level of drench frequency has been considered to be an important contributing factor for the increase in genetically resistant worms in New Zealand, which lead to treatment failure. This has resulted in anthelmintic resistance being the biggest challenge for Parasitologists in New Zealand. To date, no commercially successful nematode vaccine has been developed.

In growing animals, nutrients are partitioned between physiological functions according to their priorities, which vary depending on the acquisition phase and expression of immunity. Nutritional costs of nematode infections in the gastrointestinal tract in animal production have been well described. Extra nutrients are used to replace the losses of endogenous proteins into the alimentary tract, supporting an increased gastro intestinal protein metabolism which is associated with the responses to parasitism, leading to reduced carcass protein deposition and to reduced body growth.

However, there are still no clear values to determine net protein loss for farmers to determine how much extra nutrient needs to be supplied to these parasitized animals. Research has shown that an increase in highly metabolisable protein content in the diet of growing lambs may improve their resistance and resilience to gastrointestinal nematodes.

Alternative anthelmintic control methods in animal production systems are growing in importance worldwide. Certain forages containing CT (and other secondary compounds) such as sulla, chicory, lotus and quebracho have an effect on nematode gastrointestinal parasites and have been shown to improve animal performance. Several *in vitro* studies have been conducted to asses the efficacy of CT-containing forages as sustainable alternative nematode control methods. However, fewer *in vivo* studies have been conducted to date, resulting in strong discrepancies amongst the apparent efficacy of these forages. Grazing management, biological and chemical anthelmintic alternatives have become relevant using an integrated approach to combating anthelmintic resistance worldwide.

Studies on the nematode-nutrition interaction are plentiful; however, the possible mode of action of CT-containing forages is still unknown. Some research suggests that CT-containing forages can reduce the motility of infective L_3 larvae, disrupt the development of eggs or reduce worm burdens.

Forage willow is a good source of CT (45-50g/kg DM). Evaluation of grazing willow fodder blocks as an alternative to anthelmintic drenching needs to be carried out, to evaluate if such a system can give a degree of parasite control whilst also increasing the rate of growth in weaned lambs.

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CHAPTER 2

EXPERIMENTAL CHAPTER

1. Introduction

Sheep were first introduced into New Zealand from Australia and England and are an integral part of the country's meat and wool export industry. Sheep production is based on year round grazing of perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pastures that have high organic matter digestibility (OMD; 0.60-0.80), and are high in metabolisable energy (ME; 8.8-11.7MJ/Kg DM) and crude protein (CP; 155-220 g/Kg DM; Waghorn and Barry, 1987). These pastures support low cost, efficient production of meat and wool. However, almost 200 years after the introduction of sheep to New Zealand, gastrointestinal nematodes are still causing clinical and sub-clinical diseases, resulting in economic losses in sheep livestock production systems (Vlassoff and McKenna, 1994; Vlassoff *et al.*, 2001).

However, control of gastrointestinal nematodes almost inevitably requires use of anthelmintics. In 1999, 53% of expenditure for animal remedies worldwide was for anthelmintics (Coles, 2002). Anthelmintic resistance has become a major worldwide problem (Waller, 1985; Leathwick, 2004). The continuous use of the same anthelmintics and increased administration frequency, are key factors that have lead to the development of resistance to some anthelmintics (Waller, 1985). Furthermore, the pharmaceutical industry is struggling to find new efficient anthelmintics (Waller, 1985; Terrill *et al.*, 2001; Leathwick, 2004).

Willow (*Salix* spp.) trees have been cultivated for more than 160 years in New Zealand to control soil erosion, used as an animal welfare resource (shade and shelter) and as supplementary feed for livestock (Van Kraayenoord, 1995; Wilkinson, 1999). Willows contain high concentrations of secondary compounds, particularly condensed tannins (CT; McWilliam *et al.*, 2005a, b; Thi Mui *et al.*, 2005). Condensed tannins have the potential to help control parasite infections and to reduce dag formation (accumulation of faeces on the wool surrounding the anus; Leathwick and Atkinson, 1995), which could potentially lead to a reduced use of anthelmintics (Niezen *et al.*, 1995; 1998b). The aim of this study is to evaluate if grazing on willow fodder blocks can be used to sustainably control internal parasites in lambs, whilst also stimulating lamb growth rates.

2. Materials and Methods

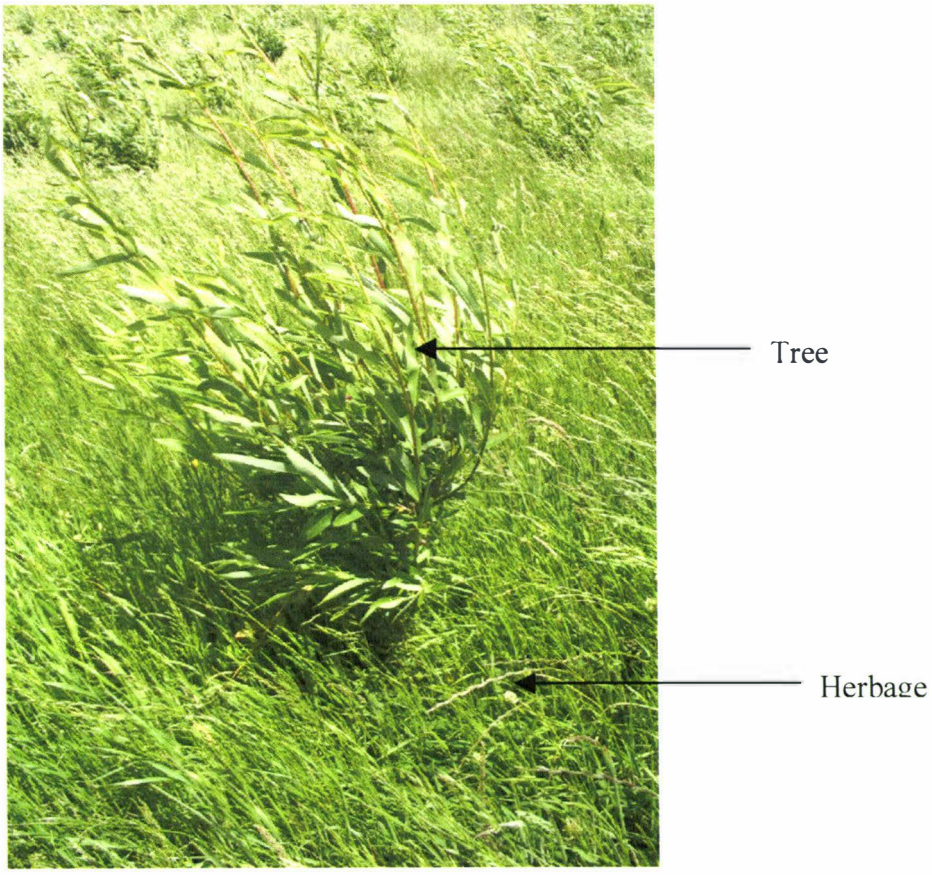
2.1 *Experimental Design*

A rotational grazing experiment was conducted over a 14-week period from 6 December 2004 to 6 April 2005, (i.e., summer/autumn) at Massey University's Riverside farm, near Masterton, New Zealand, on the East Coast of the lower North Island. The experimental areas were grazed in order to compare the efficacy of using willow fodder blocks containing condensed tannins for sustainable control of internal parasites in grazing lambs, relative to control lambs grazing perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. Six groups, each of 30 lambs were allocated the same dry matter (DM) allowance each break, which increased as the experiment progressed. The mean daily allowance was 4.9 Kg DM/lamb/day, with minimum values of 4.0 (when the experiment commenced) and maximum values of 7.0 Kg DM/lamb/day (as the experiment concluded). One hundred and eighty weaned Suffolk x Romney lambs were randomly allocated to six groups, being: Treatment 1 was the "Control Pasture" group (non-CT containing); lambs grazed perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture only. Lambs in Treatment 2 were given restricted access to willow fodder blocks i.e., lambs grazed pasture for 3 weeks followed by access to willow fodder blocks for 1 week (i.e. lambs did not have access to outside pasture during that week) and the rotation then repeated ("Restricted Access"). Treatment 3 was "Full Access" to willow fodder blocks (i.e., lambs were grazed on willow fodder blocks) for the duration of the experiment. The three forage treatments were further divided into undrenched and drenched lambs every 4 weeks, with each group grazing separate areas.

The feed in the willow fodder blocks consisted of trees and herbage grown underneath the trees and was managed as a pasture/tree association (Plate 1). An additional group of 12 lambs was slaughtered at the start of the experiment, to predict initial carcass weight.

The most important measurements were considered to be liveweight gain (LWG) and carcass weight gain (CWG) of the lambs, and identification of worm nematode numbers effectively present at the end of the experiment.

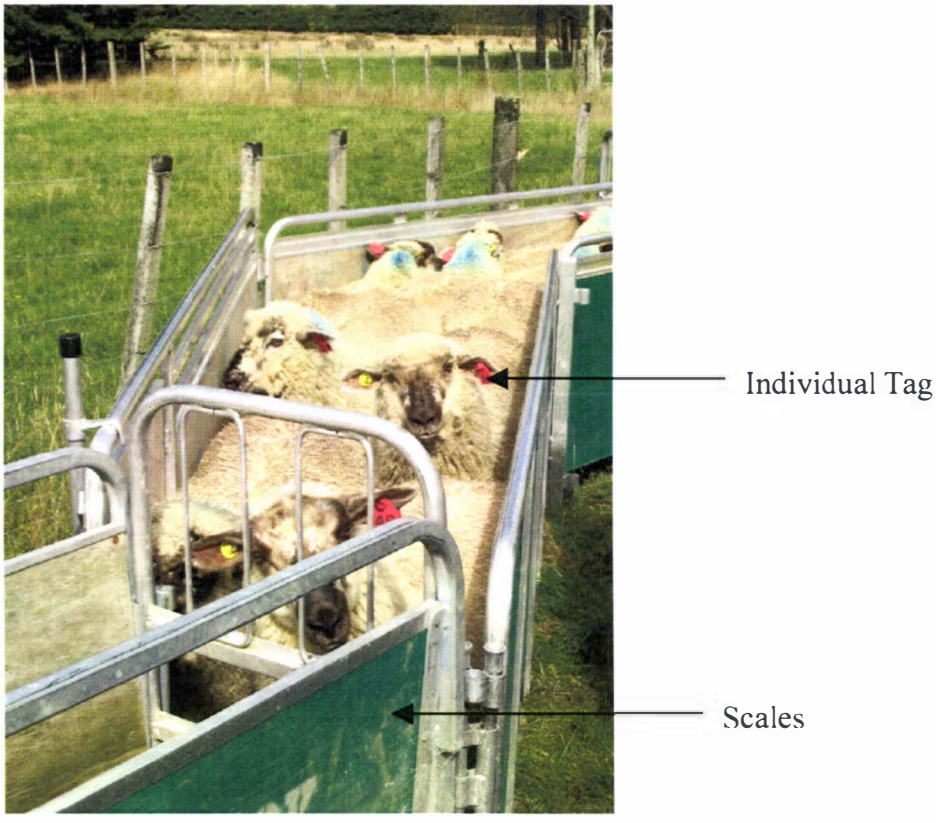
Plate 1: Willow fodder block herbage/tree association



2.2 *Animals*

Weaned Suffolk x Romney lambs of similar age, size and live weight (LW) were randomly assigned to the six treatment groups, individually tagged and weighed to ensure that the initial average LW of each group was similar (Plate 2).

Plate 2: Lambs individually tagged and weighed on scales



Rectal faecal samples for faecal egg counts (FEC) and larval counts (LC) and visual dag formation (Dag Score; DS) were assessed initially and at regular intervals throughout the experiment. All lambs were slaughtered at the end of the experiment and gastrointestinal digestive organs were collected from subgroups in the three undrenched treatments to determine total worm burdens.

From the 12 lambs in the initial slaughter group, carcass weight (CW) was related to LW by equation (1).

(1) $CW = -2.69 + 0.54\,LW$ $\qquad R^2 = 0.742$

S.E. = 2.876 0.101

NS ***

Equation (1) was then used to predict initial CW of the 180 experimental lambs from their initial LW.

2.3 *Pasture and willow fodder block management*

Pasture was rotationally grazed in 14 breaks (i.e. areas) each lasting 7 days in control pasture and 7 to 11 days in the fodder blocks, using front and back semi permanent electric fences (Plate 3). Treatment groups 1 and 2 were moved to each new break on the same day. Breaks 1 to 6 were primary growth and breaks 7 until 14 were secondary growth. Treatment 3 was moved to each new break based on the number of days needed to eat the feed available in each area. Water was provided *ad libitum* to all groups from portable water troughs.

