The Effect of Feeding Willow Upon the Death of Established Parasites and Upon Parasite Fecundity

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Declaration

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

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

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Abstract

Two indoor feeding experiments were conducted at the Animal Physiology Unit (APU) of Massey University, involving young sheep, to investigate the effect of feeding forage willow upon the death of established parasites and upon parasite fecundity, using chaffed lucerne as the control diet.

Experiment 1: Twenty-four (24) parasite-free weaned hoggets weighing 29.9 ±1.2 kg (SD) were individually penned and fed chaffed lucerne *ad libitum* during a pre-experimental adaption period of 5 weeks. They were then fed either lucerne chaff or chopped willow for a further 5 weeks (n = 12/group) and intakes were adjusted such that the DMI of the two groups was similar during weeks 9 & 10. All lambs were infected with L_3 larvae parasites comprising 20,650 *Teladorsagia*, 1,320 *Trichostrongylus* and 330 *Cooperia* through oral drenching 12 days before willow feeding started. This was done after confirmation that the sheep were free of nematodes through FEC analysis. Total faeces were collected for 3 day periods towards the end of weeks 9 & 10, to measure diet digestibility and total faecal egg excretion. The sheep were slaughtered at the end of week 10. Voluntary feed intake (VFI), FEC and liveweight were measured weekly, whilst burdens of individual parasites and carcass characteristics were measured after slaughter. Duplicate samples of each feed offered and individual animal refusals were taken daily and pooled weekly per animal for chemical analysis. Female worm fecundity was calculated by two methods. Blood samples for immunological analysis were collected on days 20, 34, 51 and 70, and analysed for components of white blood cells (WBC) and for lymphocyte subsets.

Experiment 2: A 2 x 2 changeover experiment was conducted, involving two time periods (Period 1 and Period 2 each of 14 days) with the same diets as used in Experiment 1, fed to 9 individually penned parasite-free young sheep randomly allocated to experimental diets. The parameters investigated were FEC and larvae hatching. Initially, a period of 7 days was allowed for acclimatisation in which both groups were fed on half willow and half lucerne chaff. This was followed by Period 1 with 4 lambs fed lucerne and 5 fed willow, after which the diets were changed over

for Period 2. Total faeces produced were collected from all animals on the last day of each period using bagged sheep. A known number of *Teladorsagia* eggs (500 epg) was then added to faecal samples from these sheep and faeces-egg mixtures were made from which FEC was determined, to see if egg recovery was affected by these diets. Faecal samples for Period 2 with added eggs were also incubated for 10 days to measure hatchability.

The recovery of added *Teladorsagia* eggs in Experiment 2 was 85% in lucerne-fed lambs and 53% willow-fed lambs ($P<0.001$); these were used as correction factors for Experiment 1 data. Larvae that hatched per gram of wet faeces in Experiment 2 tended to be lower for sheep fed willow than lucerne chaff (71% vs 83% of eggs added; $P=0.08$).

Willow feed offered had lower DM ($P<0.001$) and CP ($P<0.05$) content, but had a significantly higher OM content ($P<0.01$) than lucerne chaff. Condensed tannin content of chopped willow was 27 g/kg DM, with only traces for lucerne. Apparent digestibility for DM (62.4% vs 59.5%; $P\leq0.05$), OM (64.8% vs 59.9%; $P\leq0.001$), DOMD (58.1% vs 55.0%; $P\leq0.01$) and calculated ME (9.48 MJ/kg vs 8.96 MJ/kg; $P\leq0.01$) were higher for the willow diet. VFI was similar for both groups during the adaption period ($P>0.05$) but declined with the introduction of willow in week 6 ($P<0.001$) and then progressively increased until it was similar to lucerne-fed sheep in weeks 9 & 10 ($P>0.05$). Calculated DM intake per head/day during the last two weeks of Experiment 1 was similar for the two groups ($P>0.05$); while the willow group had higher ME ($P<0.01$) and CP ($P<0.001$) intake per animal/day. Liveweight increased for the two groups during the adaption period ($P>0.05$), then declined for willow-fed lambs in week 6 ($P<0.001$) but later increased and by week 10 was similar to that of lucerne-fed lambs. The willow-fed lambs had lower carcass GR than the lucerne-fed lambs ($P<0.01$) when carcass weight was used as a covariate. Adjusted total daily egg production in Experiment 1 was lower in willow-fed sheep than lucerne-fed sheep, due to reductions for *Haemonchus* spp. ($P<0.05$) and *Teladorsagia* spp. ($P<0.05$). The per capita fecundity for *Haemonchus* worm spp. ($P<0.05$) and the *in utero* fecundity in both abomasal *Teladorsagia* spp. and small intestinal *Trichostrongylus* spp. ($P<0.001$) were lower for willow-fed sheep. There was reduced production of larvae for both *Haemonchus* spp. and *Teladorsagia* spp. ($P<0.05$) in willow-fed sheep.

Feeding willow reduced the burden of *Haemonchus* adult worms in the abomasum ($P<0.01$) but reduced female worm burden only in *Teladorsagia* spp. ($P<0.05$) and reduced *Cooperia* spp. in the small intestines ($P<0.01$). Total WBC, total lymphocytes, subsets of lymphocytes and other white-cell groups were not affected by willow feeding ($P>0.1$).

It was concluded that feeding chopped willow to young sheep reduced nematode worm burdens in the abomasum, especially both male and female *Haemonchus* spp., and reduced female worm burdens of *Teladorsagia* spp. Female worm fecundity of both species was also reduced by willow feeding. These reductions have been associated with CT content in the willow feed and the reduced worm burdens have been attributed to the death of the established worms by CT, since there was no evidence of immune priming in willow-fed sheep. Compounds present in the faeces of willow-fed sheep have been found to mask some of the nematode eggs, making them invisible by microscopic examination while keeping their viability. It is postulated that this could be due to binding of nematode eggs to insoluble CT associated with indigestible fibre in the faeces of willow-fed sheep. Conventional methods of measuring FEC therefore underestimated nematode eggs present in the faeces of willow-fed sheep and this needs to be checked for other CT-containing forages.

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Finally, a special mention is due to my loving wife Florence and our four wonderful children, Kerry, Bornwell (Jr), Nchimunya and Lushomo. Each of them has made a sacrifice in order for me to reach my potential and I thank them. All of us will miss New Zealand, it is with great sadness that we leave however, it is with great joy, new knowledge and improved understanding of the world that we take our leave – ***mucaale cibotu!***

Dedication

I dedicate this work to my Chief Supervisor Prof. T.N. Barry (Tom) who, even in his time of illness, was able to encourage me to keep working. To you I say: Bravo!!

NDALUMBA KAPATI

List of Abbreviations

AA	Amino Acids
AAD	Amino-acetonitrile derivatives
ADF	Acid Detergent Fibre
AFRC	Agriculture and Food Research Council
AOAC	Association of Official Analytical Chemists
ARDOM	Apparently Rumen Digested Organic Matter
ATP	Adenosine Triphosphate
Ca	Calcium
CCK	Cholecystokinin
CH_4	Methane
CHO	Carbohydrate
CO_2	Carbon dioxide
CP	Crude Protein
CSIRO	Commonwealth Scientific and Industrial Research Organization
CT	Condensed Tannins
CV	Coefficient of variation
CW	Carcass weight
DM	Dry Matter
DMI	Dry Matter Intake
DOMD	Digestible Organic Matter in the Dry Matter
DP	Digestible Protein
EAA	Essential Amino Acids
epg	Eggs per gram
FEC	Faecal Egg Count
FV	Feeding value
GDP	Gross Domestic Product
GI	Gastrointestinal
GIN	Gastrointestinal nematodes
GIT	Gastro-intestinal tract
GR	Girth Rib (measurement of carcass fat thickness)
HCL	Hydrochloric Acid

HT	Hydrolysable Tannins
K	Potassium
Kcal	Kilocalories
LDA	Larval Development Assay
LMI	Larval Migration Inhibition
LW	Liveweight
LWG	Live weight Gain
M	Million
MAF	Ministry of Agriculture and Forestry
MAFF	Ministry of Agriculture, Fisheries and Food
ME	Metabolisable Energy
Mg	Magnesium
MJ	Mega Joules
MP	Metabolisable Protein
MW	Molecular Weight
N	Nitrogen
NAN	Non-Ammonia Nitrogen
NDF	Neutral Detergent Fibre
NEAA	Non-Essential Amino Acids
NH_3	Ammonia
NV	Nutritive Value
OM	Organic Matter
OMD	Organic Matter Digestibility
OR	Ovulation Rate
P	Phosphorus
PA	Proanthocyanidins
PEG	Polyethylene glycol
SAS	Statistical Analysis System
SEM	Standard error of the means
SI	Small intestine
SolCHO	Soluble carbohydrates
SSH	Sward surface height
Spp.	Species
UDP	Un-degradable Protein

UoB	University of Aberdeen
VFA	Volatile Fatty Acids
VFI	Voluntary Feed Intake
WBC	White Blood Cells

Table of Contents

List of Figures

List of Tables

List of Plates

CHAPTER 1
REVIEW OF THE LITERATURE

The objectives of this research are (1), to investigate the effect of willow upon the death of established worm populations and, (2), to better understand how willow affects the fecundity of those worm populations. New Zealand faces a continuing and growing problem with worm infestation and there have been wide reports of anthelmintics resistance by these worms making the control methods very difficult. The question this research report seeks to answer is twofold: can the novel use of willow –as a natural remedy - help to control existing worm populations in established lamb flocks and, can the use of willow reduce chemical usage – a by-product which can find its way into the food chain of one of New Zealand's major exports.

1.1 INTRODUCTION

New Zealand (NZ) is mainly a pastoral country (pastoral farming being the backbone of the economy) with nearly $23.5 billion (B) of the gross domestic product (GDP) coming from agriculture, with livestock (about $17.8B) being the largest contributor (Ministry of Agriculture and Forestry (MAF), 2009). The sheep industry alone contributes slightly more than $3B, accounting for 18.5% of total pastoral exports.

Pastoral farming covers approximately 13.5M hectares from a total of 27.1M hectares. The major farmed animals are dairy, sheep, beef cattle and deer. The sheep population has continued to show a downward trend since the peak of 1982 with a population of 70.3 M to 38.5 M in 2007 (Table 1.1), while that of dairy cattle has increased from 3 to 5.3 million and the deer from a 100 thousand to about 1.7 million in 2006 (MAF, 2009). This is largely due to the removal of all forms of livestock subsidies in the mid 1980s and land use changes in favour of dairy, deer and forestry (MAF, 2003). Despite the fall in sheep numbers over the last 20 years, the number of lambs weaned per 100 females mated increased from 100 in 1987 to 127 in 2007. This is attributed to improved genetic selection, ultrasound pregnancy scanning, and better flock management i.e., improved nutrition (Kenyon et al., 2004). During the same period, there was an increase in the number of ewe hoggets put to the ram (Table 1.1; MAF, 2009). Mating of ewe hoggets (6-9 months old female lambs) has been identified as one way of increasing net profits

for the sheep industry, due to the extra generation of lambs produced (Kenyon et al., 2004). New Zealand sheep production and management is based on an annual cycle, with the breeding season in March/April (autumn), lambing in August/September (late winter/early spring), and weaning in November/December (early summer; Matthews et al., 1999).

Table 1. 1: Productivity and the New Zealand Sheep Population, 1982-2008.

Year	1982	1987	1992	1997	2002	2007
Total sheep (M)	70.3	64.2	52.6	47.4	39.6	38.5
Breeding ewes put to rams (M)	50.8	45.4	36.7	33.4	26.8	26.1
Ewe hoggets put to ram (M)	2.1	1.6	1.7	0.9	2.4	2.5
Lambing %	98.7	100.4	104.3	116.1	123.7	127.0
Lambs weaned (from breeding ewes; M)	48.1	46.4	38.7	35.1	31.5	31.6
Lambs weaned (from hoggets; M)	-	-	-	-	1.1	1.4
Lambs slaughtered/annum (M)	28.7	25.1	26.7	26.1	26.0	26.9
Lamb carcass weight (kg)	13.6	13.7	14.8	16.6	16.9	16.8
Wool production/ewe (kg)	5.5	5.3	5.5	5.7	5.7	5.6

Source: Ministry of Agriculture and Forestry (1971-2009)

The profit margins from sheep in NZ are low, with one problem being losses due to parasites. It is estimated that more than $300 million per annum is spent on control of nematodes alone (Rattary, 2003). Besides, due to the continued use of oral anthelmintics (i.e., drenches) there has been increased development of nematode populations with resistance to anthelmintics in the recent years in NZ (West et al., 2002). While chemical methods of control of nematodes can be very effective (Ketzis et al., 2006), the danger of chemical resistance suggests there is a need to develop more sustainable methods for nematode control (Diaz-Lira, 2008; West et al., 2002). Furthermore, there has been increasing concern over chemical use going into the food chain (Niezen et al., 1993) posing a danger to man, hence there a greater need to find other means to control parasites so as to minimize drug use. It has been recognised that forages containing condensed tannins (CT) would help maintain parasite levels below those causing economic losses (Barry, 1998; Diaz-Lira, 2008; Ketzis et al., 2006; Musonda et al., 2009).

Willow trees have been grown in NZ for the past 160 years primarily for erosion control and animal welfare uses such as provision of shelter and shade (Wilkinson, 1999). In the recent years willow has been evaluated as a supplement browse for livestock, particularly in the drier regions of the East Coast of the lower North Island (Barry et al., 2006). Supplements of willow browse to ewes grazing low quality drought pasture during mating increased reproductive performance by approximately 20-25% (McWilliam, et al. 2005a). In some experiments, better management of internal parasites has also been achieved (Diaz-Lira, et al. 2008).

This review will therefore discuss the feeding and nutritive values of forages and their role in sheep productivity and welfare, including limitations caused by parasite infections, and will review alternative methods of parasite control. Particular emphasis will be placed on the role of plant secondary compounds, especially CT, and ways by which they can increase nutritive value and give more sustainable management of internal parasites as an alternative to drenching with anthelmintics.