The break area (ha) for each pasture break was calculated as:

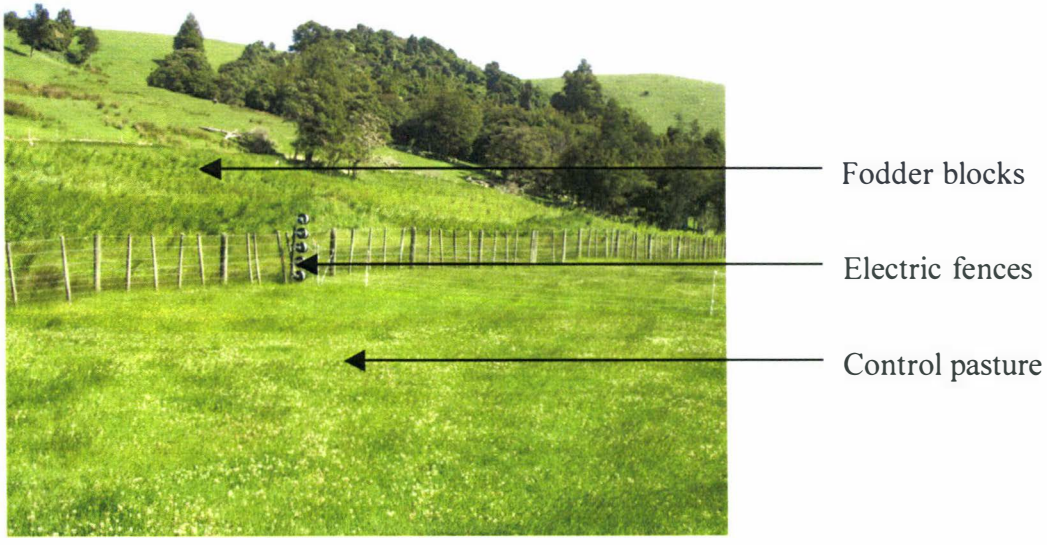
$$BA = (n \times DM\ allowance \times DB) / PPM$$

Where: BA is the break area in ha, n is the number of lambs, DM allowance in kg DM/lamb/day, DB is the total grazing days for each break, and PPM is the pre-grazing pasture mass in kg DM/ha. In the case of lambs grazing willow fodder blocks,

DM allowance used in the formula refers to the combination of both trees and herbage growing in the willow fodder blocks.

Five willow fodder blocks were established on Riverside Farm in 2000 and 2001, each with 6,000 trees/ha spaced 1.2 metres apart, in areas previously identified as rush-infested swamps and low-lying wet areas not suitable for grazing livestock production (Plate 3). The cultivars planted were *Salix matsudana* Koidz. × *alba* L. (hybrid willow) clone 'Tangoio' (NZ 1040), a drought–tolerant, hybrid willow developed in New Zealand, and *Salix matsudana* Koidz. × *alba* L. clone 'Moutere' (NZ 1184). Fodder blocks were established by planting unrooted willow stakes (0.7 m long) with 0.35 m below the surface. Further details of willow fodder block establishment were given by Pitta *et al.* (2005a, b).

Plate 3: General view of fodder blocks/electric fences /control pasture



Pasture and fodder blocks grazed in the experiment were previously contaminated with sheep parasites by grazing with undrenched ewe hoggets during winter (May-July) and lambing ewes in spring (August-November) for two consecutive years.

Non-experimental livestock were excluded from all fodder blocks in early October (Spring) 2004, since tree growth started in early spring. Vegetation present in the fodder blocks consisted of tree growth (approximately 1.0-1.2 metres tall) and herbage comprising grass, with legumes and herbs in the base.

2.4 *Forage measurements*

2.4.1 *Pasture*

Pre-grazing and post-grazing herbage mass were determined for the six groups before and after lambs grazed each break, by cutting 8 random quadrats ($0.180m^2$) samples per break to soil level, washing, and then drying the herbage overnight for 16 to 20 hours in a forced-air oven (Contherm; Thermotec 2000; Petone, New Zealand) at 80°C. Six wire-mesh exclusion cages measuring approximately 1.0 × 0.5 × 0.5 m were placed in each break including willow fodder blocks (Plate 4), immediately before the lambs were introduced for grazing.

Plate 4: Pasture cages for diet selected

At the end of grazing each break, the cages were removed and the forage was hand-plucked corresponding to the simulation of what lambs were observed to have eaten (diet selected). Diet selected samples were stored at -20°C for subsequent chemical composition determination. Representative samples of pasture diet selected were collected before and after grazing each break for dissection into green and dead matter content. Green matter was further dissected into legumes, herbs and grasses.

2.4.2 *Willow*

Willow herbage mass was estimated before grazing each break by cutting 4 trees/break, selected at random to stump level, cutting the material into approximately 2 cm lengths and drying. Willow material remaining after grazing was similarly estimated. Four rounded exclusion cages (2m height × 0.7 m diameter) per break/group were placed around individual trees, for both willow treatments. At the end of grazing each break, samples were collected that corresponded to the willow diet selected (Plate 5) by the grazing lambs and pooled per break (Plate 6; Plate 7).

Plate 5: Willow cages for diet selected

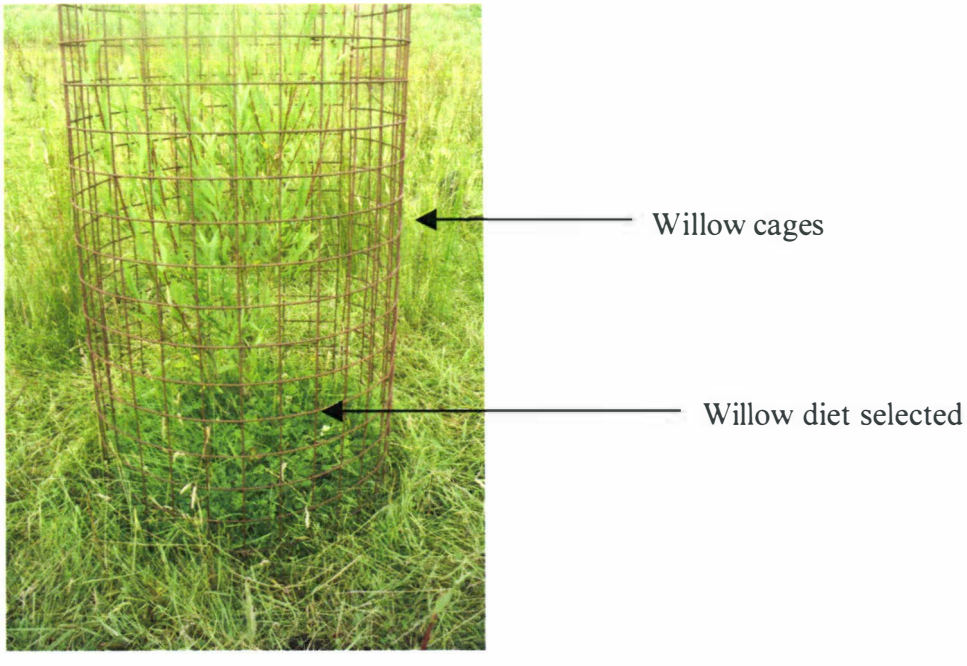


Plate 6: Willow fodder block before grazing

Plate 7: Willow fodder block after grazing

Representative samples were cut into 2 cm lengths and were stored at -20°C for nutrient analysis. DM percentage was estimated from the diet selected samples taken from each break. Diameter of the willow residue eaten was also determined for each treatment after grazing each break, using electronic callipers, with 150 measurements made per plot (75 measurements for leader shoots and 75 measurements for basal shoots).

2.5 *Animal and parasitological measurements*

Lambs were weighed using electronic scales (Tru-test, Auckland, New Zealand) and were evaluated for DS on a scale of 1–5 (1= no dags, 5= highest incidence of dags), at the start of the experiment and at 14 day intervals.

Half of the undrenched lambs in each group (n=15) were monitored for FEC at the start of the experiment and fortnightly thereafter and LC were performed monthly. For animal welfare reasons, the criteria for trigger drenching lambs in the undrenched groups was set when the FEC geometric mean of the group exceeded 1000 eggs/g wet faeces and/or LWG was reduced to zero and/or any one individual lamb exceeded 2500 eggs/g wet faeces.

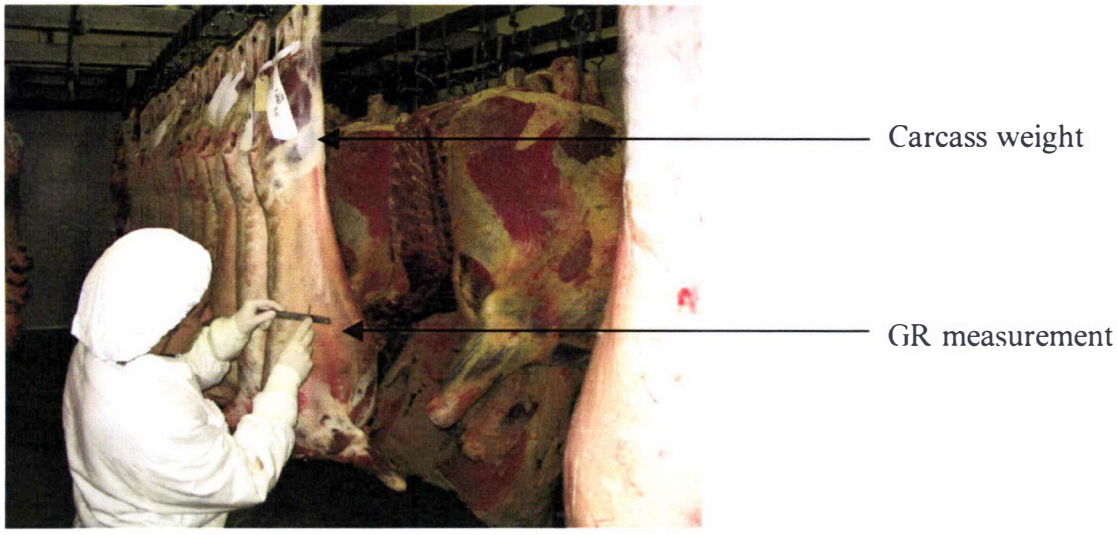
None of the above scenarios developed in the undrenched groups until 2 weeks before slaughter; therefore, the undrenched treatments were never trigger drenched due to meat withholding period of the anthelmintic. All lambs were drenched at the start of the experiment and drenched lambs were drenched at monthly intervals with "Erase MPC plus Scanda" (Coopers®, Schering-Plough, Upper Hutt, New Zealand), a combination of ivermectin, albendazole and levamisole anthelmintics.

A triple combination of anthelmintics was used, due to past records of sheep anthelmintic resistance on the farm. Half of the drenched lambs (n =15 per group) were monitored for FEC and LC at the start of the experiment and two weeks later to monitor the effectiveness of the anthelmintic. Thereafter, they were monitored and sampled for FEC and LC at monthly intervals before drenching ocurred. At all times during the experiment, drenched and undrenched groups were grazed in separate areas (Plate 8) and the same lambs were sampled on each occasion.

Plate 8: Drenched and undrenched lambs grazing separate areas

At the end of the experiment, all lambs were weighed on the farm and slaughtered in a commercial abattoir. The GR method was used to measure subcutaneous fat depth over the 12th rib at a point of 11 cm from the dorsal midline towards the lateral flanks (Kirton, 1989) and carcass weight was recorded for all lambs (Plate 9).

Plate 9: Post-mortem measurements of GR and carcass weight



The abomasum, small intestine and large intestine were collected from a representative random selection of 10 of the 15 lambs that had been sampled for FEC in each undrenched group. No worm counts were performed in drenched animals due to financial constrains. These were dissected and individually identified (Plate 10), tied and stored in bags at -20°C. Subsequently, they were processed at Massey University Parasitology Laboratory for estimation of total worm nematode numbers.

Plate 10: Dissection of gastrointestinal tract

2.6 *Laboratory Analyses*

Willow and pasture samples of diet selected were stored at -20°C, freeze-dried using a Cuddon 0610 freeze drier (W.G.G. Cuddon Ltd., Blenheim, New Zealand), and ground to pass a 1mm diameter sieve (Wiley mill, Swedesboro, USA). Total N concentration was determined using the Dumas method (Leco CNS 2000 analyser, Model 602 600 200, USA). *In vitro* organic matter digestibility (OMD) was determined by the Roughan-Holland (1977) enzymatic method, using separate standard curves prepared from *in vivo* values for forages and willow fed to sheep. Metabolisable energy (ME) content in the diet select samples was calculated as 16.3 x *in vitro* digestible organic matter/ 100g DM (DOMD; Drew and Fennessy, 1980).