1.2 SHEEP PRODUCTION UNDER GRAZING IN NEW ZEALAND (GRAZING SYSTEMS, NUTRITIONAL AND PARASITIC LIMITATIONS)

Animal production systems throughout the world have evolved and been exploited and developed to harvest and convert plant material into food and fibre products for the owners of the systems, and for making a profit through the sale of the products to other communities (Caple, 1985). The harvesting potential and conversion of the plant material into food products by ruminants will depend on the grazing system in use, nutritional limitations, and limitations due to parasites. Sections 1.2.1 to 1.2.3 below summarise some of the aspects of sheep production under grazing in NZ as they relate to parasite burden.

1.2.1 Sheep grazing systems

Grazed pasture is the main source of nutrients to livestock in NZ, providing around 90% of the total nutrient requirements of ruminants (Hodgson, 1990), typically comprising mixtures of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Kemp et al. (1999) stated that the percentage of white clover in mixed pastures was

normally 20 to 30% but could be as low as 2% in infertile hill country pastures. The role of white clover in a mixture is to fix nitrogen and to provide a high nutritive value compared to ryegrass alone (Ulyatt et al., 1988). However, excess consumption of white clover can cause bloat problems in cattle. Ryegrass, due to its rapid establishment, persistence (tolerant of treading damage and hard grazing) and steady growth throughout the year (winter-active grass) is preferred and widely used.

A grazing system is an integrated combination of animal, plant, soil and other environmental components designed to achieve specific results or goals of a producer (Clark & Kanneganti, 1998). Livestock performance can be affected by a grazing system in use, which may determine the level at which the pasture is utilised and may also determine extent of parasitic worm burden. Broadly, three main types of grazing system will be defined, although in practice there are many variations around each. These grazing systems are known as "set stocking or continuous stocking," "rotational paddock grazing," and "strip grazing."

Set or Continuous stocking system

In this system, animals remain on the same pasture throughout the season. On the extreme side of continuous grazing, there is no adjustment made to stock numbers despite changes in pasture conditions (Matthews, et al. 1999). Under continuous stocking management, pasture intake and animal performance increase at a progressively declining rate towards a maximum value as sward surface height (SSH) increases (Hodgson & Brookes, 1999). The critical SSH for grazing sheep is about 6 to 7cm. Generally, lamb producers favour a system of continuous stocking from lambing to weaning in order to maximise lamb performance (Matthews, et al. 1999). The advantages of this system are reduced input costs in fencing, water reticulation and labour. A major disadvantage is the requirement for greater skill in monitoring sward growth and in managing grazing pressure (Clark & Kanneganti 1998). Furthermore, this system encourages build up of nematode worms since there is no break in the life cycle of these parasitic worms.

Rotational paddock grazing system

Rotational paddock grazing is a system of grazing management where livestock are grazed on a rotational basis within a large number of smaller units called paddocks. Typically a paddock may be utilised for 1-3 days before the stock are moved on. Rotational paddock grazing is a more intensive management system and requires higher capital costs in fencing, water supply infrastructure, and access routes. It is often carried out on a 20-30 day cycle and allows the farmer to more accurately match the nutritional demands of the livestock with the availability of forage (Matthews, et al. 1999). Under this system, herbage intake and animal performance have often been related to variations in daily herbage dry matter (DM) allowance (Hodgson 1990; Clark & Kanneganti, 1998; Matthews, et al. 1999). Rotational paddock grazing also ensures that stock do not regraze the same area of land on a day by day basis and this can help reduce the parasitic worm burden that livestock can suffer from (Ketzis, et al. 2006). Rotational paddock grazing offers an additional advantage in the management of the grassland on a farm in that it is possible for the farmer to close up small areas of grass for conservation (i.e., silage or hay) where grass growth has exceeded livestock requirements; this helps to maintain herbage quality and nutritive value in the paddocks that continue to be grazed.

Strip grazing

Strip grazing is a grazing management system that involves giving the livestock a fresh allocation of pasture each day. It is usually organised within a rotational paddock grazing system and the animals are controlled by the use of an electric fence. Strip grazing systems are often employed where there is a significant excess of forage early in the season and where providing the livestock with access to a larger area would result in waste (e.g. through trampling or spoiling by dung). Strip grazing systems are widely used in the dairy sector, including winter grazing, and for beef and sheep where these animals are being provided with root crops as their primary forage. In a general sense, an electric line is put in front of the animals but there is often no back fence (Clark & Kanneganti, 1998).

Choice of system

In practice, grazing management systems tend to be dependant upon the overall intensity of the livestock production system. An intensive dairy unit may operate with a mix of rotational paddock and strip grazing, whilst a hill country sheep flock may have access to a few paddocks but is largely grazed on a set stocking basis. However, if all other things are equal, the difference in output of grass amongst differing grazing systems will be relatively small. Good herbage quality and nutritive value can be better maintained under rotational paddock grazing than set stocking, and strip grazing is of particular value for rationing herbage/root crops during winter grazing. On the other hand, a particular grazing system can influence the parasite status of the farm. Continuous grazing allows build up of worms since there is no break in their life cycle, whilst rotational paddock grazing tends to be effective in worm control as it allows breaks in the worm life cycle.

1.2.2 Nutritional limitation (excess/deficits - energy/protein) with fresh forage diets

Nutrition plays an important role in productivity, welfare and health of ruminants. Adequate nutrition ensures that animals meet the production targets set in terms of meat, milk or fibre (Pond et al., 2005). Nutrients are essential components needed by an animal for proper growth, production and defence mechanisms against various diseases.

Pasture based livestock production provides a low-cost feeding method (Waghorn & Clark, 2004) but has limitations in terms of pasture quality and quantity which vary according to season. Burke et al. (2002) suggest the main limitations to pasture-based diets as being: moderate energy concentrations and limited digestible DM intake; low dry matter content and excessive fibre in grass restricting feed intake; low concentration of readily fermentable carbohydrates (soluble sugars, organic acids, pectin) relative to crude protein (CP) and fibre concentrations; high CP and insufficient undegradable protein (UDP) leading to excess ammonia production which can be removed at high metabolic cost; and mineral element deficiencies, excess potassium, and incidence of endophyte and other toxins which may limit animal performance. These are general limitations to be expected from pasture-based animal production in NZ. However, these

limitations happen at different times of the year and season as outlined in the next three paragraphs for diets of fresh NZ perennial ryegrass (0.8): white clover (0.2) pasture.

Pasture during spring is predominantly green leafed; it has low concentration of fibre (NDF; 40-45%) and high metabolisable energy (ME; 11.5-12 MJ ME/kg DM), and is highly digestible (75-80% digestibility; Burke et al. 2002). However, concentrations of soluble carbohydrates are usually low (10-15% DM) relative to crude proteins (CP; 25-30%) and as such, energy in the diet is usually not enough leading to inefficient rumen microbial synthesis (Burke et al. 2002). Ulyatt & Waghorn (1993) pointed out that high CP quantities coupled with high CP degradation in the rumen (70-80%) lead to high concentration of ammonia being absorbed into the bloodstream. For animals to cope with this, energy has to be diverted from production to removal of excess ammonia as urea, and the removal of this hepatic ammonia may further deplete amino acid availability for production in pasture fed animals (Burke et al. 2002).

Because spring pastures contain only about 12-16% DM, large quantities need to be consumed to meet animal energy requirements (Burke et al. 2002; Waghorn & Clark 2004). Arguably, less saliva is produced from chewing of soft leaf which may lead to lack of ruminal pH buffering. Nevertheless, salivation associated with extensive rumination would facilitate buffering and maintenance of an optimal rumen pH (Van Soest, 1994).

Summer pastures limit productivity due to their maturing and the proportion of seedhead, stem and dead matter increase relative to leaf. This leads to high concentration of NDF (45-55% DM), lower concentration of protein (<20% DM), lower ME (<10.5 MJ ME/kg DM) and a reduced digestibility (<70%; Wilson & Moller, 1993). Burke et al. (2002) stated that slow digestion of fibre and low content of CP in mature pasture were responsible for limiting energy and protein intake and therefore, animal performance could only be sustained by substitution of mature pasture for rapidly digested forages which have adequate concentration of protein.

1.2.3 Limitations caused by parasite infections

Gastrointestinal nematodes of grazing sheep are a cause of significant morbidity and mortality in these ruminants and the resulting production losses are considerable (Brunsdon, 1988). In most cases, these nematode parasites of sheep reduce nutrients that the sheep would normally use to grow meat and wool (Kahn et al., 2000). Louie et al. (2007) state that parasitism is known to affect the host animal in at least two ways. The first induces a loss of appetite in the host, which reduces pasture consumption compared with the parasite-free animal. The second major effect of parasitism is a reduction in the metabolic efficiency of the host which decreases nutrients available for maintenance and growth (Fig. 1.1; Louie et al., 2007).

In an attempt to explain how parasitic infection depressed appetite, the use of pair-feeding studies in sheep (Sykes & Coop, 1977) and cattle (Fox et al., 1989a) were done and have revealed that inappetence may account for over 60% and 73% of the difference in weight gain between *Ostertagia-infected* and *ad libitum* fed control animals, respectively. Preliminary studies by (Fox et al., 1989a,b) established an association between elevated blood gastrin levels and depressed feed intake in *Ostertagia*-infected calves. The same authors later demonstrated a 40% depression in appetite in worm-free animals, when endogenous blood gastrin concentrations were raised indirectly by the human gastric acid secretion inhibitor, omeprazole, to values comparable with those seen in parasitised calves (Fox et al., 1989c). Work by Dynes et al. (1990) in *Trichostrongylus colubriformis*-infected sheep suggested that central satiety signals might be associated with inappetence rather than the elevated blood gastrin levels, while Roche et al. (2008) reviewed that changes in peripheral concentrations of certain peptides, such as cholecystokinin (CCK), inhibited intake since this hormone is an appetite-suppressant. Experiments have shown that CCK levels were higher in parasitised animals resulting in up to 50% reduction in intake, and once these animals were treated with anthelmintics, the level of CCK was found to drop as well (Dynes et al., 1998).

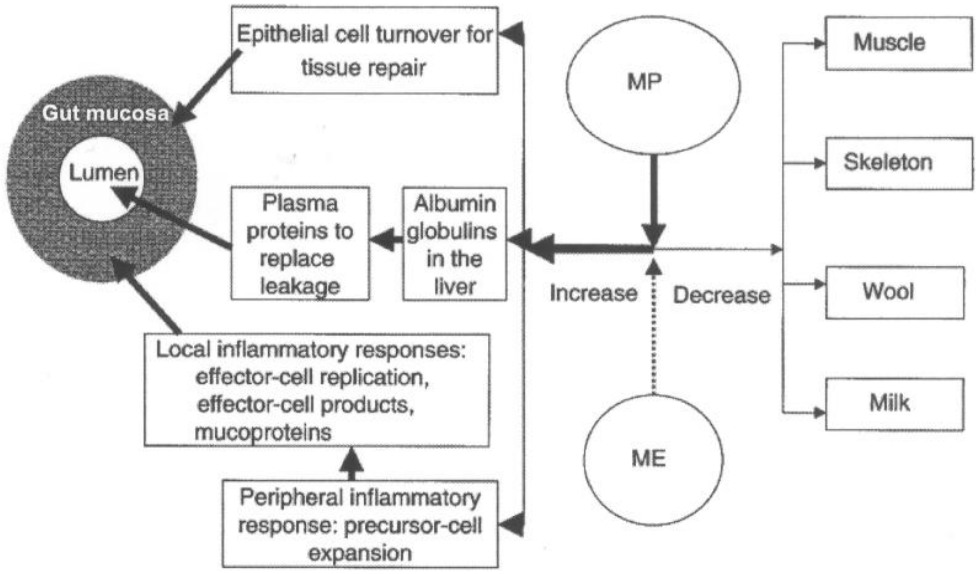


Figure 1. 1: Showing protein metabolism in sheep infected with gastrointestinal nematode (Sykes & Greer, 2003)

Parasitic infection increases the protein requirements of the animal (Bown et al., 1991; Kimambo et al., 1988; Poppi et al., 1986) and there is reduction in the deposition of protein and minerals in the carcass (Coop et al., 1982). Experiments with *T. Colubriformis* have shown that the greater protein requirement due to this infection stems primarily from an increased loss of endogenous nitrogen into the gut (Bown et al. 1991; Kimambo et al. 1988; Poppi et al. 1986), and to a lesser extent from diversion of protein synthesis away from muscle towards repair of damaged tissue (Ketzis et al., 2006; Macrae, 1993; Yu et al., 1988), and the inefficiencies inevitably associated with incomplete reabsorption of endogenous proteins (Kahn et al. 2000). Calculations based on energy balances obtained from slaughter experiments clearly show large reduction in the efficiency of using digested energy associated with parasitism (Sykes & Greer, 2003). It has been calculated that these could be reasonably explained by an increased maintenance requirement for the gastrointestinal tract, reflecting an increase in protein turnover. Yu et al. (2000) indicated a 24% increase in sequestration of amino acids from arterial pools, but also that gastrointestinal tract oxidative losses of leucine during absorption from the gastrointestinal lumen were increased by 20-40% in sheep infected subclinically with *T. Colubriformis*. The effect of all these is the shift in protein synthesis from peripheral tissues and growth to the maintenance of the alimentary tract as shown in Fig. 1.1 above (Sykes & Greer, 2003).

1.3 PLANT CHEMICAL COMPOSITION AND NUTRITIVE VALUE

Plants are the major source of ruminant feed and make their food from the process of photosynthesis taking place in the leaves, in which sunlight is absorbed by the leaves (chlorophyll) and reacts with carbon dioxide (from the air) and water to form energy (carbohydrates). Plants capture solar energy with their leaves and convert it to carbohydrates during photosynthesis. Some of the energy is converted to proteins, fibre, oils, etc., as the plant develops new leaves, stems, and seeds. The energy in the plant forms an important component for livestock nutrition.