Willow and fodder block pasture samples were analysed for acetone/water-extractable, protein-bound and fibre-bound CT fractions, using the butanol-HCL colorimetric procedure (Terrill *et al.*, 1992b) and total CT concentration was calculated by adding the three fractions. CT content of restricted access while in pasture were not analysed because they were estimated to be no different from CT present in control pasture. All condensed tannin concentrations were determined using CT extracted from *Lotus pedunculatus* as a reference standard (Jackson *et al.*, 1996).

Rectal faecal samples were collected and refrigerated (4°C) and nematode eggs per gram (epg) of wet faeces was determined using a modified McMaster technique (Stafford *et al.*, 1994) with a precision of 1 counted egg : 50 epg. (see Appendix 1). Larval cultures were prepared from pooled faecal samples for each treatment, mixed with vermiculite and water, and cultured at 25°C for 10 days (see Appendix 2a). Larvae were measured using a Baermann technique (see Appendix 2b) (Ministry of

Agriculture, Fisheries and Food, 1986). Larvae were identified (see Appendix 2e) and counted (see Appendix 2c) on a slide under a binocular microscope with the previous preheat and fixation with Lugol's solution (see Appendix 2d). Worm burdens were estimated from 10% aliquots (Wood *et al.*, 1995) from the small intestine (see Appendix 4c) and abomasums (see Appendix 4a-b) and 100% counts were obtained from the large intestine (see Appendix 4d). While counting the worms, 50 males were extracted from each genus in order to speciate them after the count was finished.

2.7 *Statistical Analysis*

All animal data was analysed using individual animals as the replicate. For logistical reasons it was not possible to use areas of land as replicates. Whilst it is realised that this results in confounding between nutritional treatments and areas of land, it is believed not to have altered the conclusions that can be drawn from the present study.

Mean and standard error of pre-grazing, post-grazing mass and chemical composition of the diet select by lambs in each of the treatments were obtained from analysis of variance (ANOVA) in the MIXED procedure of SAS (2003), fitting a linear model that considered the effects of treatment.

Regressions for change in diameter chewdown of willow diet selected with time, GR adjusted by CW and LW-CW correlation equations were estimated by analysis of variance (ANOVA) using the PROC GLM procedure of SAS (2003).

There was no replication of treatment and individual animals were each considered to be an experimental unit. Lamb LW and DS were analysed by analysis of variance

(ANOVA) using the MIXED procedure of SAS (2003). The linear model included the fixed effects of day, forage type, drenching method, and the interactions feed-drench, feed-day, drench-day and feed-drench-day and random effect of lamb within each combination of forage-drenching group.

Using the Akaike's information criterion, an unstructured error was determined as the most appropriate residual covariance structure for repeated measures over time within lambs.

Daily LW change considering the whole experimental period was analysed using a linear model that considered the fixed effects of forage type, drenching method and the interaction feed-drench. Days from initial to final LW was included as a covariable in the model because each lamb were slaughtered at different times for carcass measurements.

Lamb CW, CW gain and carcass yield were analysed with analysis of variance (ANOVA) using the MIXED procedure of SAS (2003). The linear model included the fixed effects of forage type, drenching method, the interactions feed-drench and random effect of lamb within each combination of forage-drenching group. The standard deviation of the model was not homogenous, therefore, these were assumed different between combinations of feed and drench. There was a linear relationship between initial LW and CW, so initial LW and days were used as covariables. However, carcass yield was not affected by initial LW, thus initial LW and day were not considered as covariables for CW.

Flank GR measurements were analysed by analysis of variance (ANOVA) in the MIXED procedure of SAS (2003). The linear model included the fixed effects of forage type, drenching method, flank side, the interactions feed-drench, flank-feed-drench and random effect of lamb within each combination of forage-drenching group. Days in the experiment was considered as a covariable.

Before the analysis, FEC, LC (allocated to individual animal egg counts) were square root transformed to normalise their distribution. They were analysed by repeated measurement of analysis of variance (ANOVA) using the MIXED procedure of SAS (2003). The linear model included the fixed effects of day, forage type, drenching method, and the interactions feed-day, drench-day and feed-drench-day and random effect of lamb within each combination of forage-drenching group.

Total worm burdens were square root transformed to normalise their distribution and worm burdens classified by sex were arcsin square root transformed to analyse as proportions. They were analysed by analysis of variance (ANOVA) using the MIXED procedure of SAS (2003). The linear model (nested design) included the fixed effects of forage type, sex, gastrointestinal tract, worm species nested with gastrointestinal tract and the interaction feed-worm species nested with gastrointestinal tract and random effect of lamb within each combination of forage-drenching group. Male and female worm burdens were both included in the analysis for the completion of the data, however, it was assumed that male and female proportions showed the same differences per treatment.

3. Results

3.1 *Forages and botanical composition*

Primary growth pre-grazing and post-grazing herbage mass in willow fodder blocks (full and restricted access) was higher than that of control pasture. Secondary growth of pre and post-grazing herbage mass was lower than primary growth herbage mass in all pastures treatments (Tables 1-2). Secondary growth pre and post-grazing herbage mass was similar in willow fodder blocks and control pasture (Tables 1-2). Secondary growth yield of fodder trees in the full access to willow fodder block treatment was greater than primary growth (Table 1); both values were low relative to the mass of herbage growing in the fodder blocks.

Pre-grazing herbage dead matter content was consistently higher in secondary growth than in primary growth, for both control pasture and fodder blocks. Willow fodder block full access secondary growth dead matter content was lower than that of control pasture (Table 1). Legume content was substantial (approximately 20% white clover) in both primary and secondary growth control pasture. Primary growth legume content in willow fodder blocks was similar to that of control pasture, but in secondary growth herbage, the legume content in willow fodder blocks (*lotus pedunculatus*) was much greater than that of control pasture (Tables 1-2).

3.2 *Chemical composition*

Total N concentration of herbage in the diet selected samples of lambs grazing willow fodder blocks was slightly lower than control pasture (Tables 3-4).

Table 1:

Pre and post-grazing mass (kg DM/ha) and dead matter content of primary and secondary growth from control pasture and willow fodder block full access treatment grazed by drenched and undrenched lambs (mean values with standard error).

	Control pasture		Willow fodder block Full access[a]			
	Drenched	Undrenched	Drenched		Undrenched	
			Herbage	Trees	Herbage	Trees
Primary growth						
Pre-grazing mass[b]	4243 ± 249.1	4581 ± 304.0	5070 ± 256.8	562 ± 82.7	4893 ± 395.6	544 ± 58.0
Post-grazing mass[b]	2984 ± 120.5	3248 ± 207.7	3545 ± 375.1	262 ± 56.7	3575 ± 380.1	302 ± 37.8
Botanical Composition (%)[c]						
Dead Matter content	8.6 ± 1.60	7.8 ± 1.30	9.5 ± 1.25		10.3 ± 1.66	
Grasses[d]	58.9 ± 5.02	62.6 ± 4.98	49.5 ± 6.30		55.6 ± 5.70	
Legumes[d]	19.0 ± 3.35	22.9 ± 3.93	19.4 ± 4.35		16.8 ± 2.72	
Secondary growth						
Pre-grazing mass[e]	3701 ± 296.9	4391 ± 357.3	4213 ± 405.7	775 ± 83.4	4127 ± 292.3	815 ± 91.7
Post-grazing mass[e]	2868 ± 142.1	3175 ± 163.1	3169 ± 305.5	236 ± 34.9	3190 ± 169.3	276 ± 45.9
Botanical Composition (%)[c]						
Dead Matter content	40.1 ± 6.56	38.7 ± 3.97	23.7 ± 4.61		18.0 ± 3.37	
Grasses[d]	29.6 ±4.35	32.9 ± 1.88	30.1 ± 5.28		40.8 ± 5.89	
Legumes[d]	23.9 ± 2.72	21.9 ± 3.32	37.1 ± 6.22		29.0 ± 3.06	

[a] Lambs grazed on willow fodder block for the duration of the experiment (herbage + trees).
[b] $n = 8$ measurements per control treatment and $n = 6$ measurements per willow fodder block treatment.
[c] Percentage of total forage mass.
[d] Proportion of green matter content.
[e] $n = 6$ measurements per control treatment and $n = 5$ measurements per willow treatment.

Table 2:

Pre and post-grazing mass (kg DM/ha) and dead matter content of primary and secondary growth from willow fodder block restricted access treatment grazed by drenched and undrenched lambs (mean values with standard error).

	Willow fodder block Restricted access[a]					
	Access to pasture		Access to willow fodder block			
	Drenched	Undrenched	Drenched		Undrenched	
			Herbage	Trees	Herbage	Trees
Primary growth						
Pre-grazing mass[b]	4073 ± 218.0	4575 ± 294.7	4798 ± 134.7	683 ± 24.8	4537 ± 363.4	640 ± 55.0
Post-grazing mass[b]	2924 ± 130.9	2856 ± 225.1	3236 ± 252.2	335 ± 17.9	3182 ± 120.7	328 ± 15.7
Botanical Composition (%)[c]						
Dead Matter content	7.3 ± 0.96	7.7 ± 2.34	20.6 ± 11.07		20.4 ± 14.32	
Grasses[d]	71.9 ± 2.62	62.8 ± 6.89	43.9 ± 8.23		42.0 ± 10.64	
Legumes[d]	18.1 ± 3.46	26.4 ± 2.98	22.2 ± 9.44		33.2 ± 14.49	
Secondary growth						
Pre-grazing mass[e]	4164 ± 630.7	4444 ± 532.41				
Post-grazing mass[e]	2924 ± 344.5	3074 ± 221.3				
Botanical Composition (%)[c]						
Dead Matter content	34.0 ± 3.93	31.3 ± 4.25				
Grasses[d]	38.0 ± 3.96	34.0 ± 3.62				
Legumes[d]	21.4 ± 1.83	25.6 ± 1.04				

[a] Lambs grazed on pasture for three weeks followed by limited access of one week in willow fodder block (herbage + trees).
[b] $n = 6$ measurements when grazing pasture and $n = 3$ measurements when grazing willow fodder blocks.
[c] Percentage of total forage mass.
[d] Proportion of green matter content.
[e] $n = 5$ measurements when grazing pasture.

Table 3:

Chemical composition, nutritive value and condensed tannin concentration of pasture and willow diet selected (g/kg DM) by drenched and undrenched lambs grazing control pasture and willow fodder block full access (mean values with standard errors)[a].

	Control pasture		Willow fodder block Full access[b]				Pooled
	Drenched	Undrenched	Drenched		Undrenched		S.E.M.
			Herbage	Trees	Herbage	Trees	
Total N	28.1	27.7	24.7	16.4	24.3	17.2	0.99
OMD[c]	0.66	0.65	0.64	0.70	0.65	0.71	0.013
DOMD[d]	0.60	0.59	0.58	0.64	0.59	0.65	0.010
ME[e]	9.8	9.6	9.4	10.4	9.6	10.6	0.19
Total CT[fg]	6.4 ± 1.24	6.0 ± 2.25	12.6 ± 3.58	41.6 ± 10.41	14.7 ± 0.89	42.2 ± 9.36	

Pasture measurements made on hand plucked samples of diet selected. Willow tree measurements made on hand cut samples from trees (stem diameter < 5mm) of diet selected.

[a] $n = 7$ samples per treatment.

[b] Lambs grazed on willow fodder block for the duration of the experiment (herbage + trees).

[c] OMD: Organic matter digestibility *in vitro.*

[d] DOMD: Digestible organic matter in the dry matter *in vitro.*

[e] ME: Metabolisable energy (MJ/kg DM).

[fg] CT: Condensed tannins *in vitro*; n = 3 samples per control treatment and n = 4 samples per willow fodder block treatment.

Table 4:

Chemical composition, nutritive value and condensed tannin concentration of the pasture and willow diet selected (g/kg DM) by drenched and undrenched lambs grazing willow fodder block restricted access (mean values with standard errors)[a]

	Willow fodder block Restricted Access[b]						Pooled S.E.M
	Access to pasture		Access to willow fodder block				
	Drenched	Undrenched	Drenched		Undrenched		
			Herbage	Trees	Herbage	Trees	
Total N	27.0	27.3	22.5	15.4	24.8	15.4	0.94
OMD[c]	0.65	0.66	0.64	0.71	0.66	0.72	0.016
DOMD[d]	0.59	0.60	0.58	0.66	0.60	0.66	0.013
ME[e]	9.6	9.8	9.5	10.7	9.7	10.8	0.23
Total CT[fg]			14.9 ± 6.52	51.6 ± 7.86	14.7 ± 4.92	52.0 ± 6.78	

Pasture measurements made on hand plucked samples of diet selected. Willow tree measurements made on hand cut samples from trees (stem diameter < 5mm) of diet selected.

[a] *n* =3 measurements when grazing willow fodder block and *n* = 5 measurements when grazing pasture.