Pasture chemical composition is the measure of its nutritive value (NV) and is usually expressed on a DM basis (Waghorn & Clark, 2004). The NV of a diet is a measure of available nutrients that are required by an animal and should be measured on the forage eaten rather than that on offer (Waghorn & Clark, 2004). A plant will basically be composed of primary and secondary chemical compounds as shown in Fig. 1.2 below.

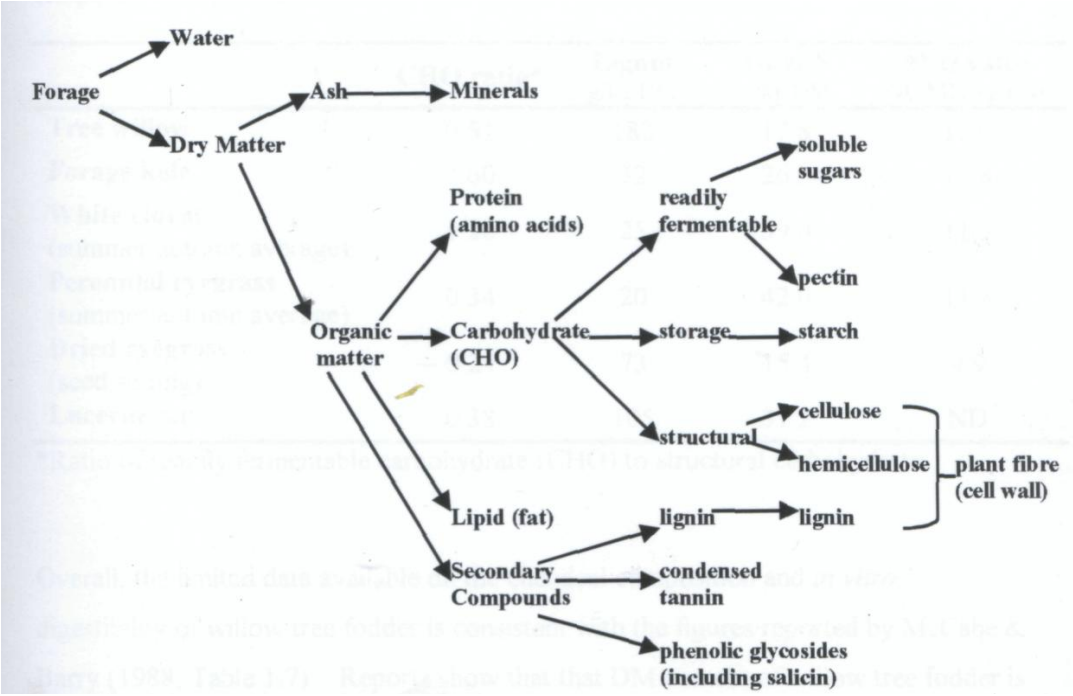


Figure 1. 2: Showing chemical composition of pastures (McWilliam, 2004; PhD thesis)

1.3.1 Primary compounds in the plant

The major primary compounds in the plant are carbohydrates, proteins, lipids and minerals. Most forages typically contain 12-30% DM, but will have varying concentrations of CP, fibre and non-structural carbohydrates as shown in Tables 1.2a & b., (Waghorn & Clark, 2004). The amount of each of these primary compounds an animal must eat depends on the physiological state of that animal. For example, CP concentration must exceed 10% of DM for livestock maintenance and about 19% of DM for high producing dairy cows or young growing stock (Waghorn & Clark, 2004).

1.3.1.1 Concentration in plants

Table 1. 2a: **Dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) composition of fresh leaf forages (adapted from Burke et al., 2000; McWilliam et al., 2005a).**

Forage	DM (%)	Composition(% of DM)	
		CP	NDF
Chicory	14	19	23
Plantain	13	25	28
White clover	15	27	26
Lucerne	24	30	30
Perennial ryegrass	19	16	48
Tall fescue	25	16	42
Willow	39	17	37
Poplar	35	17	40

Table 1. 2b: Seasonal variation in the mean digestible organic matter in the dry matter (DOMD), acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), soluble carbohydrates (SolCHO), pectin, phosphorus (P), calcium (Ca), and magnesium (Mg) concentration (g/100g DM) of pastures (adapted from Penno, 2002).

Season	Winter	Spring	Summer	Autumn
DOMD	74.3	73.7	74.5	73.5
ADF	26.7	28.8	29.0	28.1
NDF	36.4	38.6	38.5	38.3
CP	24.6	23.2	23.8	23.4
SolCHO	10.9	10.4	11.1	10.6
Pectin	2.05	2.04	1.81	1.93
P	0.37	0.35	0.33	0.37
K	2.65	2.85	2.72	2.79
Ca	0.70	0.78	0.73	0.70
Mg	0.20	0.20	0.20	0.20

1.3.1.2 Digestion in animals

Rumen fermentation gives ruminants the capacity to use certain feeds that cannot be metabolised by monogastrics. In the rumen, cell constituents are broken down in order to supply about 60% of the animal's energy requirements (Waghorn & Barry, 1987a). Carbohydrates (cellulose, hemicelluloses, pentosans, starch, etc.) are converted into volatile fatty acids (VFA) and absorbed rapidly through the rumen wall in order to be further metabolised by tissues (Reece, 2004). However, CO_2 and CH_4 are released into the environment as by-products mostly via eructation.

The digestive physiology of ruminants is distinguished by the development of the rumino-reticulum (rumen; Van Soest, 1994). The rumen contains bacteria, protozoa and fungi capable of hydrolysing cellulose, hemicellulose and other substances resistant to enzymatic digestion by the host animal (Minson, 1990). Hydrolysis is a slow process therefore the rumen is a large organ with a small outlet. The rumen and contents account for 10 to 20% of a ruminant's liveweight.

The rumen is responsible for approximately 55 to 65% of apparent organic matter (OM) disappearance from the digestive tract (Waghorn & Barry, 1987a). Volatile fatty acids (VFA) are formed as the end products of microbial fermentation. During microbial fermentation, energy is conserved as adenosine triphosphate (ATP) and subsequently used for maintenance and growth of the microbial population (AFRC, 1993). Volatile fatty acids, waste products of microbial fermentation, provide the primary source of energy for the host animal and account for approximately 75 to 88% of the energy absorbed from the rumen, caecum and colon (AFRC, 1998).

Dietary carbohydrates form the predominant fermentation substrates, with cellulose, starch, pectin and soluble sugars degraded to hexose, and hemicellulose and some pectin degraded to pentose, before being converted to VFA via pyruvate (Chesworth et al., 1998). Most soluble carbohydrates, starch, and pectins are rapidly and completely degraded in the rumen, with the exception of starch from maize grain. As much as 35% of starch from maize grain can escape rumen fermentation (AFRC, 1998), but this is almost totally digested and absorbed in the small intestine as glucose. Dietary protein can also be a source of VFA particularly in diets such as fresh pasture, which contain

relatively large amounts of rumen degradable protein (Van Soest, 1994). The proteins are hydrolysed to amino acids, which are then deaminated before conversion to VFA.

Forage diets typically result in a mixture of VFA containing 65 to 75% acetate, 15 to 25% propionate, and 8 to 15% butyrate (AFRC, 1998; Mackle et al., 1996). As the amount of starch and soluble carbohydrate in the diet increases, the proportion of propionate formed usually increases at the expense of acetate (Murphy et al., 1982; Sutton, 1985). These changes in the relative proportions of VFA are the result of shifts in microbial metabolism and species (Russell & Hespell, 1981).

During ingestion and rumination most cell walls are ruptured, exposing the protein from cell contents to rapid microbial degradation (Van Soest, 1994). Rumen microbes hydrolyse proteins to amino acids (AA), which are then either directly incorporated into microbial protein or further degraded to form ammonia (NH_3; Waghorn & Barry, 1987a; Lobley, 2003). The ammonia is either incorporated into microbial protein, or is absorbed before being either recycled or excreted after conversion to urea. In fresh pasture diets, approximately 70% of protein is degraded in the rumen (Waghorn & Barry, 1987a), with the remainder escaping to the small intestine whence it is absorbed. Degradation of protein is reduced by any factor that slows the processes of microbial degradation or reduces the time spent in the rumen by the forage (Minson, 1990; Waghorn & Barry, 1987a).

Microbes utilise ammonia, amino acids and energy derived from the hydrolysis of plant carbohydrates to grow and multiply, hence forming microbial protein (Pond et al., 2005). Microbes washed from the rumen form the predominant source of protein available to ruminants. The availability of nitrogenous and energy yielding compounds control the rate of production of microbial protein (Clark et al., 1992). The efficiency of microbial CP synthesis ranges from 98 to 308g/kg apparently rumen digested organic matter (ARDOM; Minson, 1990). In an extensive review, Minson (1990) showed that the mean efficiencies of microbial protein synthesis from fresh forage, dried forage and ensiled forages were 206, 177, and 152 g/kg ARDOM, respectively. The higher efficiency of microbial protein production from fresh forage has been attributed to the higher VFA yield from each kg of DM apparently digested in the rumen (Walker et al., 1975). For the same reason, it could also be expected that forages of higher digestibility

result in greater efficiency of microbial protein production than low digestibility forages. For optimum efficiency of microbial protein production forages should contain about 170g CP/kg DM, and forages containing less than 100g CP/kg DM usually result in low efficiency (McMeninman & Armstrong, 1977).

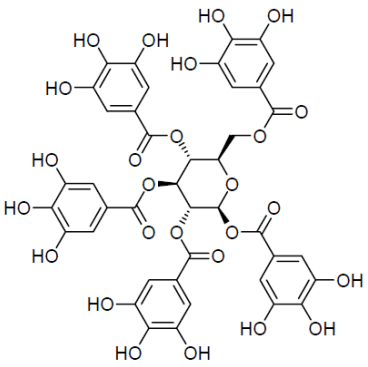
The total CP flowing to the small intestine is the sum of the undegraded plant protein, microbial protein, and any endogenous protein sloughed from the walls of the rumen (CSIRO, 2007). When fresh forage contains more than 130g CP/kg DM, less non-ammonia CP leaves the rumen than enters it, the difference being adsorbed as ammonia (Minson, 1990). Of the non-ammonia CP entering the small intestine, about 80% is true protein and the remainder is predominantly nucleic acids. The net absorption efficiency is 0.7 therefore the yield of AA has been estimated as 560g/kg non-ammonia CP entering the small intestine (Minson, 1990). Undigested CP enters the large intestine and is either deaminated and fermented to VFA, or is excreted in the faeces (Minson, 1990; CSIRO, 2007).

1.3.2 Secondary compounds in the plant

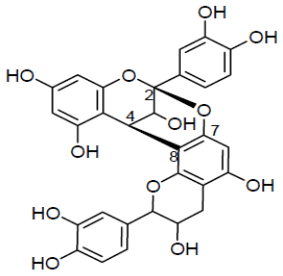
The major secondary compounds found in most temperate and tropical forage plants are condensed tannins (CT) and hydrolysable tannins (HT), phenolic monomers and lignin (Barry et al., 2001; Haslam, 1989). Tannins and lignin are synthesised from monomeric phenols (Barry et al., 2001; Gessner & Steiner, 2005) and these include cinnamates and flavanoid monomers.

Two groups of tannins have been classified according to their structural types (Fig. 1.3); HT and CT or proanthocyanidins (McLeod, 1974; McSweeney et al., 2001; Waghorn, 2008). HT are molecules with a carbohydrate, generally D-glucose, as a central core (McSweeney et al., 2001; Waghorn, 2008). The hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid (gallotannins) or ellagic acid (ellagitannins; Haslam, 1989; McSweeney et al., 2001; Waghorn & McNabb, 2003). Hydrolysable tannins are usually present in low amounts in forage plants (Mueller-Harvey, 2001). These tannins are found in oak (*Quercus* spp.), Acacia, Eucalypts and a variety of browse and tree leaves (Waghorn & McNabb, 2003). HT mainly occur in fruit pods and plant galls and, unlike CT, their degradation products are

absorbed from the small intestine of animals and are potentially toxic to ruminants (McLeod, 1974). Condensed tannins are complexes of oligomers that comprise ten to twelve polymerised flavan-3-ol-units, linked by carbon-carbon bonds (Barry & McNabb, 1999; Min et al., 2003; Waghorn & McNabb, 2003). These will be dealt with in more detail in the following subsection.



(a) Structure of 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucose, a HT



(b) Structure of CT, A-type

Figure 1. 3a & b: Chemical structures of (a) hydrolysable (HT: Mueller-Harvey, 2001) and (b) condensed (CT: Saito et al., 2002) tannins.

1.3.2.1 Condensed tannins (CT)

Of the tannins, CT are the most widely distributed and are normally found in cell vacuoles of some forage legumes, trees and shrubs (Min et al., 2003). They are called condensed tannins because of their condensed chemical structure. CT are also termed proanthocyanidins (PA), which is derived from the acid catalysed oxidation reaction that produces red anthocyanidins through heating of PA in acidic alcohol solutions (Haslam, 1982). Cyanidin (procyanidin) and delphinidin (prodelphinidin) are the most common anthocyanidins produced (Reed, 1995).

Condensed tannins have a complex chemistry. The heterocyclic C-rings can be formed via 2,3-cis or 2,3-trans, which determine "how monomeric units are attached relative to one another" (Barry & McNabb, 1999). CT are polymers of flavanol (flavan-3-ol) units, connected by carbon-carbon bonds that are not susceptible to anaerobic enzyme degradation (Lowry et al., 1996; McSweeney et al., 2001). The number of monomeric units are variable (Foo et al., 1996, 1997) making an "infinite variety of chemical structures, which in turn affect the biological properties of the condensed tannins"

(Barry & McNabb, 1999, p. 264). For example, *Lotus corniculatus* and *Lotus pedunculatus* are considerably different concerning their chemical structure (Foo et al., 1996, 1997).

Table 1. 3: **Condensed tannin concentration in temperate forage species which are of significant value to New Zealand farming systems. CT measured by butanol-HCL method[*] and is a sum of extractable, protein-bound and fibre-bound CT fractions**

Forage	Total condensed tannin (g/kg DM)
Grasses	
Lolium perenne (perennial ryegrass)	1.8
Legumes	
Lotus corniculatus (birdsfoot trefoil)	47
*Lotus pedunculatus (*big trefoil*)*	77
Hedysarum coronarium (sulla)	
Spring	84
Autumn	51
Trifolium repens (white clover)	
Normal	3.1
High CT selection	6.7
Trifolium pratense (red clover)	1.7
Medicago sativa (lucerne)	0.5
Herbs	
Chicorium intybus (chicory)	4.2
Plantago lanceolata (plantain)	14
Forage trees	
Willow *(salix spp)*	33
Poplar *(Populus)*	14

Adapted from (Kemp et al., 2001; Ramirez-Restrepo & Barry, 2005b)
[*] (Terrill et al., 1992a)

The reactivity of CT depends on its concentration, molecular weight (MW) and chemical structure (Barry et al., 2001). The total CT concentration in a range of grazed temperate grasses, legumes, herbs and forage trees are summarized in Table 1.3. The average molecular weight (MW) of CT from *L. corniculatus* is 1900 comprising predominantly procyanidin subunits with epicatechin (67%) the dominate subunit, while the average MW of *L. pedunculutas* is 2200, with the polymer being of the prodelphinidin type, with epigallocatechin (64%) as the major extender unit (Foo et al., 1996, 1997). In addition, *L. pedunculatus* also contains a very high MW CT polymer

(13,200) which may contribute to the differences in biological activity observed between the two *Lotus* spp. (Meagher et al., 2004).

1.3.2.2 Role of CT

When cells break up during chewing (which ruptures about 60% of plant cells; Waghorn, 2008), CT binds to salivary and plant protein by pH-reversible hydrogen bonding. Stable and insoluble CT-protein complexes are formed in the neutral environment of the rumen (pH 6.0–7.5), which disassociate and release protein in the acidic environment of the abomasum (pH < 3.5; Frutos et al., 2004; Jones & Mangan, 1977) and when pH is greater than 8 (Frutos et al., 2004). Addition of polyethylene glycol (PEG; MW 3350) can be used to quantify the effect of CT, in both *in vitro* and *in vivo* studies including CT-containing forages, as PEG causes an exchange reaction where protein is released from the CT-complex and CT binds with PEG to form an insoluble complex (Barry & Manley, 1986; Jones & Mangan, 1977). This releases protein in the forage for normal rumen degradation. The detailed roles are discussed below.

1.3.2.2.1 Protein digestion

The AA requirements of ruminants are essentially provided by microbes which are synthesised in the rumen and also from dietary protein that is not degraded in the rumen but is digested by enzymes in the abomasum and intestines. Of the dietary protein, about 60-90% is degraded by rumen microorganisms (Mackie & White, 1990), and in plant protein, there is usually extensive and rapid protein degradation leading to loss of AA and production of ammonia (Waghorn & Clark, 2004). Some of the ammonia so produced becomes precursor for microbial protein synthesis and is utilized by ruminal bacteria for growth. However, with fresh forages, the rate of ammonia release often exceeds what the microbes are capable of utilising and the excess is absorbed and detoxified by the liver through conversion to urea and passed out as urine (Barry, et al., 2001; Min, et al., 2003; Waghorn & Clark, 2004). There is a 20-35% loss of nitrogen (N) from this process that will therefore be unavailable for use by the ruminant (Barry, et al., 2001; Min, et al., 2003). At the same time, there is also energy loss that goes with protein degradation and also energy cost associated with the process of ammonia detoxification by the liver (Ulyatt, 1997). Waghorn & Wolff (1984) stated that during

the hepatic synthesis of urea, there was a significant use of ME which accounted for up to 4% of available ME, and Van Soest, (1994) indicated an energy cost of 12 Kcal/g of excess ammonia detoxified in the liver. There is also the issue of inefficient duodenal flow of non-ammonia nitrogen (NAN) and absorption of essential amino acids (EAA) in the small intestine. In a review Barry & McNabb (1999) concluded that duodenal flow of NAN in high quality fresh temperate grasses (with trace or no CT content) was only about 75% of the N eaten and that the absorption of EAA in the small intestines was limiting production. There is therefore need to minimise protein losses and inefficiencies in order to increase productivity.

Numerous studies have revealed that CT are able to minimise protein losses in the rumen by binding it into by-pass proteins (Leng, 1997). Waghorn & Barry, (1987a) have indicated that low concentrations of CT present in the diet (10-40g/kg DM) were enough to protect plant protein from degradation in the rumen thereby increasing the NAN flux to the small intestines. Feeding of low and high CT containing *Lotus pedunculatus* (46 and 106g/kg DM, respectively) recorded a total N gain across the rumen of 1.8 and 10.5g/day respectively versus predicted losses of 3.7 and 2.1g/day for non-tannin containing fresh forages at the same total N intake (Barry & Manley, 1984). They concluded that feeding of *Lotus* spp. in the range of 3-106g/kg DM increased AA supply. Barry & McNabb (1999) concluded that duodenal NAN flow increased linearly with increasing concentration of CT and equated the N intake at an approximate CT concentration of 40g/kg DM (Fig. 1.4).

Condensed tannins have also been reported to have an effect on plasma essential AA (EAA) concentration including the branched chain AA. Waghorn et al. (1987b) and Waghorn et al. (1994) reported an increased absorption of EAA from the small intestine by up to 62% units from feeding *L. corniculatus* with about 22g/kg DM CT as opposed to no effect from feeding *L. pedunculatus* containing 55g/kg DM CT (Table 1.4). Although the CT in both *L. corniculatus* and *L. pedunculatus* increased the abomasal/duodenal NAN flow, compared to polyethylene glycol (PEG) supplemented sheep, the increased absorption of EAA in the small intestines was only seen from CT in *L. corniculatus* (Min et al., 2003). The reason for this difference from the two plants is that CT in sheep fed with *L. pedunculatus* reduced the apparent digestibility (% abomasal flow) of EAA in the small intestine, whereas CT in *L. corniculatus* fed sheep did not affect apparent digestibility in the small intestines (Min, et al. 2003).

Bermingham et al. (2001) found similar results by demonstrating that 38g/kg DM CT in sainfoin (*Onobrychis viciifolia*) did not affect apparent absorption of EAA from the small intestine but that 64g/kg DM CT in sulla (*Hedysarum cornarium*) markedly increased the absorption of AA in the small intestine. It was also observed that abomasal flux of total and EAA were increased by the presence of CT in sainfoin and sulla (Min, et al. 2003).

The differing effects upon digestion and absorption of amino acids in the small intestines seen above just confirm that reactivity of CT depends upon its chemical structure, MW and concentration.

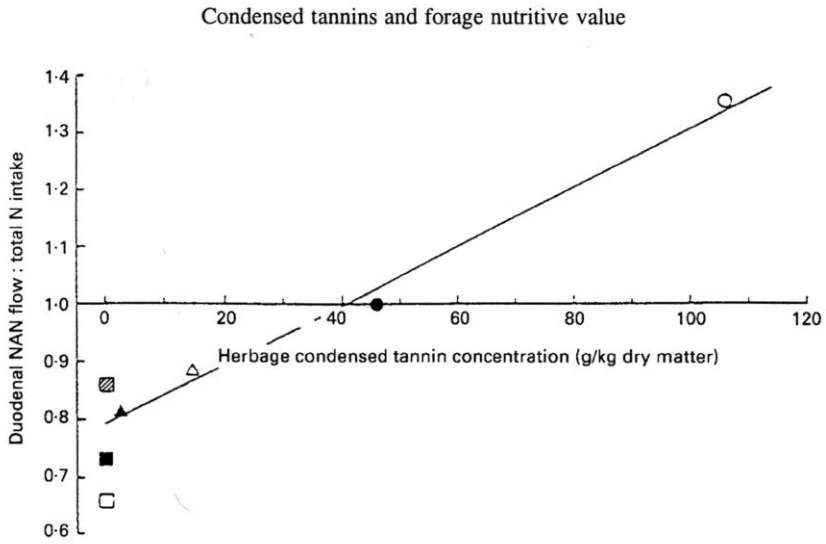


Figure 1. 4: Duodenal non-ammonia nitrogen (NAN) flow per unit total nitrogen intake as a function of herbage condensed tannin concentration in sheep fed on *Lotus* species. (O), High- and (●) low-tannin *Lotus pedunculatus*; (Δ), high- and (▲) low-tannin *Lotus corniculatus*. Results are compared with 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)

Table 1. 4: **Effect of condensed tannins (CT) on the digestion of amino acids in the small intestine of sheep fed *L. corniculatus* (22 g CT/kg DM) or *L. pedunculatus* (55 g CT/kg DM) with (-CT) or without (+CT) a continuous intra-ruminal infusion of polyethylene glycol**

	L. corniculatus		***L. pedunculatus***	
	+CT	–CT	+CT	-CT
Rumen ammonia (mgN/l)	367	504	175	460
CT intake (g/d)	98.9	98.9	103.2	116.8
N digestibility	0.70	0.78	0.67	0.81
Abomasal NAN flux (g N/d)	29.50	25.80	34.00	31.30
Abomasal EAA flux (g/d)	95.60	63.90	121.00	105.60
Apparent EAA absorption (g/d)	58.80	36.20	81.40	83.50
EAA digestibility in small intestine	0.69	0.65	0.66	0.79
Abomasal NEAA flux (g/d)	68.50	60.00	84.30	77.70
Apparent NEAA absorption (g/d)	37.40	41.30	50.80	57.20
NEAA digestibility in SI	0.55	0.69	0.59	0.73

From Waghorn et al., (1987, 1994)

N, nitrogen; NAN, non-ammonia nitrogen; EAA, essential amino acids; SI, small intestine; NEAA, nonessential amino acids

1.3.2.2.2 Carbohydrate digestion

As with protein digestion, CT have also been found to affect carbohydrate digestion at varying concentrations. It has been reported that CT concentration in *L. corniculatus* between 25 and 35g/kg DM did not affect carbohydrate digestion (Barry & McNabb, 1999), while high CT concentration in *L. pedunculatus* (95 to 106 g/kg DM) depressed rumen digestion of readily fermentable carbohydrates (soluble sugar + pectin) and hemicellulose (Barry et al., 1986). The depressed rumen digestion was however offset by increased post-ruminal digestion (Barry & Manley, 1984; Barry et al., 1986).

1.3.2.2.3 Voluntary feed intake

Tannin consumption was believed to reduce the voluntary feed intake (VFI) until in the recent past. It is now largely understood that amount of CT consumed by an animal may elicit either a positive or negative effect on the animal (Frutos et al., 2004). Research work and studies have shown that in general terms, consumption of plant species with high CT contents above 50g/kg DM significantly reduced VFI while consumption of plants with low to medium CT content (< 50g/kg DM) had no effect on VFI (Barry & Duncan, 1984; Barry & Manley, 1984; Barry & McNabb, 1999). While there is a positive flow of duodenal N and NAN utilization post ruminal in the presence of high levels of CT concentration (Barry & Manley, 1984; Barry & McNabb, 1999), VFI is negatively affected (Barry & Manley, 1984). Barry & Duncan (1984) found a substantial depression of VFI by as much as 27% from consumption of *L. pedunculatus* with CT concentration between 63 and 106g/kg DM, while Waghorn et al. (1994)

reported a 12% VFI depression in sheep consuming a CT concentration of 55g/kg DM of the same forage. However, there was no VFI depression in sheep consuming *L. corniculatus* (34-44g/kg DM; Barry & McNabb, 1999). This shows that VFI is affected by the level of concentration of CT in forages.

1.3.2.3.4 Animal production

Since CT consumption affects the VFI and its digestive utilisation, animal productivity will be affected as well. The reported areas in which CT have affected productivity of animals include body growth, reproduction, wool growth, lactation and animal health (Barry et al., 2001; Min et al., 2003; Frutos et al., 2004; Ramirez-Restrepo et al., 2005b).

Sheep grazing willow fodder blocks had better liveweight gain (LWG; 97 g/day vs 86 g/day, respectively) than control pasture (Musonda et al., 2009). Diaz-Lira et al., (2008) also reported better LWG in sheep grazing full access willow fodder blocks as compared to the restricted access willow fodder or control pasture groups. Hoskin et al. (2003) found better LWG in deer fed on chicory (drenched or trigger drenched; 208 and 175 g/day, respectively) as compared to pasture (drenched or trigger drenched; 134 and 60 g/day, respectively). However, *L .pedunculatus* containing 76 to 90 g CT/kg DM significantly reduced LWG in lambs (Frutos et al., 2004).

Reproduction efficiency of sheep, measured by ovulation rate (OR) or lambing percentage, has been found to be affected positively by the action of CT. Sheep mated on *L. pedunculatus* showed a 22% increase in OR over the perennial ryegrass/white clover pasture (Min et al., 2003). Sheep grazing on willow folder blocks recorded better reproductive efficiency than those that were on control pasture (Musonda et al., 2009), and Min et al., (2003) report an increase in lambing percentage due to the action of CT over no CT-containing forages (1.70 vs 1.40 respectively).

Wool is predominantly protein and an action to increase the protein increases the quality of the wool. The beneficial effects of CT from *L. corniculatus* was found to be between 22 and 38 g CT/kg DM (Min et al., 2003; Wang et al., 1996a). The wool growth was negatively affected at CT levels of above 50 g/kg DM and for CT levels below 22 g/kg DM the response was variable (Min et al., 2003).

The action of CT in *L. corniculatus* fed to dairy cows showed a 60% more milk than those fed on perennial ryegrass, and milk protein concentration was increased by about 10% (Min et al., 2003). Whole milk, lactose and protein secretion were increased by 21, 12 and 14% respectively when lactating ewes grazed *L. corniculatus* (Wang et al., 1996b).