[b] Lambs grazed on pasture for three weeks followed by limited access of one week willow fodder block (herbage + trees).

[c] OMD: Organic matter digestibility *in vitro*;

[d] DOMD: Digestible organic matter in the dry matter *in vitro.*

[e] ME: Metabolisable energy (MJ/kg DM).

[fg] CT: Condensed tannins *in vitro*; n = 3 samples per treatment when access to willow fodder block.

Herbage selected in willow fodder blocks was similar to control pasture, in OMD and ME (0.65; 9.7 MJ/kg DM respectively). However, the selected tree browse in the willow fodder blocks had a higher OMD of approximately 0.71 and was higher in ME concentrations (10.7 MJ/kg DM; Tables 3-4) than herbage diet selected. Herbage CT concentration in willow fodder blocks was consistently higher than the trace CT levels detected in the control pasture diet selected (14.5 g/kg DM versus 6.2 g/kg DM respectively; Tables 3-4). Trees in willow fodder blocks were particularly different from the rest of the herbage in the treatments due to their higher concentration of CT (approximately 45.5 g/kg DM; Tables 3-4).

As an overall *in vitro* analysis, OMD, DOMD and ME concentrations were all higher for selected tree fodder in willow fodder blocks than herbage selected in either willow fodder blocks or control pasture. Diameter (D; mm) chewdown of leader shoots in samples of willow selected by full access drenched and undrenched (Equation 2) treatments linearly increased with time (t; days) and individual regressions were not significantly different (Fig.1). Diameter chewdown of basal shoots in willow fodder block full access undrenched and drenched treatments (Equation 3) increased during the experiment and individual regressions were not significantly different (Fig.1).

(2) $D = 3.065 + 0.013\ t \qquad R^2 = 0.6660$

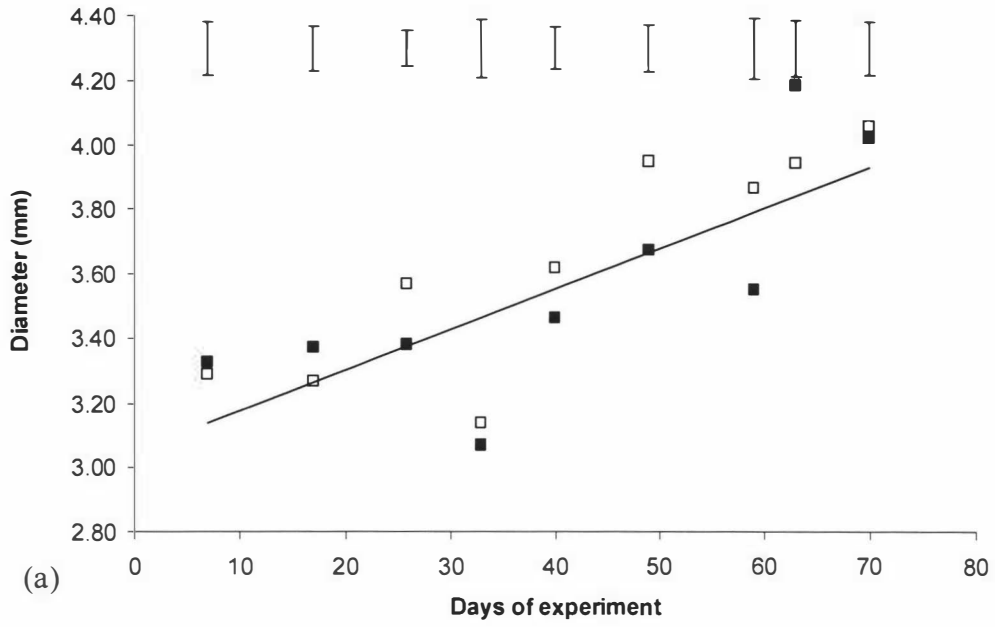
S.E.= 0.1051 0.0023

*** ***

(3) $D = 2.121 + 0.005\ t \qquad R^2 = 0.5510$

S.E. = 0.0512 0.0011

*** ***



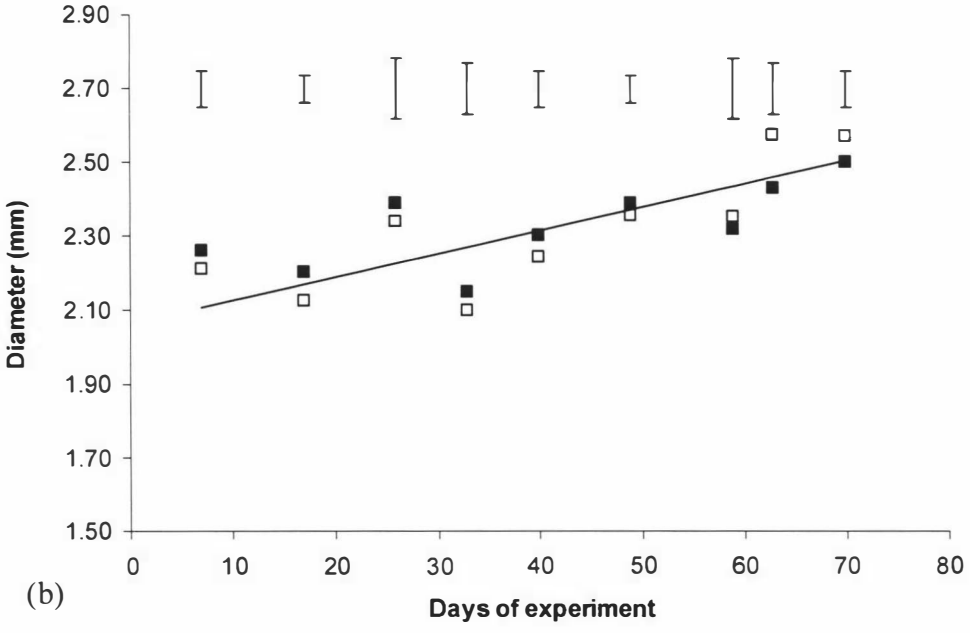


Figure 1: Change in (a) leader shoot diameter chewdown and (b) basal shoot diameter chewdown in samples of willow selected by drenched or undrenched lambs grazing willow fodder block (full access). (□) full access drenched; (■) full access undrenched. Vertical bars represent standard error of the mean.

3.3 *Live weight, liveweight gain, carcass weight and fatness values*

When expressed over the entire experiment, lamb growth generally followed trends of greatest for willow fodder block full access drenched, intermediate and similar for undrenched willow fodder block full access and drenched control pasture and least for the two groups grazing willow fodder block restricted access and the undrenched control pasture treatment.

All growth rates declined in the second half of the experiment as herbage nutritive value declined due to the hot and dry summer conditions, with the most pronounced decline being for undrenched lambs grazing willow fodder block restricted access and control pasture (Table 5); the decline was less for undrenched lambs with full access to willow fodder blocks.

Final LW for anthelmintic drenched lambs was significantly higher than undrenched lambs in the willow fodder block full access and control pasture groups ($p = 0.0005$, $p < 0.0001$ respectively), but was similar for both groups grazing willow fodder block with restricted access ($p = 0.0761$; Table 5). Full access willow fodder block drenched and undrenched groups had the highest final LW of all groups, whilst undrenched willow fodder block restricted access and undrenched control pasture groups had the lowest final LW. Undrenched lambs grazing willow fodder block with full access had similar final LW to drenched lambs grazing control pasture ($p = 0.7666$; Table 5).

Over the whole experiment, regularly drenched lambs had significantly higher LWG than undrenched lambs in all treatments ($p = 0.0199$). Lambs in willow fodder block full access had an increased LWG in drenched compared to undrenched lambs of 182 g/day versus 154 g/day ($p = 0.05$; Table 5).

During the first half of the experiment (days 1-42) most of the treatments had a similar gain of approximately 200 g/day, whereas in the second half of the experiment weight gain dropped due to drought conditions and differences between treatments became more prominent. Nevertheless, LWG of willow fodder block full access lambs had a much lower reduction compared to lambs grazing control pasture or willow fodder block with restricted access, particularly for undrenched lambs (Table 5).

Table 5:

Live weight and carcass characteristics of drenched and undrenched lambs grazing perennial ryegrass/white clover (control) and willow fodder blocks (full and restricted access)(mean values and pooled standard errors)[1]

	Control		Willow fodder block Full access[2]		Willow fodder block Restricted access[3]		Pooled S.E.M.
	Drenched	Undrenched	Drenched	Undrenched	Drenched	Undrenched	
Initial live weight (kg)	28.5	28.3	28.6	28.4	28.5	28.6	0.38
Final live weight (kg)	43.7 b	39.3 c	46.4 a	43.4 b	40.6 c	39.0 c	0.60
Live weight change (g/day)							
First half (days 1-42)	217	184	218	191	183	194	7.5
Second half (days 43-98)	116	56	158	129	80	42	7.2
Mean (days 1-98)	158 b	111 cd	182 a	154 b	123 c	107 d	4.7
Carcass weight (kg)	18.9 a	16.3 cd	18.3 b	16.9 c	17.7 b	16.0 d	0.24
Carcass weight gain (g/day)	58 a	34 cd	52 ab	40 c	46 b	31 d	2.4
Carcass yield (proportion)	0.41ab	0.42 a	0.42 a	0.43 a	0.42 a	0.40 b	0.042
GR (mm; right flank)	8.5 a	6.1 c	6.3 c	6.5 c	6.0 c	5.0 b	0.41
GR (mm; left flank)	7.8 a	5.9 c	5.9 c	6.1 c	6.2 c	5.0 b	0.41

Means within the same row with different letters (abcd) differ significantly ($p < 0.05$).

[1] $n = 30$ samples per treatment.

[2] Lambs grazed on willow fodder block for the duration of the experiment (herbage + trees).

[3] Lambs grazed on pasture for three weeks followed by limited access of one week to willow fodder blocks (herbage + trees).

There was a linear relationship between initial LW and CW 0.098 ± 0.014 ($p < 0.0001$; so initial live weight was used as a co-variate). CW and CWG were increased by regular anthelmintic drenching, with the effects being apparent in all 3 grazing treatments ($p < 0.0001$; Table 5).

The reduction in CWG of undrenched lambs compared to drenched lambs in the full access to willow fodder block group (12 g/day) was half of the reduction for the control pasture group (24 g/day; $p < 0.05$). CW was highest for drenched control pasture group, followed by drenched willow fodder block full access and drenched willow fodder block restricted access groups. Undrenched willow fodder block full access had the highest CWG amongst all undrenched treatments (Table 5).

Carcass yield was not affected by initial LW, thus initial LW was not considered as a covariable. Carcass yield was lower for undrenched than for drenched willow fodder block restricted access lambs ($p = 0.0084$), but otherwise was not affected by the treatments applied (Table 5).

When mean values of right (Equation 4) and left (Equation 5) flank GR (mm) measurements were adjusted by carcass weight (cw; kg), it showed a linear increasing correlation ($p < 0.05$; Fig. 2).

(4) $GR = -0.08 + 0.08\ cw$ $\qquad R^2 = 0.705$

S.E. = 0.456 0.026

NS *

(5) $GR = -0.06 + 0.07\ cw$ $\qquad R^2 = 0.740$

S.E. = 0.349 0.020

NS *

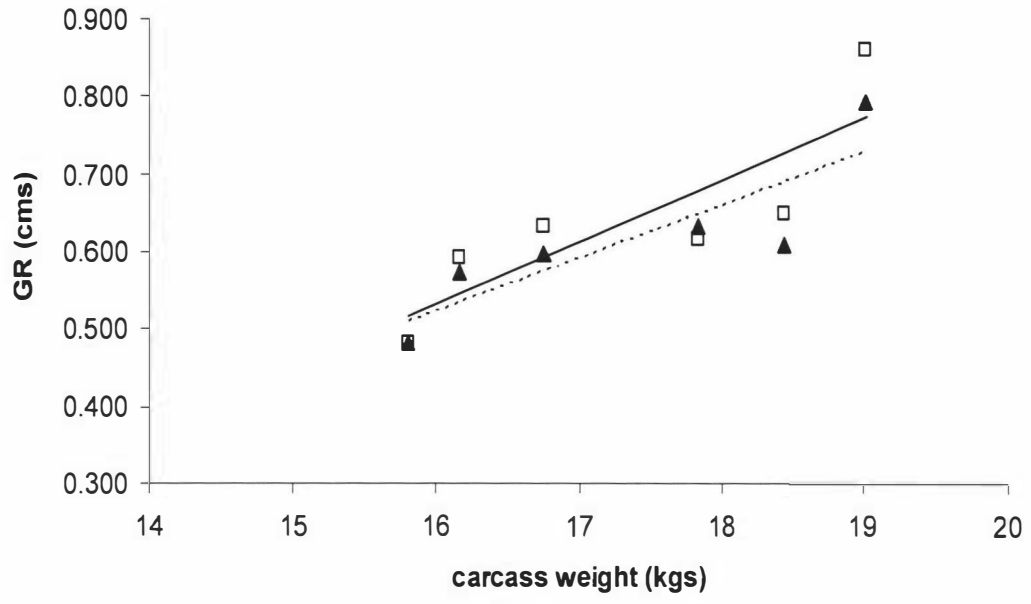


Figure 2: Mean values of right and left flank GR measurements adjusted by carcass weight. The solid line (—) indicates regression in left flank GR measurements. The broken line (--) indicates regression in right flank GR measurements. (▲) left flank GR measurement; (□) right flank GR measurement.

Carcass GR was significantly lower ($p = 0.001$) for undrenched lambs than for drenched lambs, when grazing either willow fodder block restricted access or control pasture (Table 5). However, for the willow fodder block full access treatment, there were no significant differences between drenched and undrenched lambs. Drenched lambs fed control pasture had the highest GR, followed by willow fodder block full access and the restricted access (Table 5).

3.4 *Dag score, faecal egg count and larval count*

For dag score, there was a significant effect of time ($p < 0.0001$) and a significant interaction between time and feed ($p = 0.0012$), as well as between time and drench ($p = 0.0007$). These effects are outlined as follows.

Dag score generally increased with time in all groups until Day 70 of the experiment (Fig. 3), with no differences between the six treatment groups. From Day 70 until the end of the experiment, mean DS significantly decreased ($p < 0.0001$) from 1.58 to 1.39 units. The control pasture group had the highest DS in the last 30 days, in both drenched and undrenched groups (Fig. 3). DS of lambs grazing willow fodder block full access were consistently lower ($p = 0.001$) than the DS of lambs grazing willow fodder block restricted access or control pasture. At the end of the experiment, willow fodder block full access undrenched lambs had a similar DS to control pasture drenched lambs.

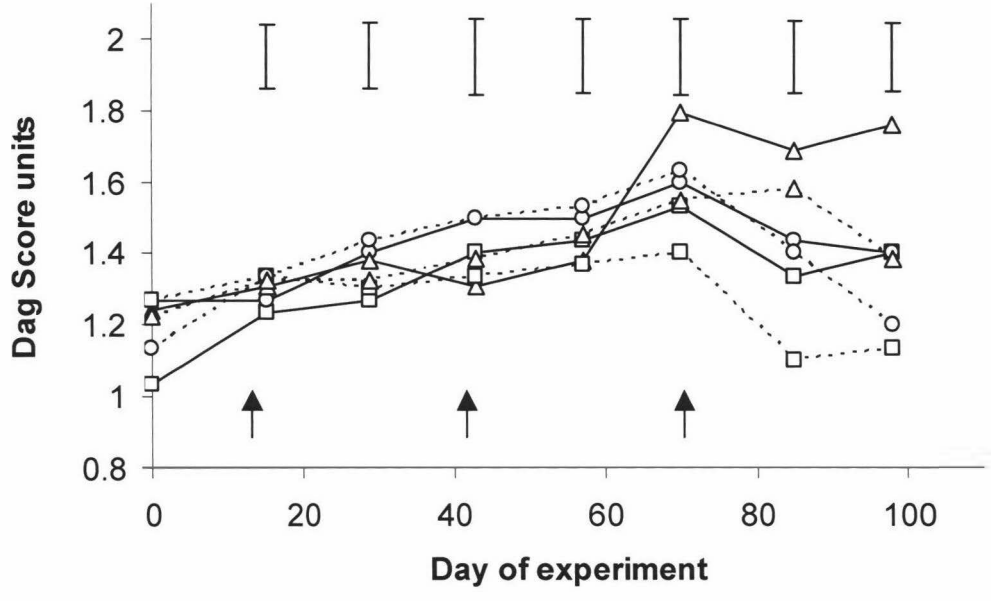


Figure 3: Changes in least square mean values of dag score units in drenched or undrenched groups grazing control pasture and willow fodder blocks (restricted and full access). The solid line (_) indicates undrenched lambs. The broken line (--) indicates drenched lambs. (△) control pasture; (□) full access; (O) restricted access. Vertical bars represent standard error of the mean. ▲ Indicates oral anthelmintic given.

Post-treatment 14 days FEC reduction test efficacy to confirm that the anthelmintic used was working was of 100%. Regular administration of oral anthelmintic maintained low FECs in the drenched groups (Fig. 4), whereas FECs of undrenched groups tended to increase with time and were significantly different from each other ($p < 0.0001$).

Although, FEC values of willow fodder block full access undrenched lambs were high at Day 42 of the experiment, they significantly decreased over time ($p < 0.0001$) until Day 70 when they started increasing again, although never reaching the highest levels obtained at Day 83 of the experiment by the control pasture undrenched group (approximately 1400 eggs/g.; Fig. 4).

Similar trends were observed in undrenched lambs grazing willow fodder block restricted access and undrenched lambs grazing control pasture, both having their lowest FECs at Day 56, significantly increasing to the maximum levels at Day 83 ($p < 0.0001$; Fig. 4). In the overall analysis, day of the experiment, type of forage, drench treatment and their interactions all had significant effects on FEC values.

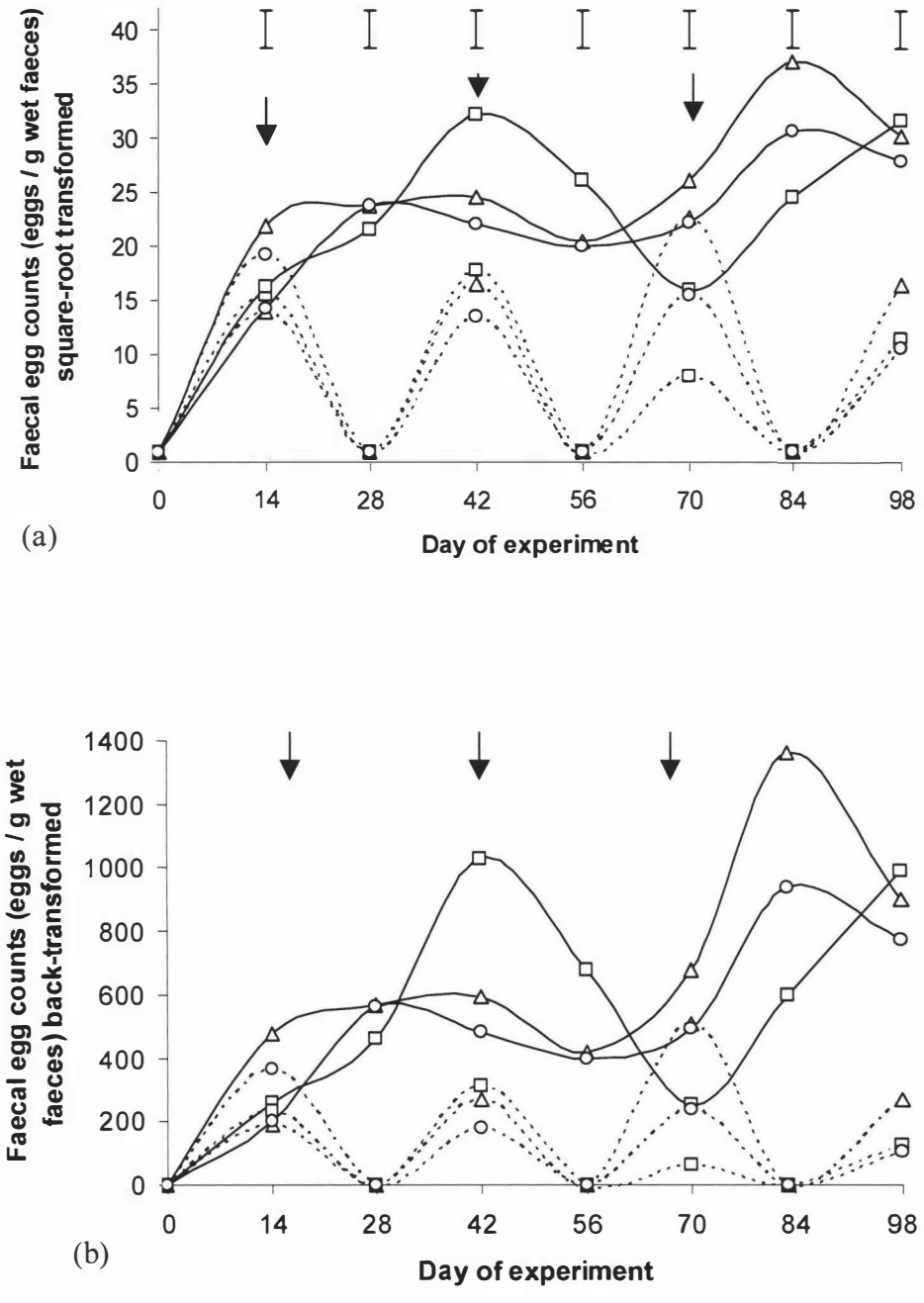


Figure 4: Changes in (a) least square mean values and (b) back-transformed square root values of FECs (eggs/g wet faeces) in drenched or undrenched groups grazing control pasture and willow fodder blocks (restricted and full access). The solid line indicates undrenched lambs. The broken line indicates drenched lambs. (△) control pasture; (□) full access; (O) restricted access. Vertical bars represent standard error of the mean. ↓ Indicates oral anthelmintic given.

The number of eggs allocated to different genera shown in Fig.5 changed significantly ($p = 0.0140$) with time (30, 60, 90 and 120 days). There were no obvious differences between drenched groups as they tended to stay similar and low over time for all treatments. Undrenched FECs allocated by genera tended to increase with time in all grazing treatments, with *Haemonchus*, *Cooperia* and *Trichostrongylus* being the main sources of the rise (Fig. 5).

As an overall analysis, undrenched control pasture-fed lambs had the highest *Cooperia*, *Teladorsagia* and *Trichostrongylus* FECs and the lowest *Chabertia* and *Oesophagostomun* faecal egg counts compared to the rest of the drenched and undrenched treatments throughout the entire experiment.

Undrenched lambs grazing willow fodder block full access tended to have the highest *Haemonchus*, *Chabertia* and *Oesophagostomun* FECs and the lowest *Trichostrongylus* faecal egg counts, whereas undrenched lambs grazing willow fodder block restricted access lambs had intermediate levels compared to other treatments at almost all times of the experiment (Fig. 5).

The number of *Cooperia* eggs increased throughout the experiment ($p< 0.05$). Day 120 had the highest levels in undrenched lambs grazing control pasture (Fig. 5).

Haemonchus eggs tended to significantly increase ($p < 0.0001$) over time in all undrenched groups, although there was a significant decrease at Day 90 in willow fodder block full access which increased again by Day 120 (Fig. 5). *Haemonchus* egg

counts were significantly higher in willow fodder block full access undrenched group than control pasture drenched and undrenched ($p < 0.001$).

Teladorsagia egg counts significantly increased until Day 60 of the experiment in almost all treatments, with the exception of willow fodder block full and restricted access drenched groups which decreased significantly ($p < 0.0001$; Fig. 5) compared to earlier times. After Day 60 *Teladorsagia* egg counts in all treatments tended to decrease (Fig. 5).

Trichostrongylus eggs increased significantly ($p < 0.0001$) for all undrenched treatments after Day 90, whilst drenched groups remained low. Control pasture undrenched had the highest counts at all times of the experiment except Day 60, where willow fodder block full access undrenched group had its peak (Fig. 5). Willow fodder block restricted access undrenched treatment significantly ($p < 0.0001$) increased through time.

Chabertia and *Oesophagostomun* egg counts increased with time in all undrenched groups with willow fodder block full access being the highest at all times, followed by willow fodder block restricted access and control pasture undrenched groups (Fig. 5).