Studies have shown that some forage species containing CT may reduce the degree of parasite infestation and improve growth rates in young sheep (Diaz-Lira, 2008; Musonda et al., 2009; Niezen et al., 1993; Waghorn et al., 1995; Waller, 1999). To find out the effect of CT on growth rates of parasitised lambs, regularly drenched (parasite free) lambs grazing CT-containing forage (sulla) were compared with regularly drenched lambs on non CT containing lucerne. The two groups had similar growth rates (200g/d vs 184g/d, respectively). However, better growth rates were reported on undrenched (parasitised) lambs grazing sulla than undrenched lambs grazing lucerne (129g/d vs 39g/d, respectively), making an indication that they could better tolerate the parasite infection ((Niezen et al., 1995). Similarly, Musonda et al. (2009) found that lambs on willow fodder block that were trigger drenched had better growth rates and reproductive performance than those on control pasture either regularly or trigger drenched, and they also had reduced dag formation. Willow fodder blocks failed to replace the need to administer anthelmintics (Diaz-Lira et al., 2008; Musonda et al., 2009) but were successful in reducing the rate of anthelmintic administration (Diaz-Lira et al., 2008).

Bloat is another health problem affecting animals consuming highly fermentable forages containing high levels of protein. Inclusion in the diet of 5 g/kg CT is enough to eliminate rumen frothy bloat through reducing protein solubility (Barry & McNabb, 1999).

1.3.2.3 Mechanisms by which condensed tannins might affect parasites

The antiparasitic effects of CT are believed to be mediated via either a direct anthelmintic and/or indirect nutritional mechanism (Ketzis et al., 2006). CT directly react with the proteins on the surface of the parasites and disturb the normal physiological functions of the nematodes like motility, development, viability and migratory ability of the parasite larvae (Athanassiadou et al., 2001; Molan et al., 2002).

In the indirect mode of action, when tannin-rich forages are consumed, the then released CT build complexes with proteins and protect these from ruminal degradation (tannins have a higher affinity to proteins than any other substances). These complexes dissociate in the abomasum and release protein, ready for absorption. Since nematode parasitism leads to a loss of protein and decrease protein absorption, the intake of tanniferous forages may balance the protein loss and thereby increase resilience (Min et al., 2003). Increased supply of protein is known to enhance immunity to parasites (Ketzis et al., 2006).

The concentration of the CT may determine the mode of action of that CT. Hoste et al. (2006) suggested that for sheep, goats and deer, a concentration threshold of 30-40 g CT/kg DM had to be reached if the antiparasitic effects were to be seen.

1.4 FORAGE FEEDING VALUE

The feeding value (FV) of forage can be defined as the nutritive value (NV) of that forage and the amount of that forage an animal can eat (FV = NV x intake; Waghorn et al., 2007; Waghorn & Clark, 2004) or the production response to grazing that forage under unrestricted conditions (Barry, 1998). The NV of forage is the measure of the ability to meet the animal's requirements for maintenance and production. This ability is dependent upon the nutrient composition, forage type, VFI, digestibility and how the digested nutrients are utilised (Barry, 1998; Waghorn et al., 2007). The FV will also depend on the stage of growth and level of maturity of the forage. Feed intake, which is the difference between the feed on offer and feed refusal, is the driving force of production (Waghorn et al. 2007).

1.4.1 Grasses

Grass (especially perennial ryegrass) as the main source of feed for NZ ruminants has high FV when it is tender and fresh. However, as it matures, its fibre concentration increases, the rate and extent of digestibility decreases and VFI declines, consequently its FV drops (Table 1. 5; Waghorn & Clark, 2004). During its tender stage (especially during spring), the CP content is so high that there is high production of ammonia in the rumen resulting in inefficient use of protein. Energy is usually the first limiting nutrient

and the microbes cannot mobilise enough energy to handle the high ammonia production. When grass has matured, there is usually channelling of the nutrients towards reproduction (flower and seed head formation) and as such the feeding value is lowered. Grazing management is the tool frequently used by farmers to maximise the proportion of leaf and legume and minimise stem development (Lambert et al., 2004).

Table 1. 5: **Chemical composition and nutritive value of perennial ryegrass with maturity as % of DM. Adapted from Waghorn & Clark (2004).**

Perennial ryegrass	Weeks after cutting			
	2	4	6	8
Green leaf	82	63	37	25
Stem	18	37	57	63
Inflorescence	0	0	3	6
Dead leaf	0	0	3	6
NDF	40	45	47	60
Crude protein	15	12	11	6
In vitro digestibility	86	83	79	62
ME (MJ/kg DM)	12.0	10.8	10.9	8.9

1.4.2 Legumes

The FV of legumes is often higher than that of grasses because of their more rapid particle breakdown, quicker rumen fermentation, higher outflow rate and hence greater VFI (Barry, 1998; Ramirez-Restrepo & Barry, 2005b). Besides, legumes tend to remain in vegetative form for a longer time than grasses and hence will provide more nutrients. Legumes will often have higher ME, CP, soluble sugars and less crude fibre content (Table 1.6) than grasses. Whilst the legumes have high FV, a higher proportion in the diet will often limit VFI due to excess production of ammonia and volatile fatty acids during digestion especially when there is insufficient fibre (Waghorn et al., 2007). For this reason, and also the fact that legumes produce less feed per hectare than grass (Ramirez-Restrepo & Barry, 2005b), a mixture of grass and legumes becomes an ideal system of presenting these to animals. In NZ most pastures are composed of 80% ryegrass and 20% white clover mixtures.

Table 1. 6: **A comparison of chemical composition in perennial ryegrass, red clover and chicory. Adapted from Barry (1998).**

	Perennial ryegrass	Red clover	Chicory
ME (MJ/kg OM)	12.3	13.4	13.7
Total N (g/kg DM)	45.2	46.9	19.7
Soluble sugars (g/kg DM)	74	95	111
Ash (g/kg DM)	105	104	149
CT (g/kg DM)	0.9	1.7	1.7

1.4.3 Herbs

Herbs are considered to be annual or perennial crops that are grazable by ruminants within a season or more. The two main herbs that have found their way into the feeding system in NZ are chicory (*Cichorium intybus*) and plantain (*Plantago* spp).

Table 1. 7: **Comparison of feed break down and outflow from the rumen in red deer fed perennial ryegrass and chicory under indoor conditions (Barry, 1998).**

	Perennial ryegrass	Chicory
Composition		
Dry matter (g/kg)	247	161
Total N (g/kg DM)	30.4	26.9
Ash (g/kg DM)	102	180
Apparent digestibility		
Organic matter	0.74	0.82
NDF	0.76	0.68
Rumen pH	6.44	5.63
Particle breakdown efficiency		
Eating	0.37	0.27
Ruminating	0.47	0.65
Chewing time (minutes)		
Eating	221	209
Ruminating	257	30
Rumen fractional outflow rate (%/h)		
Liquid	13.6	18.9
Particulate (lignin)	2.78	4.08
Particulate (ADF)	2.02	4.30
Rumen mean retention time (h)		
Liquid	8.9	6.4
Particulate (lignin)	49.0	37.7
Particulate (NDF)	52.5	27.9

Chicory has higher organic matter digestibility as compared to perennial ryegrass but the NDF digestibility is lower (Table 1.7; Barry, 1998). The lower NDF digestibility is

believed to be due to the lower rumen pH when fed chicory which restricts the rumen microbial degradation of cellulose and hemicelluloses. One good attribute of chicory is that it remains relatively green over the summer period providing fresh vegetation to ruminants despite the fact that it has a strong tendency to go into reproductive state (Barry, 1998). In some studies, chicory maintained in the vegetative state during the summer/autumn period was superior in promoting high growth rates (by over 70%) in lambs than those that grazed perennial ryegrass during the same period (Barry, 1998; Kusmartono et al., 1996; Min et al., 1997). When compared with the legumes lucerne and red clover, there was faster body growth on chicory but not as fast as for animals grazing white clover. Therefore, during the summer/autumn period chicory has better FV than either lucerne or red clover.

1.4.4 Tree forage

Tree forage is more and more recognised around the world as a potentially high feed resource for ruminants, particularly to supply crude protein (Leng, 1997). This is especially true in harsh and arid conditions of the tropical and subtropical regions (Leng, 1997), and also in temperate regions that experience summer droughts (especially in the eastern region of NZ; McWilliam et al., 2004; Moore et al., 2003). The FV of tree leaves is often high because they provide more edible biomass than pasture and this biomass remains green and high in protein, even when pastures dry off and senesce (Leng, 1997). The popular forage trees in NZ are willow (*Salix* spp) and poplar (*Populus* spp) and have been successfully used as source of emergency fodder for sheep and cattle during summer/autumn feed shortage and droughts (McWilliam et al., 2004; Moore et al., 2003). Willow has more edible DM/tree than poplar (Kemp et al., 2003) and as such it is more preferred in animal feeding trials and research.

1.4.4.1 Willow as a tree forage

Willow trees are an inexpensive feed source and provide an annually renewable source of feed for animals (McWilliam et al., 2004). The main edible parts of willow tree fodder are the leaves and fine stems (of less than 5 mm diameter) and these are generally adequate for nutritional maintenance of sheep, goats, cattle and red deer, and the NV is said to be higher than low quality summer pasture (Kemp et al., 2001; McCabe & Barry 1988; Moore et al., 2003). The chemical composition and the low

toxicity of willow compounded by its high FV make it a good feed source for animals especially in the drier regions of NZ.

Table 1. 8: **Chemical composition of pasture diet selected by ewes grazing low quality drought pastures which have been supplemented with willow cuttings. Adapted from McWilliam et al. (2005c).**

	Control drought pasture	Willow supplementation
Metabolisable energy (MJ/kg DM)	7.2	10.4
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

The total N and ME content of willow falls within the range of 16-24 g/kg total N and > 7.5 MJ/kg ME respectively which is enough to support moderate growth in livestock (Ulyatt et al., 1980). It is highly palatable and its digestibility is moderate. In one experiment conducted, it was shown that total N, OMD and ME for willow diet selected by sheep was higher than low quality drought pasture (Table 1.8; (McWilliam et al., 2005c). Furthermore, the same authors found that supplementary willow had lower neutral detergent fibre (NDF; 355 g/kg DM) content compared with drought pasture which had 603 g/kg DM, and the level of CT in willow (Pitta et al., 2007) was as much as 38.3 g/kg DM. Pitta et al. (2007) found similar trends in chemical composition of willow and pasture (Table 1.9). The low content of NDF suggests higher digestibility for willow than low drought pasture. The VFI of willow is appreciably high especially when the CT concentration is lower than 55 g CT/kg DM (Barry, 1985; Barry & McNabb, 1999) as it usually is in willow. McCabe & Barry (1988) reported a 29% lower VFI in willow than that of lucerne hay but at this level, willow was still able to provide more than the maintenance requirement of sheep. This stems from its high digestibility (Pitta, et al., 2007). Willow and poplar supplementation of ewes mated on drought pastures substantially increases reproductive rate (McWilliam et al., 2005a).

Table 1. 9: **Chemical composition of control drought pasture and willow folder block selected diet grazed by sheep. Adapted from Pitta et al. (2007).**

	Control drought	Willow folder block	
	Pasture	Herbage	Trees
Metabolisable energy (MJ/kg DM	8.2	8.8	9.9
Total nitrogen	24.2	20.0	13.6
NDF (g/kg DM)	551	512	417
Organic matter (g/kg DM)	885	916	943
OMD *in vitro*	0.57	0.60	0.67
DOMD *in vitro*	0.50	0.59	0.61
CT (g/kg DM)	1.9	3.6	38.3

1.5 INTERNAL PARASITES AND THEIR ROLE IN SHEEP PRODUCTION IN NZ

Internal parasites are a significant threat facing today's small ruminant producers (Athanassiadou et al., 2001). Problems associated with parasites, particularly those of the gastrointestinal tract of sheep and goats can cause irreversible damage or even death to grazing animals, reduced performance and economic loss for the producer. Animals that are overburdened with parasites can be hindered in their reproductive performance, experience reduced growth rates, and become less productive overall, whether their purpose be meat, fibre, or milk. Prevention and control of the parasites that infect grazing sheep and goats are becoming increasingly difficult in some countries due to excessive use of the available anthelmintics over many years, which has resulted in increasing resistance by the parasites to common anthelmintics.

1.5.1 Gastrointestinal nematode classification

The introduction of sheep into NZ brought with it the whole ecosystem surrounding the animal. Nematodes are not exceptional and about 29 species were unknowingly introduced together with the sheep into NZ (Table 1.10; (Pomroy, 1997; Vlassoff et al., 2001; Vlassoff & McKenna, 1994). Of these species, the abomasal nematodes (*Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus axei*) and the small intestinal species (*Trichostrongylus* spp., *Nematodirus* and to a lesser extent *Cooperia* spp.), have generally been associated with causing production losses and clinical disease (Pomroy, 1997; Rattray., 2003; Vlassoff et al. 2001). Epidemiologically, some of these species tend to be regionally prevalent in NZ. However, *Teladorsagia*

spp. and *Trichstrongylus* spp. can be found in all areas while *Haemonchus contortus* occurs mainly in the North Island and to a lesser extent, the northen part of the South Island (Vlassoff & McKenna, 1994; Vlassoff et al., 2001).