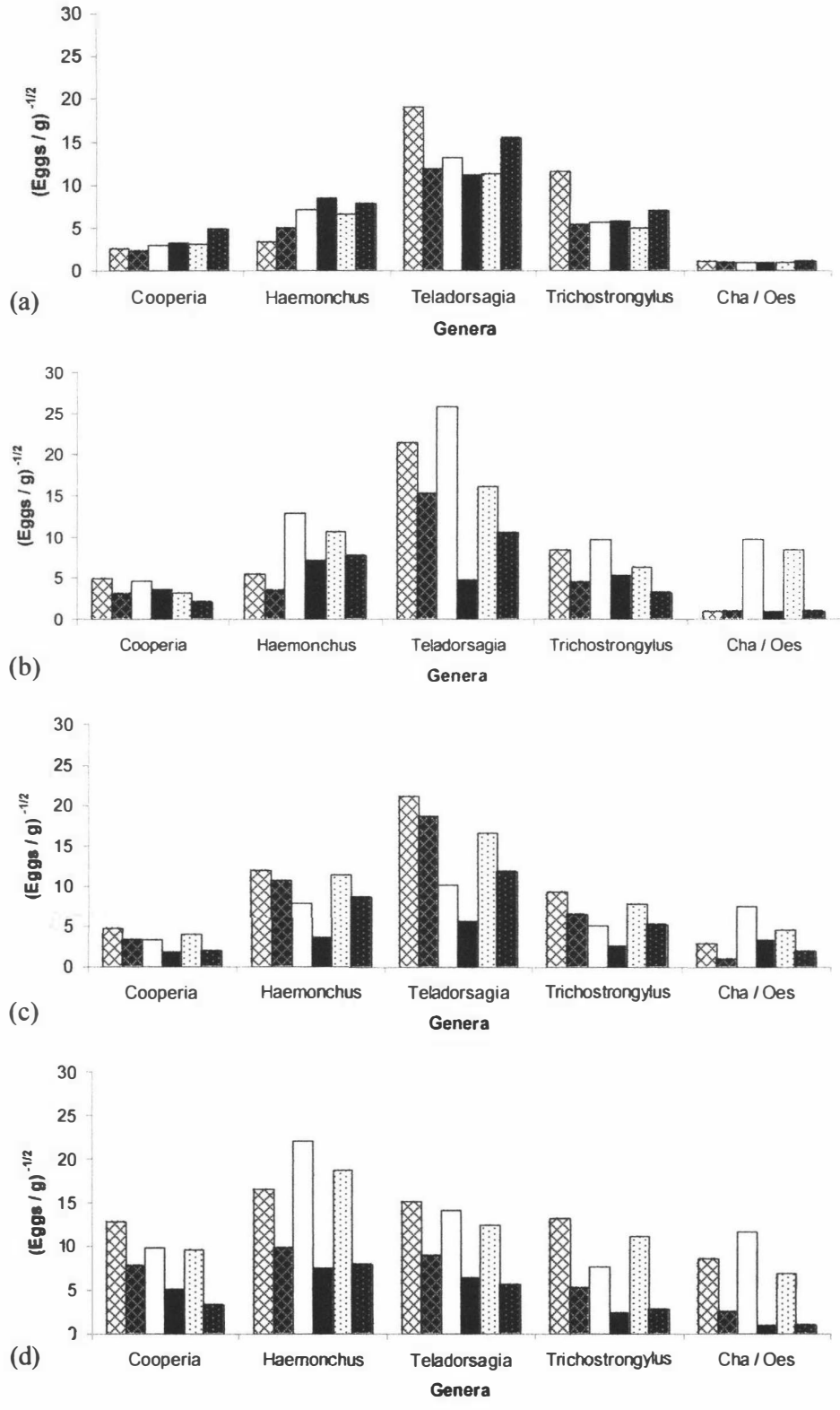


Figure 5: Comparative square root transformed means of FEC allocated to genera in drenched and undrenched lambs at 30 days (a), 60 days (b), 90 days (c) and 120 days (d). (◊) control undrenched; (♦) control drenched; (□) willow undrenched; (■) willow drenched; (▒) restricted undrenched; (▓) restricted drenched.

3.5 *Gastrointestinal nematode burdens*

The parasites established in the greatest numbers in undrenched lambs grazing control pasture were *Teladorsagia trifurcata*, *Teladorsagia circumcincta*, *Nematodirus spathiger*, *Trichostrongylus vitrinus and Trichostrongylus colubriformis* followed by *Trichostrongylus axei* (Table 6). Undrenched willow fodder block full access fed lambs had significantly lower ($p < 0.05$) *Nematodirus spathiger*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* worm burdens at slaughter than undrenched lambs grazing control pasture, but greater burdens of *Haemonchus contortus* ($p = 0.0299$; Table 6). They also had significantly lower *Nematodirus spathiger* and *Trichostrongylus vitrinus* ($p < 0.0001$; $p = 0.0371$ respectively) worm burdens than willow fodder block restricted access fed lambs, but significantly higher *Teladorsagia circumcincta* and *Teladorsagia trifurcata* worm burdens than restricted access ($p < 0.001$; $p = 0.0134$ respectively; Table 6).

Undrenched lambs grazing willow fodder blocks with restricted access had significantly ($p < 0.0001$) lower *Teladorsagia circumcincta*, *Teladorsagia trifurcata*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* worm burdens at slaughter than undrenched lambs grazing control pasture ($p < 0.05$; Table 6).

Trichostrongylus axei, *Nematodirus filicollis*, *Cooperia curticei*, *Cooperia oncophora*, *Chabertia ovina*, *Oesophagostomun venulosum* and *Trichuris* spp. showed no significant differences between treatments ($p > 0.05$; Table 6).

Table 6:

Square root transformed worm counts in undrenched groups grazing control pasture and willow fodder blocks (restricted and full access) over the summer/autumn season of 2005 on the East Coast of the North Island, New Zealand (mean values and pooled standard errors)[1]

	Control Pasture	Willow fodder block Restricted access[2]	Willow fodder block Full access[3]	Pooled S.E.M
Abomasum				
Haemonchus contortus	6.68 b	8.12 ab	16.79 a	1.174
Teladorsagia circumcincta	85.52 a	49.79 b	87.33 a	5.077
Teladorsagia trifurcata	24.23 a	11.29 b	23.10 a	1.690
Trichostrongylus axei	14.87 a	10.83 a	14.02 a	0.676
Small intestine				
Nematodirus spathiger	53.77 a	50.25 a	16.52 b	4.205
Nematodirus filicollis	9.87 a	12.44 a	6.41 a	1.039
Trichostrongylus vitrinus	62.97 a	34.38 b	24.68 c	3.119
Trichostrongylus colubriformis	31.22 a	15.26 b	16.99 b	1.498
Cooperia curticei	20.71 a	16.92 a	17.06 a	1.457
Cooperia oncophora	2.58 a	1.98 a	1.98 a	0.065
Large intestine				
Chabertia ovina	2.16 a	3.09 a	3.89 a	0.127
Oesophagostomun venulosum	6.40 a	7.46 a	6.24 a	0.277
Trichuris spp.	3.67 a	4.24 a	4.99 a	0.172

Means within the same row with different letters (abc) differ significantly ($p < 0.05$).

[1] $n = 10$ samples per treatment.

[2] Lambs grazed on pasture for three weeks followed by limited access of one week to willow fodder blocks (herbage + trees).

[3] Lambs grazed on willow fodder block for the duration of the experiment (herbage + trees).

There were consistently more female than male worms established for all parasite species except *Nematodirus* spp., which showed a significant lower proportion of females than males in all treatments ($p < 0.05$; Table 7). Generally different treatments had no significant effect on the proportion of males and females for abomasal and small intestine parasites ($p = 0.2669$; $p = 0.0001$ respectively; Table 7). However, the proportion of male *Chabertia ovina* in willow fodder block restricted and full access treatments was significantly ($p = 0.0326$; $p = 0.0268$ respectively) higher than the proportion of males in control pasture. The proportion of male *Trichuris* spp. worms in willow fodder block full access was significantly higher than the proportion in willow fodder block restricted access and control pasture treatments ($p < 0.05$; $p = 0.0304$ respectively; Table 7).

Table 7:

Arcsin square root transformed proportion of male and female worm counts in undrenched groups grazing control pasture and willow fodder blocks (restricted and full access) over the summer/autumn season of 2005 on the East Coast of the North Island, New Zealand (mean values and pooled standard errors)[1]

	Control pasture		Willow fodder block Restricted access[2]		Willow fodder block Full access[3]		Pooled S.E.M
	Male	Female	Male	Female	Male	Female	
Abomasum							
Haemonchus spp.	0.78 a	0.79	0.74 a	0.83	0.71 a	0.86	0.041
Teladorsagia spp.	0.54 a	1.03	0.51 a	1.06	0.61 a	0.96	0.034
Trichostrongylus axei	0.67 ab	0.90	0.52 b	1.05	0.68 a	0.89	0.033
Small Intestine							
Nematodirus spp.	0.97 a	0.60	0.88 a	0.69	0.83 a	0.74	0.027
Trichostrongylus spp.	0.66 a	0.91	0.62 a	0.95	0.69 a	0.88	0.025
Cooperia spp.	0.52 a	1.05	0.40 a	1.17	0.40 a	1.17	0.055
Large Intestine							
Chabertia ovina	0.38 b	1.19	0.61 a	0.96	0.62 a	0.95	0.058
Oesophagostomum venulosum	0.70 a	0.87	0.70 a	0.87	0.62 a	0.95	0.022
Trichuris ovis	0.53 b	1.05	0.55 b	1.02	0.76 a	0.81	0.032

Means within the same row with different letters (abc) differ significantly ($p < 0.05$).

[1] $n = 10$ samples per treatment.

[2] Lambs grazed on pasture for three weeks followed by limited access of one week to willow fodder blocks (herbage + trees).

[3] Lambs grazed on willow fodder block for the duration of the experiment (herbage + trees).

4. Discussion

The objectives of this study were firstly to evaluate if grazing on willow fodder blocks could be used to sustainably control internal parasites in weaned lambs and secondly to investigate if this could be achieved whilst also stimulating lamb growth rates. The most significant finding in this study was that undrenched lambs grazing on willow fodder block full access had lower worm burdens of *Nematodirus spathiger*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* at slaughter, but higher *Haemonchus contortus*, relative to undrenched lambs grazing control pasture and had increased in lamb growth rates compared to the rest of the undrenched treatments. Furthermore, undrenched lambs grazing on willow fodder block with restricted access had fewer *Teladorsagia circumcincta*, *Teladorsagia trifurcata*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* worm burdens relative to undrenched lambs grazing control pasture, but did not have increased lamb growth rates. The parasites reduced were those of economic importance that were present in the highest numbers in undrenched lambs grazing control pasture as defined by Gardner and Craig (1961).

The second most important finding is that, if anthelmintic is withdrawn there is a loss of productivity on all treatments; however, the loss on full access willow fodder block treatment (12g CWG/day) was half the loss of control pasture undrenched (24g CWG/day). Therefore, grazing full access on willow fodder blocks was successful in achieving the original objective of the study, whilst grazing willow fodder blocks with restricted access was partly successful.

The pick up and establishment of parasites had a large effect on animal productivity in this experiment, as shown by the reductions in LWG and CWG in all three undrenched groups relative to regularly drenched lambs. The most relevant comparison is considered to be between drenched lambs grazing control pasture and undrenched lambs grazing willow fodder blocks full access. From a productive point of view, as the two groups had similar LWG and final DS, this indicates that anthelmintic use can be reduced in lambs grazing fodder blocks but that this will incur some reduction in CWG.

Results from the current experiment can be compared with the results obtained by Ramírez-Restrepo *et al.* (2005; Table 8), that showed highest rates of both LWG and CWG for drenched lambs grazing *Lotus corniculatus*, a high quality forage legume, and similar lower growth rates, as found in this study, for drenched lambs grazing either control pastures or willow fodder blocks. Lamb growth generally followed the same trend over time for Ramírez-Restrepo *et al.* (2005) and for the current experiment, tending to decline as time passed due to severe drought conditions that lead to lower pasture nutritive values.

Withdrawal of anthelmintic drench dramatically reduced LWG for lambs grazing control pasture. Experimental evidence summarized by Ramírez-Restrepo and Barry (2005) also indicated that withdrawal of anthelmintic drench had least effect in reducing LWG for lambs grazing forages containing CT and other secondary compounds such as *Lotus corniculatus*, the legume sulla or the herb chicory; this may be the same observed in undrenched lambs grazing willow fodder block full access.

Table 8: The effect of grazing on willow fodder block full access, *Lotus corniculatus* or perennial ryegrass/white clover (control pasture) on liveweight change and carcass weight gain with regularly anthelmintic drenched lambs, in two Experiments conducted on Riverside Farm over the same season (summer) but in different years.

	Lotus corniculatus	Willow fodder block	Control pasture
Liveweight change (g/day)			
Ramirez-Restrepo *et al.*, 2005	298		200
Current experiment		182	158
Carcass weight gain (g/day)			
Ramirez-Restrepo *et al.*, 2005	133		66
Current experiment		52	58

The results summarised in Table 8 are in agreement with the generally higher feeding value of legumes than grasses (Waghorn and Barry, 1987). They also suggest that some grazing management changes may be needed if the growth of lambs grazing willow fodder blocks is to exceed that of lambs grazing control perennial ryegrass-based pastures.

The higher productivity of lambs grazing on willow fodder block full access could be explained due to the higher OMD, DOMD, ME and CT values present in the trees, together with the higher legume content of fodder block herbage and its higher CT content compared to control pasture (Table 9), resulting in improved efficiency of protein digestion (Waghorn *et al.*, 1987).

The CT-protein complex formed during the digestion of willows in the rumen may reduce protein degradation in the rumen, increasing EAA absorption from the small

intestine as occurred in sheep fed *Lotus corniculatus* (Waghorn *et al.*, 1987), improving the overall nutritional and productive performance of the animal (Ramírez-Restrepo *et al.*, 2002; 2004). However, effects of other secondary compounds in willows needs further research.

Table 9: Chemical composition (g/kg DM) and nutritive value of the diet selected for lambs grazing pasture and willow fodder blocks in the Experiments conducted on Riverside Farm.

	Total N[a]	OMD[b]	DOMD[c]	ME[d]	CT[e]
Control pasture					
Ramirez-Restrepo *et al.*, 2005	26.5	0.65	0.59	9.6	1.6
Pitta *et al.*, 2005b	25.5	0.65	0.59	9.6	2.0
Current experiment	28.1	0.66	0.60	9.8	6.4
Willow fodder block herbage					
Pitta *et al.*, 2005b	20.0	0.60	0.59	8.8	3.6
Current experiment	24.7	0.64	0.58	9.4	12.6
Willow fodder block trees					
Pitta *et al.*, 2005b	13.6	0.67	0.61	9.9	38.3
Current experiment	16.4	0.70	0.64	10.4	41.6

Pasture measurements made on hand plucked samples of diet selected.
Willow tree measurements made on hand cut samples from trees (stem diameter <5mm) of diet selected.
[a] N: Nitrogen; [b] OMD: Organic matter digestibility *in vitro*; [c] DOMD: Digestible Organic matter in the dry matter *in vitro*; [d] ME: Metabolisable energy (MJ/Kg DM).

Whilst the willow fodder blocks were effective in supplying CT, it is evident that the herbage growing in them was only average OMD and ME (approximately 0.62 and 9.0 MJ ME/kg DM respectively), being slightly different from control pasture (Table 9). A reasonable target for good animal growth is an OMD of 0.72 and a ME value of 10.5 MJ/Kg DM.

A major limitation of willow fodder blocks was the high pregrazing mass (primary and secondary growth) and high dead matter content (secondary growth) of the herbage that accumulated in them (Table 10), which can be compared to previous results obtained by Pitta *et al.*, (2005b); mature grasses under these conditions are the principal cause of the low OMD and ME. It is extremely important to improve the quality of herbage in the willow fodder blocks through better pasture management, through more frequent grazing to maintain lower pasture mass levels (2500-3000 Kg DM/ha) as reported by Ramírez-Restrepo *et al.* 2005; Table 10). In the future, three grazings per season are suggested.

Table 10: Pre-grazing and Post-grazing mass (kg DM/ha) and dead matter content (%) of control pasture and willow fodder block grazed during different experiments.

	Pre-grazing		Post-grazing
	Mass (Kg DM/ha)	Dead matter content (%)[a]	Mass (Kg DM/ha)
Control pasture			
Ramirez-Restrepo *et al.*, 2005	2680	10	1740
Pitta *et al.*, 2005b	4256	27	2401
Current experiment			
primary growth	4243	9	2984
secondary growth	3701	40	2868
Willow fodder block herbage			
Pitta *et al.*, 2005b			
primary growth	5724	31	3605
secondary growth	3369	15	1333
Current experiment			
primary growth	5070	10	3545
secondary growth	4213	24	3169
Willow fodder block trees			
Pitta *et al.*, 2005b			
primary growth	814		470
secondary growth	226		101
Current experiment			
primary growth	562		262
secondary growth	775		236

[a] Percentage of total forage mass

Results presented in this experiment show differences in parasite worm burdens between lambs grazing all three forage systems, as reported by Marley *et al.* (2003; 2005) who observed that different forages may affect worm burdens species in different ways. There are no published reports investigating anti-parasitic activity of the CT in willows grazed by livestock. However, there are a number of studies which have investigated the effect of CT from other sources on nematodes, although some of these have shown contradictory results. It has been suggested that CT are more likely to have an effect on abomasal and small intestine parasites, which is consistent with results obtained in the present experiment (Niezen et al., 1995; Butter et al., 2000).

Terrill *et al.* (1994) observed that during the processes of digestion, the CT from *Lotus corniculatus* became less extractable as it passed through the digestive system of sheep, even though most C^{14}-CT was still present in ileal digesta and faeces. This suggests that CT was converted to other compound(s) during digestion and was more strongly bound (and therefore less reactive) as the digesta continued down the gastrointestinal tract. This may help explain why the effects of grazing willow fodder blocks were more pronounced for parasites of the abomasum and small intestine than for parasites of the large intestine.

Results obtained in the current experiment, show undrenched lambs grazing on willow fodder blocks (restricted and full access) had lower worm burdens at slaughter of the small intestinal species of both *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis* compared to undrenched lambs grazing control pasture. Both of these two species are considered to be pathogenic and of economic importance (Gardner and Craig, 1961) and the reduction in their numbers is consequently of likely benefit.

Similarly, Butter *et al.* (2000) found that lambs had lower *Trichostrongylus colubriformis* burdens when fed 50 grams of quebracho extract per kilogram diet, indicating a direct anti-parasitic effect of CT on *Trichostrongylus* species *in vivo*. Niezen *et al.* (1995) also found that lambs fed CT-containing forages, in this case sulla, for 6 weeks had lower *Trichostrongylus* worm burden compared to lambs grazing lucerne. In contrast, Niezen *et al.* (1998b) reported higher *Trichostrongylus* burdens in lambs grazing *Lotus corniculatus* compared to lambs grazing pasture. These authors explain the differences between these two reports partially due to differences in the CT concentration and to differences in structure of CT present in sulla and lotus (120 g/kg DM and 48 g/kg DM respectively).

Most experiments which have investigated the effects of CT on *Trichostrongylus* species have been for a short period of time, with minimal opportunity for any effects on larval development and reinfection to be investigated. Lambs in the report by Niezen *et al.* (1995) were set stocked for 6 weeks and the Niezen *et al.* (1998b) experiment was conducted for 42 days using back-fenced strip-grazing to prevent reinfection. In the present experiment animals had continued rotational grazing for 14 weeks, grazing each area three times, which provided an extended opportunity for any direct effects on the reduction of some parasites due to reduced reinfection rates plus any effects on larval development to be expressed as a result of restricted or full access grazing on willow fodder blocks.

It is interesting to note that lambs with restricted access to willow fodder blocks also had fewer *Teladorsagia circumcincta* and *Teladorsagia trifurcata* worm burdens at slaughter than lambs grazing on control pasture, although lambs grazing willow

fodder block full access showed no differences from those on control pasture for these two parasites. This genus is also considered to be pathogenic and of economic importance (Gardner and Craig, 1961).

Contradictory results have been reported for *Teladorsagia circumcincta* and *Teladorsagia trifurcata.* Ramírez-Restrepo *et al.* (2005) found that lambs grazing *Lotus corniculatus* had lower *Teladorsagia circumcincta* worm burdens at slaughter than lambs grazing control pasture, which is similar to the results reported in the present experiment with the willow fodder block restricted access lambs. In contrast, Tzamaloukas *et al.* (2005) and Athanasiadou *et al.* (2001) reported no reduction of *Teladorsagia circumcincta* establishment in lambs when fed either sulla or *Lotus pedunculatus* for 2 weeks or 80 g/kg DM of quebracho extract for 3 days. A possible explanation may relate to the duration of the experiments as discussed above for *Trichostrongylus* spp.

Haemonchus contortus burdens were relatively low in the present experiment and curiously, there were higher burdens in lambs grazing willow fodder blocks. Paolini *et al.* (2003) and Athanasiadou *et al.* (2001) found no differences in *Haemonchus contortus* worm populations in animals fed either tannin group (quebracho diets) or control pasture based diets. In contrast, Ramírez-Restrepo *et al.* (2005) found that lambs grazing on *Lotus corniculatus* had lower *Haemonchus contortus* worm burdens compared to control pasture fed lambs. The reason for the higher burdens in the present experiment are unclear, but warrant further investigation.

There was no difference in the large intestine nematode worm burdens between treatments. Previous reports have generally not commented on antiparasitic effects on large intestinal species. The lack of difference may reflect the reduction in extractable CT in the lower intestinal tract as suggested by Terrill *et al.* (1994).

There are no published reports demonstrating a differential effect of CTs on the proportion of male versus female nematodes. Why there were more male *Chabertia ovina* in the willow fodder blocks (restricted and full access) compared to control pasture is not clear.

The preceding discussion has not considered any possible difference in antiparasitic activity between different CTs for different forages. However, a possible explanation for differences between experiments using various CT-containing forages is that the CTs from different plants are present in different concentrations, have different chemical structures, molecular weight and reactivity (Barry and McNabb 1999).

Another way in which willow could effectively reduce parasite problems in lambs is indirectly via nutritional effects. It is well established that CT protect protein from ruminal degradation, which enables the host animal to have a better nutritional status (Aerts *et al.*, 1999) and this may play an important direct or indirect effect in parasitism. Sykes (1994) suggested that CT may counteract protein losses caused by nematode infections, by increasing by-pass protein supply. If lambs are fed with higher protein levels, there is an enhancement of the lamb's capability to overcome enteric loss and improve the utilization of food due to an increase efficiency of protein digestion (Sykes and Coop, 1976), which will stimulate the immune system (Coop

and Kyriazakis, 2001). The possible alternative of indirect effects through enhanced immunity should be considered because lambs had enough time to develop a level of immunity during the course of the present experiment (Coop and Kyriazakis, 2001; Waller and Thamsborg, 2004; Thi Mui *et al.*, 2005).

Reports have shown that pasture environment can influence larval development and survival (Scales *et al.*, 1994; Niezen *et al.*, 2002). Plant morphology and sward characteristics play an important role in the dynamics of the free-living stages of larvae. Pasture mass under the willows was very dense and had high humidity, which may have created a favourable environment for larval development, whereas control pasture conditions appeared less favourable. However, the presence of infective larvae (stage L_3) would be negligible in the actual willow trees and this provided approximately 20% of the DM intake. Although not measured, the overall ingestion of infective larvae may have likely decreased compared to control pasture.

Control pasture had shorter grazing heights, which combined with the absence of anti-parasitic effects of CT-containing forages, may have lead to an increase in larval intake and establishment in the lambs. However, control pasture did dry out as drought conditions prevailed in the second half of the experiment, which would have been expected to reduce larval ingestion due to desiccation of eggs and larval stages. By contrast, herbage in the willow fodder blocks remained green and the relative humidity in the sward, although not measured, is likely to have been higher than for control pasture and thus larval survival may have been higher. Pasture larval levels were not measured so the relative importance of these factors is unknown.

Apart from the differences in chemical and nutritional composition of willows and pasture, there might be a direct effect of CT in inhibiting larval development (Min et al., 2004) and larval motility (Molan *et al.*, 1999), which needs to be studied for willow CT. *In vitro* experiments suggested that CT extracted from *Lotus pedunculatus*, *Lotus corniculatus*, sulla, chicory and sanfoin reduced egg hatching, development from eggs to L_3 larval stages as well as reduced L_3 motility of *Trichostrongylus colubriformis* (Molan *et al.*, 1999; 2000). It is also possible that interruption of larval development by CT and/or reduced L_3 larval intake due to tree morphology may have reduced reinfection rates in the latter half of this experiment, thus causing a reduction in the establishment of some parasites in lambs grazing willow fodder blocks.

It seems that grazing willow fodder blocks had little direct effect in rapidly killing established parasites, as if this had happened there would provably have been a progressive decrease in FEC in the first half of the experiment, before potential effects on reinfection could have occurred.

Even though FEC from undrenched groups tended to increase with time, examination of the FEC data showed that these results were variable between groups. Firstly, there was a lack of consistency on the different forages on FEC throughout time. As an example, lambs grazing on willow fodder block full access had higher FEC compared to lambs grazing control pasture by Day 42. However, by Day 70 lambs grazing willow fodder block full access had lower FEC than the lambs grazing control pasture, but by Day 98 FEC had again increased relative to control lambs and were very similar. In order to understand the dynamics of these findings, factors affecting

FEC need to be considered as well as the underlying CT-containing mechanisms that could potentially affect egg counts. In contrast to the present experiment, Paolini *et al.* (2003) found that lambs grazing 50 g/kg DM quebracho diet for 8 days had a 64% decrease in FEC than lambs grazing control pastures with no quebracho supplement. However, Hoskin *et al.* (2000) showed that farmed deer fed with either lucerne, birdsfoot trefoil or sulla had no statistical differences in FEC. A factor that might have influenced FEC between groups in the current experiment was the total faecal output and the potential differential effect in dilution of FEC as a result. Digestibility of willows was greater than control pasture but there was no mechanism for correcting FEC to account for these differences. This is why, in future experiments it is recommended that if FEC is the measure of interest the total faecal output be measured to avoid the variation presented by different intakes and digestibility of various forages as seen in this experiment.