Table 1. 10: **Important NZ nematode parasites of sheep**

Lung	
Dictyocaulus filaria	*Muellerius capillarius*
Protostrongylus rufescens	
Abomasum	
Haemonchus contortus[a]	*Ostertagia (Teladorsagia) circumcincta*[a]
Ostertagia trifurcata[a]	*Ostertagia pinnata*
Ostertagia crimensis	*Ostertagia ostertagi*
Trichostrogylus axei[a]	
Small intestine	
Bunostomum trigonocephalum	*Capillaria bovis*
Cooperia curticei[a]	*Cooperia mcmasteri*
Cooperia oncophora	*Cooperia punctata*
Nematodirus abnomalis	*Nematodirus filicolis*[a]
Nematodirus furcatus	*Nematodirus helvetianus*
Nematodirus spathiger[a]	*Strongyloides papillosus*
Trichostrongylus capricola	*Trichostrongylus colubriformis*[a]
Trichostrongylus vitrinus[a]	
Large Intestine	
Charbertia ovina	*Trichuris ovis*
Oesophagostomum columbianum	*Oesophagostomum venulosum*

[a] currently recognised as important in causing disease and loss of production. Compiled after Tetley (1934), Brusdon (1960), McKenna and Ozock (1971), and Townsend (1993)

1.5.2 Life cycle

The life cycle of almost all strongylid gastrointestinal nematodes infecting sheep is similar. This comprises the adult, egg and four larval stages which basically will take at least 5-6 weeks to complete the whole cycle (Charleston, 1982; Vlassoff, 1982). The cycle starts with adult worms mating in the gastro-intestinal tract (GIT) and females lay eggs (Fig. 1.5a & b) that contain multi-celled embryos which are passed out in the sheep's faeces (Vlassoff et al., 2001).

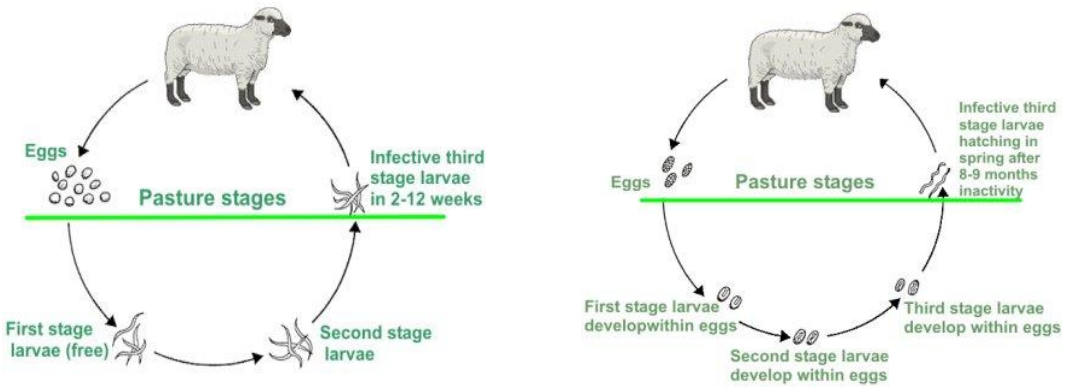


Figure 1. 5: (a) **General life cycle of nematodes** (b) **Life cycle of Nematodirus. (From University of Aberdeen; UoA, 2006)**

With conducive environmental conditions (temperature of 15-30^{o}C), the first stage larva (L_1) develops in the egg, emerges, grows and then moults into the second stage larva (L_2) which develops into the non-feeding infective third larva stage (L_3) and this stage is more resistant to adverse environmental conditions as it retains the cuticle of the L_2 to provide an additional layer of protection (Vlassoff, et al., 2001). The L_3 migrates from the faeces onto the pasture, given a suitable temperature and a moisture film. During cool nights these larvae stay at the base of a grass sward near the ground or in the upper layer of the soil. When sunlight warms the pasture, the larvae migrate up wet blades of grass to settle near the top, utilising the surface tension layer as medium to travel. In this position they are most likely to be eaten by sheep.

Once inside a suitable host (Charleston, 1982; Georgi, 1985), infective larvae become established in the site of predilection appropriate to their species and become adult worms. Before becoming adult worms, a number of processes take place. Upon being ingested, the L_3 larvae shed off the L_2 cuticle (sheath) in response to the stimuli from CO_2 concentration, temperature and pH (Vlassoff et al., 2001). Two further moults will complete their development into mature adult worms which takes about 15-21 days from ingestion. This occurs in their site of predilection (Familton & McAnulty, 1997; Vlassoff et al., 2001). The *Nematodirus* species life cycle is slightly different from the other nematodes (Fig. 1.5b). The ensheathed L_3 larvae of *Nematodirus* species develop within the egg (Charleston, 1982) and hatch in spring after being inactive for 8-9 months (UoA, 2006).

1.5.3 Role of nematodes on farm production and profit

Nematodes have no beneficial effects on farm animals. With large infections they cause clinical disease and possibly death of their host. More commonly smaller infections lead to ill thrift of the host.

These subclinical infections in grazing sheep in NZ have brought about substantial economical losses (Howes et al., 1992). Rattray (2003) has outlined the economically important consequences of nematodes infections which include: reductions in LWG, wool growth or quality, fertility, and milk production; increased mortality; predisposing animals to other diseases; and the possibility that carcasses may end up as unfit for human consumption. The weight loss comes as a result of the reduction in the efficiency of utilization of ME for maintenance and growth owing to the need for the host to mount an immune response and the increased cost of protein synthesis to maintain tissue integrity and function (Bown et al., 1991; Poppi et al., 1986; Van Houtert & Sykes, 1996).

Apart from the effect of parasites on the health and wellbeing of ruminants, there are also cost implications. In his report, Rattray (2003) estimated the losses in meat production incurred by the NZ sheep industry due to parasitism as $92M each year. In addition to the outlined costs incurred, Kempthorne et al. (1996) suggest a further $26M per annum was spent on purchase of anthelmintics of which the largest proportion was spent on lambs. It is estimated that these costs outlined by Rattray in 2003 will not have declined in the intervening years. Thus parasitism with GI nematodes continues to present a challenge to farmers and researchers to find ways of reducing these losses.

1.5.4 Anthelmintic control and drench resistance

The control of parasitic infections has been based on the well implemented use of synthetic broad spectrum anthelmintics (Hounzangbe-Adote, et al., 2005a; 2005b; Vlassoff et al., 2001; Waller, 2006; Watson, 1994). A number of different products have been developed to combat this problem, but they have been used primarily to rid an existing infection rather than prevent infection (Ketzis et al., 2006). There are three major classes of anthelmintics used against gastrointestinal nematodes: macrocyclic lactones (avermectins), benzimidazoles, and imidothiazoles (Watson, 1994; Waller,

2006). In the recent past, various combinations of products have emerged which combine two or three of these products. This has increased efficacy thereby increasing chances of controlling some multiple resistances seen in all action groups (Watson, 1994). Within the last few months an additional broad spectrum anthelmintic group, the amino-acetonitrile derivatives (AADs; Kaminsky et al., 2008a; 2008b; Leathwick et al., 2009), has been released with the single active compound being monepantel (Kaminsky et al., 2008a; 2008b). The mode of action of these products differs from each other. Avermectin binds the glutamate-gated chloride channel to cause increased permeability to Cl^- ions resulting in and hyperpolarisation of nerve and muscle cells which leads to flaccid paralysis (Watson, 1994; Wolstenholme & Rodgers, 2005). Benzimidazoles will kill both worms (interference of intracellular microtubule formation) and the egg. Imidothiazoles are nicotinic agonists and affect the parasites' movement by causing rigid paralysis resulting in worms being flushed down the GIT (Watson, 1994). With imidothiazoles and macrocyclic lactones, eggs remain unaffected and are passed into the environment whence forth they develop into infective larvae (Watson, 1994). The AADs are also nicotinic agonists but act against acetylcholine receptor subunits different to that of the imidothiazoles. They cause hypercontraction of the body wall muscles leading to paralysis, spasmodic contractions of the interior portion of the pharynx and ultimately death of the parasite (Kaminsky et al., 2008b).

Strategic use of these drugs is now being compromised by the increasing resistance to numerous anthelmintics in nematode populations (Lawrence et al., 2006; 2007; Waghorn et al., 2006). Resistance to anthelmintics, defined as 'failure to reduce nematode faecal egg count (FEC) by at least 95%' (McKenna, 1994), is increasing and becoming a global problem (Leathwick & Hosking, 2009). A number of studies have reported nematode resistance to drugs in all three classes of anthelmintics (Gopal et al., 1999; 2001; Leathwick et al., 2001; Macchi et al., 2001; McKenna, 1994; Pomroy, 2006; Scherrer et al., 1989; Waller, 2006). Information from animal health laboratories in NZ has suggested that prevalence of resistance to anthelmintics on sheep farms surveyed indicated the following: benzimidazoles was about 66%, levamisole about 42% and the combination of benzimidazole together with levamisole about 39% (Waghorn et al., 2006). The drug resistance has resulted from a number of factors including the continuous use of the same anthelmintics, a high frequency of use,

exploitation of drugs from various families within the same year, and inappropriate dose rate (Leathwick & Hosking, 2009; Leathwick et al., 2009).

An important concept recommended to delay the emergence of anthelmintics resistance is to maintain a population of unselected parasites within the general ecosystem of the farm. These are referred to as a population *in refugia* (Leathwick et al., 2009; Leathwick et al., 2006b). The resistance to anthelmintics is delayed by maintaining a relatively small proportion of susceptible genotypes in the population to be parents of the next generation (Leathwick & Hosking, 2009). This population *in refugia* should encourage resistant nematodes to mate with non-resistant nematodes to produce viable offspring that are heterozygotes and generally non-resistant to anthelmintics. The principle of creation of a population *in refugia* is a complex one and confusing and several authors approach this issue differently. Leathwick et al. (2008) found that the number of parasites required in the refugia to achieve a level of dilution of resistant parasites surviving anthelmintic treatment was dependent upon the efficacy of the drug used. For instance, a source of unselected parasite in randomly selected untreated animals for an efficacy of 99.9% would require about 1% of the animals left untreated to achieve a 10-fold dilution of resistant survivors. On the other hand, for an anthelmintic treatment of 95% effectiveness it would require about 34% of the flock left untreated to achieve the same level of dilution. This clearly indicates that the population *in refugia* will depend on the efficacy of the drug (Leathwick et al., 2009). Early on, Leathwick et al. (2006a) concluded that if 10% animals were left unexposed to anthelmintics it was sufficient to maintain a susceptible population. Conversely, a study in Western Australia found that leaving 10% of the nematodes unexposed to anthelmintics while maintaining lower levels of anthelmintics resistance, had also potentially increased the parasitism (Besier, 2001) hence lowering productivity (Leathwick et al., 2009). For this concept to work, care has to be exercised not to over- or under-brow the population *in refugia*. The principle behind the population *in refugia* is to create non-resistant nematodes to anthelmintics that are able to infect sheep and dilute the population of resistant nematodes (Le Jambre et al., 2000) and this becomes a tool in delaying resistance.

Other measures that could be taken to avoid development of resistant population include: avoiding treating adult sheep when there is no foreseeable need as this leads to selective for anthementic resistant (Leathwick, et al., 2009); avoiding use of long-acting

anthelmintics especially in ewes pre-lambing (Leathwick, et al. 2001; Leathwick 2004; Le Jambre et al., 1999); ensuring that treated sheep are not put on clean pastures immediately because doing so will lead to strong selection for resistance as any worms surviving the treatment become the major source of subsequent contamination of pasture (Leathwick & Hosking, 2009; Van Wyk, 2001); and by quarantining and effectively drenching all bought-in animals leads to avoid introduction of highly resistant parasites (Lawrence et al., 2006; Pomroy, 2006).

The increasing resistance to all three classes of anthelmintics (Waghorn et al., 2006) has necessitated some farmers to use a combination of anthelmintics just to achieve some level of parasite control. Two reasons have been advanced on the use of combination anthelmintics: 1) to maintain the efficacy when multiple drug resistance has developed; and 2) to slow or delay resistance development (Leathwick et al., 2009). Combination of drugs in this context refers to formulations that combine 2 or more classes of anthelmintics with similar actions rather than combinations targeted to control different species of nematodes. Documented evidence is in favour of use of combination anthelmintics and this treatment option has received wide usage in both Australia and New Zealand (Dobson et al., 2001; Leathwick, 2004).

While there has been success in the use of combinations of anthelmintics to deal with parasites resistant to individual anthelmintics, resistance has also been recorded in these combination products as well (Ducray et al., 2008; Kaminsky et al., 2008a) prompting the need for an anthelmintic with a new mode of action (Kaminsky, et al., 2008a). Monepantel (AADs; Kaminsky, et al., 2008b) was introduced to the market in 2009. It is from a new anthelmintic class which comprise low molecular mass compounds with different aryloxy and aroyl moieties on an amino-acetonitrile core (Ducray, et al., 2008). AADs meet the requirements of a new anthelmintic for ruminants in that: they are low in toxicity; they have broad-spectrum efficacy against nematodes for sheep and cattle; they possess favourable pharmacokinetic properties; and they are able to kill multidrug-resistant parasites. Despite all these good attributes, Kaminsky et al. (2008b) conclude that nematodes can develop resistance to any new drug, including the AADs especially if the resistance problem had not been monitored.

"Prevention is better than cure" and so Anderson (1990) convincingly argued that preventive control was the best way to deal with gastrointestinal parasites. In line with this, Brunsdon & Vlassoff (1982) and Leathwick et al. (1995) both reported that a basic five-drench programme for lambs and hoggets at intervals of 21 to 28 days beginning at weaning in November/December resulted in few worms contaminating pasture with eggs over summer and practically eliminated the autumn larval peak. However, Beckett (1993) suggested increasing the number of drenches to 6 since the observation was that the five-preventive drench programme which commenced at weaning ceased too early to prevent the build up of some genera during late autumn. Furthermore, Beckett (1993) stated that an increase to 6 drenches resulted in further gains in productivity.

In general terms, farmers need to be aware that control of nematodes is a complex issue and the use of anthelmintics poses a challenge, especially when anthelmintic resistance has developed (Pomroy, 2006; Lawrence et al., 2007). Despite this, Lawrence et al. (2007) came to a conclusion that management of anthelmintic resistance was both practical and achievable. However, it should also be noted here that in the foreseeable future a completely parasite-free environment is utopia and effort needs to be directed towards sustainable control systems which don't result in aggressive selection for anthelmintic resistance. Waller (2006b) sees a paradigm shift from the aggressive control with chemicals to more sustainable parasite management systems by living with parasites.

1.5.5 Alternative nematode control strategies

These are control mechanisms or strategies other than the use of chemoprophylaxis (Athanasiadou et al., 2000) aimed at combating helminth problems. The development of resistance against nearly all anthelmintics (Leathwick et al., 2009; Waller, 2006; 2006b; Waller & Thamsborg, 2004), coupled with increasing demand for organic agriculture which strictly prohibits the use of synthetic products (Hordegen et al., 2003; Waller, 2006b) combined with rising consumer concerns about chemical use on farms, has encouraged research into alternative strategies for control of internal parasites particularly in sheep in NZ (Niezen et al., 1993). These include the amalgamation of chemotherapy with grazing management, especially alternate grazing with different stock classes; pasture spelling and renovation; making hay or silage; use of tannin-

containing forages in a grazing rotation; immunomodulants; use of nematophagous fungi; vaccines and targeted inactivation of genes regulating nematode development (Leathwick et al., 2009; Molan et al., 2002; Pomroy, 2006; Sangster, 1999; Sykes & Coop, 2001; Vlassoff et al., 2001; Waller, 1998, 2006; Williams, 1997).

One way to reduce the parasite burden on pastures is through grazing management. This involves a planned rotation programme aimed at limiting exposure to infective larvae on pasture. Knowledge of the life cycle of parasites becomes paramount here. In wet tropical climates, studies have shown that peak larval concentration of *H. contortus* and *Trichostrongylus* spp. occurred on pasture about 7 days post contamination but fell to hardly detectable levels within 28 – 42 days (Barger et al., 1994; Waller, 2006). In investigating this, Barger et al. (1994) set up a grazing trial consisting of 10 paddocks in which each paddock was grazed in a sequence for less than 7 days (3.5 days) to avoid auto-infestation and then spelled for 31.5 days (parasite larvae die within this period). The result showed that the egg counts of goats which grazed in the rotation system were less than half those that grazed outside this rotation system. In the temperate regions, larvae do not necessarily die this quickly. An alternative is interchange grazing between sheep and cattle which has proved to be considerably beneficial in worm control. In this system, grazing management systems exploit host specificity in which case species that are pathogenic to one host will not affect the alternative host or will be less pathogenic or prolific (Waller, 2006). Specifically, this involves alternation of the separate host species at intervals of 60 – 180 days with use of anthelmintic (although not always) at times of alternation (Waller, 2006). On the other hand, there is a possibility in the interchange grazing system of cattle nematodes showing clinical disease in sheep or vice-versa and so relying heavily on this system may prove disastrous.

Biological control of nematodes is another alternative method gaining ground in some parts of the world (Waller, 2006). Several types of biological control mechanisms have been recognized, but the interest here has been the nematophagous fungi (*Duddingtonia flagrans*; (Larsen et al., 1994; Waller & Faedo, 1996; Waller & Larsen, 1993). The fungus exploits the free-living stages of parasites as a source of food (Waller, 2006). It has very important attributes in that chlamydospores of this fungus are able to survive gut passage of livestock and are able to grow rapidly in freshly deposited dung and have nematophagous abilities (Waller, 2006). Control is achieved by the fungus capturing

and killing the infective larval stages before they migrate from dung to pasture where they could be ingested by grazing animals to complete their life cycle (Ketzis et al., 2006). In one experiment, Chandrawanthani et al. (2002) found that dose rates of approximately $1x10^6$ *D. Flagrans* spores/animal/day reduced the percentage of infective larvae development in faecal cultures by more than 90%. This could become a very useful tool where anthelmintics resistance is pronounced.

Plants have recently been studied in connection with antiparasitic effects against nematodes and other beneficial effects on livestock. It has been shown through studies that some plant species that contain CT have the potential to reduce the degree of parasite infestation, allowing the sheep to withstand helminth infection and improve growth rates in sheep (Waghorn, 2008). It was shown that drenched lambs grazing CT-containing legumes (Sulla spp.) grew at a comparable rate to lambs grazing non-CT-containing Lucerne (226 g/day vs 243 g/day) but faster than lambs grazing perennial ryegrass/white clover pasture (166 g/day; Niezen et al., 1998b). On the contrary, undrenched lambs grazing CT-containing legumes had higher daily gains (Sulla; 175 g/day) than when grazed either lucerne (121 g/day) or pasture (88 g/day). Furthermore, Niezen et al. (1998a) reported that parasite burdens at slaughter were similar for undrenched lambs that grazed *Lotus* spp. and pasture but were nevertheless always lower for growing animals grazing sulla. This clearly shows that forages containing CT when grazed by ruminants are able to sustain growth even in a parasitised state.

Work done by Diaz Lira et al. (2008) showed that grazing of weaned lambs, that had not been drenched, in willow fodder blocks showed some marked reduction in the burden of some of the most important internal parasites (*Nematodirus spathiger*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*), compared with undrenched lambs grazing on conventional grass-based pastures, although grazing fodder blocks was not as effective as anthelmintic drenching. It was also shown in the same experiment that non-drenched lambs grazing in willow fodder blocks grew at comparable rates (154 g/day vs. 155 g/day) to drenched lambs grazing perennial ryegrass/white clover pasture but faster (111 g/day) than non-drenched lambs grazing perennial ryegrass/ white clover pasture. More recently, Musonda et al. (2009) concluded that grazing willow blocks played a beneficial role in sustainable farming systems in which the use of anthelmintics could be reduced.

1.6 MECHANISMS OF ALTERNATIVE NEMATODE CONTROL

The mechanisms of alternative nematode control are largely due to the action of CT contained in some forages and documented evidence suggests that the action of CT on internal parasites is in two ways; 1) the CT bind dietary protein (DP) thereby protecting them from microbial breakdown in the rumen resulting in increased proportion of UDP reaching the small intestine, consequently improving the absorption of amino acids by the small intestines (Aerts et al., 1999; Barry et al., 2001; Hoste et al., 2006; Min et al., 2003). Bown et al. (1991) and Coop & Holmes (1996) have stated that the high protein intakes have been associated with increased immunocompetence in young sheep, while Niezen et al. (1995) and Coop & Kyriazakis (2001) have associated the increased protein uptake with reduced negative effects of intestinal nematodes on productivity. 2) the presence of CT is believed to disrupt the nematode life cycle by preventing their eggs from hatching and also preventing L_1 larvae from developing to the infective L_3 larvae (Hoste et al., 2006; Molan et al., 2002). However, the exact mechanisms of action remain unclear (Hoste, et al., 2006) and could differ depending on the parasite, stage of development and the biochemical characteristics of forage species involved (Min, et al., 2003), although it is thought that CT may inactivate enzymes responsible for the hatching process (Molan et al., 2002).

1.7 METHODOLOGY FOR MEASURING EFFECTS OF INTERNAL PARASITES

There are several methods used to measure the effects of internal parasites in ruminants. Each method may have a specific measure in itself but the aims of these methods include: to monitor the overall worm status of the mob and devise a control system; to check for any drug resistance within the mob; to monitor if the previous treatments were effective or not; and also to assess the levels of contamination being put onto the next paddocks (Southwell et al., 2008).

The faecal egg count (FEC) is a technique used to assess whether an animal is infected with parasites and to what extent (MAFF, 1986). This method reveals the number of worm eggs present in the faeces measured by eggs per gram (epg) basis. The modified McMaster technique (Southwell et al., 2008) is the most widely used technique to

determine worm counts. Each egg counted using this method equates to 50 eggs/gram of faeces and the total number of eggs found in the two grids is therefore multiplied by 50 to get the total epg of faeces (MAFF, 1986). While this method provides evidence of presence of the parasites, its accuracy to deduce the concentration of eggs is questionable (MAFF, 1986). A number of factors have been cited by MAFF (1986) which affect FEC, and these are: i) fluctuation of egg counts if faecal samples are taken at different times of the day. It is therefore important to take samples at the same time of the day (Wood et al., 1995). ii) sampling errors arising from unevenly distributed eggs in the faecal sample although this error is not so great. iii) error due to the total quantity of faeces passed out by the animal which ultimately affect the number of eggs per unit faeces weight.

The most precise way of measuring faecal egg output is by total daily egg output which is determined by collecting and weighing all the faeces passed out over a 24-hour period. The epg determined on a representative sub-sample is then multiplied by the total weight of faeces to calculate the total egg output.

The total number of eggs can also be obtained by recovering the adult female nematodes at necropsy and then counting the number of eggs in the female under a microscope. The abomasal and small intestine contents have to be processed as described by Wood et al. (1995). All female worms are identified into their species and then the number of eggs per female is counted. This method is good for comparing the fecundity of female nematodes.

One other method for quantifying internal parasites in faeces is through faecal cultures to ascertain the ability of eggs to develop into infective larvae as well as to determine the proportional composition of the parasite burden. A known weight of faecal sample collected is crushed and then moisturised after which it is incubated at 20-27^{o}C for 7-14 days (MAFF, 1986). The infective larvae are then collected using a Baermann apparatus as described by MAFF (1986) and the nematodes are counted.

Counting of adult worms at necropsy is the most accurate method for determing the worm burden of sheep. This method is more reliable to determine the burden than egg count or larvae counts (MAFF, 1986). After an infected animal has died or is

slaughtered in a humane manner, a worm count is performed on individual sections of the gut (abomasum, small intestines and large intestines) to ascertain the numbers of worms of different species that are present. The screening and dilution technique (MAFF, 1986) is used for this purpose.

1.7.1 Effects of bioactive forages

Bioactive forages are plants containing CT – the bioactive substances having the antiparasitic compounds or nutraceuticals which are generally non-toxic and can consequently not be overdosed and therefore can be incorporated in the normal diet for ruminants. Different plants contain different types and levels of bioactive substances that will affect the nematodes at different stages of their development.

1.7.1.1 Nematode establishment

Condensed tannins are believed to have a profound effect on nematode establishment. Niezen et al. (1998a) found that there was an overall establishment of 26-34% of *T. circumcincta* larvae in lambs given lotus vs 43-53% in lambs fed ryegrass. This showed a reduction in the establishment of *T. circumcincta* in lotus fed lambs suggesting that CT could have been the cause of this. Other records have indicated reduction in adult worm burden. In one experiment, lambs grazing *L. corniculatus* showed fewer adult abomasal and small intestine worms than those grazing the other forages (Table 1.11; (Marley et al., 2003a). Conversely, Tzamaloukas et al. (2005) found a significant reduction in worm establishment in lambs fed on lotus, sulla and grass/clover with values of 65, 63 and 68%, respectively, while lambs fed on chicory did not show a significant reduction (37% reduction).

Table 1. 11: **Rank transformed total adult nematode intensity per gram organ weight of lambs naturally infected with parasites, after grazing chicory, lotus and ryegrass/white clover for 35 days. Adapted from Marley et al. (2003).**

		Adult nematodes	
	Abomasum	Small intestines	Large intestines
Chicory	10.8	15.0	11.0
Birdsfoot trefoil	6.9	8.1	13.1
Ryegrass/white clover	18.3	14.4	13.4

There seem to be establishment differences between male and female nematodes in sheep fed on CT containing forages. In one trial, lambs fed on lotus had fewer female *O.circumcincta* and the male: female ratio was also higher (Table 1.12; Niezen, et al., 1998b). The reason for this difference could not be determined. In the same trial, no establishment differences were found between male and female *T.* colubriformis nematodes fed the same diet (Table 1.12).

Table 1. 12: **The effects of ryegrass and lotus on adult male and female *O. circumcincta* and *T. colubriformis* establishment, eggs per female and male to female ratios. Adapted from Niezen et al., 1998b)**

	Females	Males	Eggs per female	Male to female ratio	Total
		Ostertagia circumcincta			
Ryegrass-PEG	2327 [a]	2007 [a]	27.8 [a]	0.86 [a]	4334 [b]
Lotus-PEG	1368 [c]	1978 [a]	36.1 [b]	1.69 [b]	3346 [bc]
Ryegrass+PEG	2909 [b]	2380 [a]	33.5 [ab]	0.84 [a]	5289 [a]
Lotus+PEG	1349 [c]	1236 [b]	25.2 [a]	0.92 [a]	2585 [c]
		Trichostrongylus colubriformis			
Ryegrass-PEG	3051 [a]	2538 [a]	21.4 [a]	0.84 [ab]	5589 [a]
Lotus-PEG	3349 [ab]	3058 [a]	21.9 [a]	0.91 [b]	6407 [a]
Ryegrass+PEG	3338 [ab]	2930 [a]	19.9 [b]	0.85 [ab]	6269 [a]
Lotus+PEG	3525 [b]	2716 [a]	22.4 [a]	0.77 [a]	6242 [a]

Values in columns with different superscripts indicate differences between treatments significant at the 5% level

1.7.1.2 Nematode fecundity

Fecundity refers to the egg laying capacity of female nematode measured either by counting the eggs in each female worm after recovering them from the gut or by dividing the FEC per animal by the total female worms recovered from that animal (Athanasiadou et al., 2005; Min & Hart, 2003). CT have been reported to have an effect on the nematode fecundity. Paolini et al. (2003a, b) demonstrated on goats that the administration of extracts from tanniniferous plants was able to decrease egg excretion due to a decrease in the fecundity of female worms without changes in worm burdens. The per capita fecundity of worms of *T. colubriformis* was reported reduced in sheep drenched with 16% Quebracho CT of food intake compared with worm fecundity in control sheep (0.055 vs 0.181 eggs/female worm per day; Athanasiadou et al., 2000; Athanassiadou et al., 2001), although these effects could not be conclusively attributed to action of CT (Min & Hart, 2003). However, some results from grazing sheep on

different forages with or without CT contents showed no significant difference in worm fecundity (Athanasiadou et al., 2005; Niezen et al., 1998a; Tzamaloukas et al., 2005).