Vlassoff and Brunsdon (1981) proposed the implementation of a preventive approach to control parasitism in lambs so higher production gains could be achieved. This consisted of drenching all the animals at 28 day intervals on 5 occasions to prevent an autumn peak in larvae by keeping pasture larval levels low. This is similar to the drenching protocol for the control pasture treatment. The present study compared this preventive approach (control pasture) with 2 different grazing systems as an alternative method to help control internal parasites. Grazing willow fodder blocks decreased some worm burdens but still maintained lamb growth rates equivalent to drenched lambs grazing control pasture. Further research should be conducted to understand the epidemiology of nematodes in lambs grazing CT forages such as willows, together with a better management of the pasture masses in the fodder blocks.

5. Conclusions

Responses to anthelmintic treatment in all three grazing systems (control pasture, restricted access and full access to willow fodder blocks) showed that parasitism was restricting lamb growth in undrenched lambs. This was supported by a progressive increase in FEC with time in all undrenched groups. The principal parasites established in undrenched control lambs at slaughter were *Teladorsagia trifurcata*, *Teladorsagia circumcincta*, *Nematodirus spathiger*, *Trichostrongylus vitrinus*, *Trichostrongylus colubriformis* and *Trichostrongylus axei*.

Undrenched lambs grazing on both restricted and full access willow fodder blocks had lower establishment of some parasites at slaughter. Relative to undrenched lambs grazing control pasture, those with restricted access to fodder blocks had fewer *Teladorsagia circumcincta*, *Teladorsagia trifurcata*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis,* whilst lambs with full access to fodder blocks had fewer *Nematodirus spathiger*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*. This was reflected in reductions in CWG of undrenched, relative to regularly drenched lambs, which were greatest for control lambs (24 g/day) and least for full access lambs (12g/day).

Dag score progressively increased with time up to Day 70, with no differences between treatments. From Day 70 until the end of the experiment, DS was lower for drenched than for undrenched lambs and was reduced by access to fodder blocks. Both rates of LWG and final DS were similar for full access willow fodder block

undrenched lambs and control pasture drenched lambs, showing that willow fodder blocks have the potential to be utilized in parasite control strategies.

Grazing willow fodder blocks could have reduced parasite worm burdens in undrenched lambs by a reduction in the reinfection rate, through action of CT interrupting parasite life cycles and/or through a reduction in L_3 larval consumption due to taller plant morphology of the trees. However, there seems to be no direct effect on rapidly killing established parasites, as if that had happened, there would have been a decrease in FEC in the first half of the experiment, before any effects of reinfection took place. Further research allowing time and pasture management procedures for reinfection to be expressed are needed in this area.

It is unlikely that there will be a new commercial drug to effectively combat anthelmintic resistance in the near future. Therefore, the further study of the use of bioactive forages such as willows, is an alternative that has to be seriously considered. Grazing willow fodder blocks is a very good alternative to utilize unproductive farm areas, providing palatable and nutritious foliage as a supplement during dry summers. This is a cheaper alternative in terms of labour than pruning willow trees or cutting from fodder blocks and feeding as a supplement to grazing livestock. Willow fodder blocks can play a dual role in improving soil conservation whilst also providing a sustainable source of nutrients for grazing livestock.

Anthelmintic resistance is a worldwide problem whose solution or control requires the collaboration of Researchers, Veterinarians, Parasitologists, Nutritionists and Farmers. CT-containing forages could be used in conjunction with the use of LWG monitoring,

diarrhoea and/or body condition score for the control of gastrointestinal nematodes. However, it still needs further evaluation.

Breeding for enhancement of the immune system, development of new vaccines, individual selective approach to drenching, development of resilience, use of pasture management techniques or combinations of all these need to be investigated. Nematode control is currently being managed using various natural and synthetic anthelmintics, however research needs to focus on finding more efficacious and economic alternatives to improve sustainability of anthelmintic usage.

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Appendix 1

Modified McMaster Method for counting strongyle eggs.

METHOD:

1. Weigh out 2 grams of faeces into a 100 ml container.

2. Add 28 ml of saturated NaCI solution (specific gravity 1.2) and mix well to a fine suspension using a spoon.

3. Pour mixture through a small coarse sieve (aperture approximately 0.85 mm) into a high-edged metal dish.

4. Mix well while taking out a sub sample, using a Pasteur pipette and repeat procedure to fill both chambers of a McMaster counting slide.

5. Each egg counted represents 50 eggs per g faeces.

Appendix 2a

Culturing of faecal eggs to 3rd stage larvae.

METHOD:

1. Larval cultures were set up the same day of the sampling or the following morning after obtaining faecal samples. Samples were stored at 4°C until processed.

2. Weigh out 20 g of faeces from a pooled sample of each treatment.

3. Place faeces in the base of an 85mm diameter glass container containing approximately 20 ml of distilled water.

4. Place the lid on the container (not fitted tightly in order to allow diffusion of oxygen and evaporation out of the container) to maintain the humidity as high as possible during the incubation time.

5. Incubate cultures at 25°C for 10 days (in an incubator).

6. Check the cultures every 3 days and add more distilled water if necessary, to maintain humidity levels.

7. Extract larvae as described in Appendix 2b.

Appendix 2b

Baermann procedure for extracting 3rd stage larvae from faecal cultures.

METHOD:

1. Remove larval cultures from incubator after 10 days.

2. Place a sieve on top of each glass funnel in the Baermann set-up. Put one layer of paper tissue in each sieve. And add water into the funnel.

3. Empty out the faeces into the sieve. Rinse all parts of the glass container with deionised tap water, and wash into the sieve. Make sure that faeces are covered by water. And set for 24 hours.

4. Collect approximately 200 ml of the sediment at the bottom of the funnel into a 500ml flask allowing them to settle for 24 hours at 4°C.

5. Carefully siphon off the supernatant of the sample until a volume of 50 ml is left in the flask, taking care to avoid stirring up the sample whilst doing this.

6. Samples are now ready for larval identification and counting as described in Appendix 2c.

Appendix 2c

Counting third stage larvae

METHOD:

1. Larvae are recovered from faecal cultures as described in Appendix 2b.

2. Mix the sample well and take out a sub-sample of 2 ml with an automatic pipette.

3. Place the sample in a glass counting slide and add a drop of Lugol's iodine (described in Appendix 2d) to kill the larvae.

4. Leave to settle for about 30 seconds and place the slide on the compound microscope.

5. Count and identify 3rd stage larvae in the whole area of the slide.

6. If possible, counting of 100 3rd stage larvae was performed.

Appendix 2d

Lugol's Iodine solution

Dissolve 2 g Potassium Iodide (Analar, BHD Laboratory Supply, England; KI = 166.0) in 100 ml of distilled water and add 1 g Iodine (Analar, BHD Laboratory Supply, England; I = 126.90) mixing thoroughly and keep in a cool place.

Appendix 2e

Identifying third stage larvae

METHOD:

1. While counting the larvae (Appendix 2c), larvae were identified to genera, sex and species level using the x40 and x10 objective to measure larval length.

2. Where possible, identification of 100 third stage larvae to genus level was performed.

3. The identification of the 3rd stage larvae of common gastro-intestinal nematodes of performed using the Ministry of Agriculture, Fisheries and Food (1986) techniques were:

Strongyloide	Without sheath; oesophagus - half the length of body
	With sheath; oesophagus less than 1/4 the length of body
Trichostrongylus spp.	Head of larva tapered, tail indistinctly rounded or bearing one or two tubercles without sheath, < 720 μm.
Teladorsagia spp.	Head of larva squared, tail indistinctly rounded, 'shoulders' just below head of larva, > 720 μm.
Cooperia spp.	Head of larva squared, bearing refractile bodies or band. Tail of sheath tapering almost to a filament or abruptly becoming a fine point.
Haemonchus spp.	Head of larva narrow rounded, tail of sheath off-set.
Oesophagostomum/Chabertia spp.	Head broad rounded, 32 gut cells, long tail.

Appendix 3

Necropsy procedure

The method described below is generally used for diagnostic worm counts in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

METHOD:

1. Animals were slaughtered in commercial abattoir.

2. Removal of gastrointestinal tract from the carcass was performed immediately after slaughter.

3. Locate abomasum, place string ligatures at either end removing as much mesentery as possible. Put abomasum in a labelled plastic bag.

4. Locate pylorus and place a string ligature just distal to this. Take out small and large intestines placing another ligature and place them on the dissection metal table.

5. Dissect small intestine, strip the mesentery of the small intestine until the ileo-caecal junction is reached placing another ligature here. Put small intestine in labelled plastic bag.

6. Dissect large intestine, strip mesentery off and place a ligature 15 cm before rectum finishes. Put large intestine in labelled plastic bag.

7. Store recovered organs in freezer at -20 °C until further processing.

Appendix 4a

Worm count procedure - Abomasum

The method described below is generally used for diagnostic worm counts in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

METHOD:

1. Take abomasum out of freezer and thaw overnight.

2. Open the abomasum along its length using a pair of scissors.

3. Pull the opened intestine between the fingers to scrape the contents off the mucosa into a 10-litre bucket, under a trickle of water. Make up the contents of the bucket to 4 litres with water.

4. Mix the contents by cross-stirring taking out sub-samples of 400 ml (= 10% of the total volume). Transfer the two aliquots to jars for storage.

5. The washed abomasum is then digested according to the technique in Appendix 4b.

6. Add 10% Neutral-buffered formalin to one aliquot to obtain a final formalin concentration of 5% which will be stored as a reserve sample.

7. The other 10% (400 ml) aliquot is poured through a 38 μm large sieve and washed until the water runs clear. Collect material retained in the sieve and proceed to count.

8. Count the worms of the entire volume of the sieved sample using a dissecting microscope.

9. Identify and count adult females and males, immature females and males.

10. Collect a minimum of 50 adult male worms. Keep them in formalin for later examination.

Appendix 4b

Pepsin digest technique

This method is used after the abomasum has been washed and ensures that all adult worms and younger larval stages are removed from the mucosal surface and included in the total worm count.

METHOD:

1. Pour 2.5 g of pepsin (70 FIP-U / g) with 600 m1 of distilled water and 10 ml of concentrated HCI into a large glass beaker containing one abomasum
2. Incubate in a waterbath at 37°C for 2 hours.
3. After incubation pour digest fluid into a 10-litre plastic bucket, wash the abomasum thoroughly under a trickle of water and store in jar.
4. Count the worms of the 10% volume of the sieved sample using a dissecting microscope.
5. Add 10% Neutral-buffered formalin to one aliquot to obtain a final formalin concentration of 5% which will be stored as a reserve sample.
6. Add the worm count from the digest fluid to that of the washings in Appendix 4a, to obtain a total worm count.

Appendix 4c

Worm count procedure - Small intestine

The method described below is generally used for diagnostic worm counts in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

METHOD:

1. Take small intestine out of freezer and thaw overnight.

2. Open the small intestine along its length using a pair of scissors.

3. Pull the opened intestine between the fingers to scrape the contents off the mucosa into a 10-litre bucket, under a trickle of water. Make up the contents of the bucket to 4 litres with water.

4. Mix the contents by cross-stirring taking out sub-samples of 400 ml (= 10% of the total volume). Transfer the two aliquots to jars for storage.

5. Add 10% Neutral-buffered formalin to one aliquot to obtain a final formalin concentration of 5% which will be stored as a reserve sample.

6. The other 10% (400 ml) aliquot is poured through a 38 μm large sieve and washed until the water runs clear. Collect material retained in the sieve and proceed to count.

7. Count the worms of the entire volume of the sieved sample using a dissecting microscope.

8. Identify and count adult females and males, immature females and males.

9. Collect a minimum of 50 adult male worms. Keep them in formalin for later examination.

10. Multiply the number of worms counted by 10 to obtain the total worm burdens.

Appendix 4d

Worm count procedure – Large intestine

The method described below is generally used for diagnostic worm counts in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

METHOD:

1. Take large intestine out of freezer and thaw overnight.

2. Open the large intestine along its length using a pair of scissors.

3. Pull the opened intestine between the fingers to scrape the contents off the mucosa into a 10-litre bucket, under a trickle of water. Make up the contents of the bucket to 4 litres with water.

4. Poured the total 4 litres through a 150 μm large sieve and washed until the water runs clear. Collect material retained in the sieve and proceed to count.

5. Count the worms of the entire volume of the sieved sample using a dissecting microscope.

6. Identify and count adult females and males, immature females and males.

7. Collect a minimum of 50 adult male worms. Keep them in formalin for later examination.