1.7.1.3 Egg hatching

CT have been associated with a reduction in the faecal egg output and a number of experiments have shown this (Barry et al., 2001; Barry et al., 2006; Diaz-Lira, 2008; Marley et al., 2006; McCabe & Barry 1988; Musonda et al., 2009; Niezen et al., 1998b; Ramirez-Restrepo & Barry, 2005b). If these reductions in faecal egg output were accompanied by reduced egg hatchability and/or larval development/survival, Max et al. (2007) suggest that this effect would be of epidemiological value as far as pasture contamination by infective larvae were concerned. Indeed some records have shown that CT have the ability to reduce the egg hatchability. In an *in vitro* study of *T. colubriformis* eggs using several CT containing forages, Molan et al. (2002) showed a significant reduction in the egg hatchability to all the forages used compared with the control and that hatchability was affected with concentration of CT extracts. Compared to control wells at 92% hatchability, the hatchability in CT containing forages ranged from 63 – 78% at 200µg/ml CT concentration and at 400µg/ml CT concentration, the hatchability ranged from 56 – 76%. It was significantly lower at CT concentration of 900µg/ml than it was at 400µg/ml. In another *in vitro* study, similar results were observed with *H. contortus* eggs (Fig. 1.6; Iqbal et al., 2007). Since CT are not absorbed in the digestive tract (Terrill et al., 1994), they become concentrated in the faeces, hence affecting the hatchability of nematode eggs and this leads to lower pasture infestation (Iqbal et al., 2007).

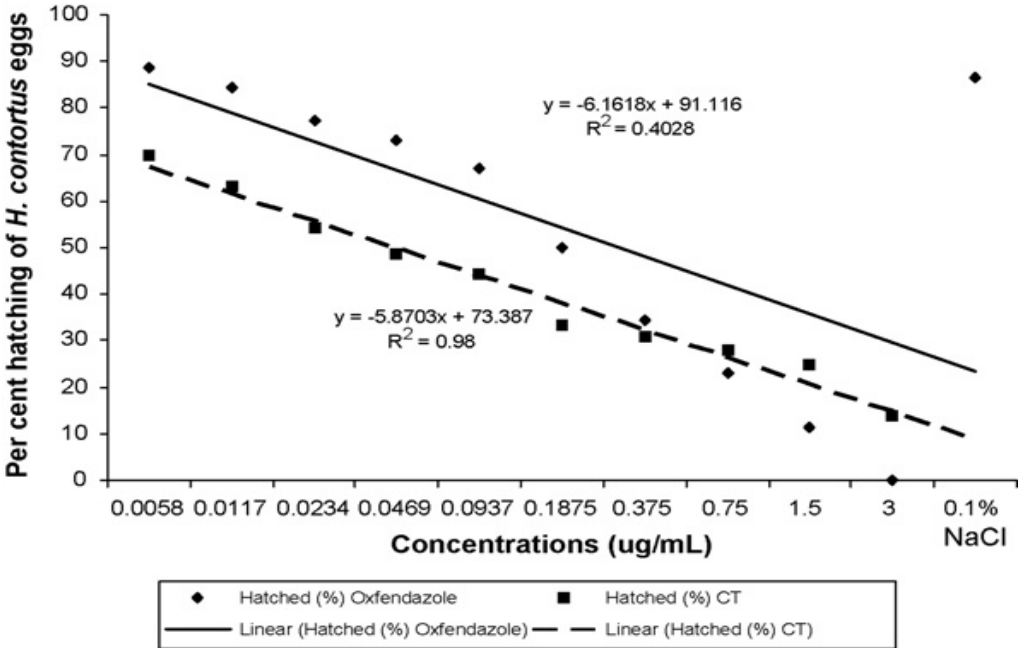


Figure 1. 6: **Showing regression analysis of percentage of eggs hatched in various concentrations of oxfendazole and condensed tannins *in vitro* (Iqbal et al., 2007).**

Molan et al. (2003b) carried out *in vitro* studies to determine whether and/or which of the monomer units of CT (flavan-3-ols) were responsible for the prevention of the egg hatching capacity of *T. colubriformis*. All the flavan-3-ols (C, EC, GC and EGC) and their galloyl derivates (CG, ECG, GCG and EGCG) showed inhibitory effects but with different trends. Among the flava-3-ols, only C and EGC showed significant reduction in hatching at low concentrations of 100µg/ml although none of them exceeded 20% inhibition of egg hatching. Conversely, all the flavan-3-ols gallets showed some inhibition of egg hatching at 100µg/ml and there was complete inhibition at 1000µg/ml (Molan, et al., 2003b).

In vitro studies seem to have shown inhibitory effects on egg hatching (Molan et al., 2002; 2003b; Iqbal et al. 2007) but it appears the grazing trials have failed to prove likewise. In a comparison of four forages (chicory, birdsfoot trefoil, ryegrass/white clover and rygrass/red clover), none of the forages showed any inhibitory effects on egg hatching (Marley et al., 2003b).

1.7.1.4 Larval development

There are suggested ways by which CT may affect the development of the larvae. One of the ways suggested by Athanasiadou et al. (2001) is that ingested CT by the larvae may bind to the intestinal mucosa thereby decreasing metabolism through inhibition of oxidative phosphorylation leading to larval death. Another suggestion is that the CT complex with available protein either in the faeces or animal itself inhibits the available nutrients for larval growth leading to starvation and death (Min & Hart, 2003; Iqbal, et al., 2007). CT may also bind with the glycoprotein found on the surface (cuticle) of the larvae leading to death (Athanasiadou et al., 2001; Molan et al., 2002). However, the actual mechanisms are not clear, but what are seen are the end results of these proposed mechanisms. For instance, Min et al. (2005) found a 69% larval development reduction in an *in vitro* study attributed to CT action.

1.7.1.5 Larval migration on plants

The effect of CT on the migration of nematodes has been investigated using the larval migration inhibition assays (LMI; Alonso-Diaz et al., 2008; Molan et al., 2003a; 2003b). All these *in vitro* trials showed that CT negatively affect the locomotive ability of the nematodes. This therefore means that the nematodes may not have the ability to migrate from the infected faeces onto the plant where they can be picked by grazing animals.

1.7.1.6 Immunological aspects

The immune system in sheep can either be innate or acquired. Innate immunity is the inherent ability of some sheep to resist infection with worms. Acquired immunity develops in sheep after they have been exposed to worms. Most adult sheep have good acquired immunity to worms, whereas lambs do not. Whatever the kind of immunity the animal may have, it is important to boost it up especially in young lambs and pregnant ewes. Proper nutrition will help boost this. CT may play a role here through their indirect antiparasitic effects (Min et al., 2004). It is suggested that the protein binding by CT help increase the protein supply which may enhance host immune response against GI nematodes (GIN; Barry & McNabb, 1999; Min, et al., 2004; Ramirez-Restrepo & Barry, 2005b; Hoste et al., 2006).

The immune responses to infection involves two T helper (Th) cells: type 1 Th (Th1) cells are responsible for cell-mediated immunity against microorganisms and other intracellular parasites while type 2 Th (Th2) cells mediate the antibody-dependent immunity against extracellular parasites and that includes the gastro-intestinal nematodes (GIN; Koski & Scott, 2001). Experimental studies have shown that functional immunity to GIN involves systemic Th2-type cytokines and effectors (Lawrence et al., 1996), which produce a dominant immunological phenotype (Koski & Scott, 2001). It is believed that the expression of the two Th cells depends on the level of nutrition and they seem to be antagonistic to each other (Koski & Scott, 2001). However, the concern here is the Th2 responsible for immunity against GIN. When the animal is undernourished, the Th2 phenotypes are prevented from expressing their immunological capacity (Koski et al., 1999) and their absence results in prolonged survival of GIN (Koski & Scott, 2001). If energy, proteins and vitamin A are in deficit they will prevent expression of Th2. In CT fed animals, the increased immunity to parasite could therefore be associated with the enhanced Th2 dominant phenotype to express their immunological abilities. This is because bound proteins released in the abomasum and small intestines help to boost immunity.

Recently, a grazing trial involving young sheep was conducted to compare the immune response from willow fodder blocks containing CT and a control comprising perennial ryegrass/white clover (Ramírez-Restrepo et al., 2010b). Half of the lambs grazing each system were regularly drenched, whilst the other were trigger drenched. The results showed that trigger drenched lambs fed on willow fodder block containing CT had increased blood platelet and eosinophil counts; had increased total white blood cells and total lymphocytes; had increased size of $CD21^{+}$ and gamma deltas TCR^{+} lymphocyte subsets compared to trigger drenched lambs grazing pasture. The increase of white blood cells, in general, is an indication of antibody formation against pathogens. Although such effects were seen, Ramirez-Restrepo et al. (2010b) concluded that the higher immunological levels seen in trigger drenched sheep grazing willow fodder block could have been due to increased larval intake and/or due to CT in willow priming the immune response.

1.7.1.7 Grazing systems studies

Grazing management is one way of controlling the parasites in grazing ruminants. A carefully executed programme will ensure less worm burdens to livestock. What is known, however, is the fact that environments favouring the establishment and maintenance of improved pastures will equally be favourable for the development, establishment and transmission of nematode parasites to ruminants (Waller, 2006; Taylor et al., 2007). Transmission of these nematodes is accelerated by the presence of the favourable host. The separation of the two (parasites and host) will ensure less worm burden to livestock and this could be achieved by preventive, evasive and diluting as proposed by (Michel, 1985). Preventive strategies aim at putting nematode-free animals onto clean pasture. However, as Leatherwick & Hosking (2009) stated, there is a danger of perpetuating resistance to anthelmintics due to lack of population *in refugia* in a clean paddock. Evasive strategies do not restrict pasture contamination by worm eggs but aim at moving livestock to another pasture just before the hatching of the eggs on the original pasture. On the other hand, diluting strategies aim at grazing susceptible animals together with a large population of animals of the same species or of different livestock species with naturally acquired resistance to parasites in order to reduce the herbage infestation with worm eggs. These grazing management strategies in combination with anthelmintic drenching were effective as far as it were concerned (Waller, 2006). However, as discussed before in section 1.5.4, this brings about danger of anthelmintic resistance by nematodes.

The height of the pasture sward has an effect on parasite infestation. Many of the worm larvae crawl only about 2.5 cm from the ground onto plants, so not allowing animals to graze below this level will cut down on a lot of infestation (Fig. 1.7; West, et al. 2002). Despite this limit in larval migration, other conditions such as moisture availability and optimum temperature will determine how far the larvae will migrate.

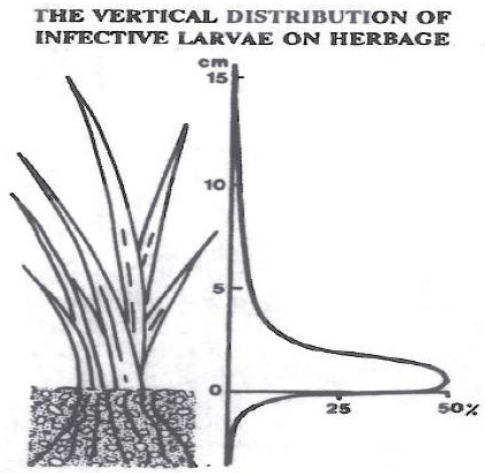


Figure 1. 7: **Showing the relative distribution of infective nematode larvae on pasture (Vlassoff, 1982)**

Where environmental conditions allow it is a good idea to move animals into a new paddock when moisture (film of water) has dried down. Equally of importance to note is that forage type and animal type will determine the infection levels. For instance, lower vegetation (such as most pasture grasses) may encourage infections than would higher vegetation forage (such as willow or poplar) and similarly, grazing herbivores are more likely to be infected than would the browsers (Apio et al., 2006).

Another very important point to note is the stocking rates effect on parasitism. In some grazing system management, pasture is best utilised with increased stocking rates which culminate into high productivity (Waller, 2006). However, increased stocking rate also increases the demand for feed and consequently there is also increased rate of parasitism in ruminants (Taylor et al., 2007; Thamsborg et al., 1996) since pasture will need to be improved and any condition favouring pasture improvements also favours nematode worm infestation.

A grazing system that includes use of CT-containing forages may help reduce the worm burden or sustain the parasitised animals either directly or indirectly as has been discussed earlier. As has been stated, "By analogy then, any specific parasite control method may be unsustainable when used in isolation and the more choices and the greater the variety of controls used in combination, rather than relying almost sorely on anthelmintics, the longer effective worm control can be expected" (Waller, 2006, p. 277). A deliberate policy of mixing the main pasture grass with forages containing CT

and high nutritious herbs would help reduce the anthelmintic use to minimum. So far, the herb chicory and the CT containing forages (*Lotus* spp. and sulla) have been found to promote fast growth in lambs and also they have reduced the parasite burdens (Ramirez-Restrepo & Barry, 2005b) and therefore should have an advantage if used in a grazing system.

1.8 CONCLUSION AND NEED FOR FUTURE RESEARCH

- New Zealand's economy is largely based on agriculture and pastoral farming is the major contributor to NZ's economy. The major farmed animals are dairy, sheep, beef cattle and deer. Sheep numbers have shown a downward trend in the recent past due to a shift of land use in favour of dairy and forestry. Despite the fall, the lambing percentage has increased.

- Grazing ruminant animals are used to harvest and convert plant materials into food and fibre products for the benefit of man. This is an efficient low cost production system. The harvesting potential of ruminants will depend on the grazing system in use. Equally, the grazing system in use will also determine the parasite burden. The main grazing systems are: set or continuous stocking system, rotational paddock grazing, and strip grazing.

- There are limitations with the use of forage diets. The perennial ryegrass/white clover mixture has moderate energy concentration, low digestible DM intake, and low concentration of soluble carbohydrates but has high CP and fibre concentrations. The low nutrient content limits animal productivity and also limits the potential for resistance against parasites and other diseases. The high CP content and insufficient UDP lead to excessive rumen ammonia production which is removed at high metabolic cost.

- Gastrointestinal nematode parasites are major limiting factors to the productivity of young grazing sheep. If not controlled they cause high morbidity and mortality in these ruminants and the control of GIN costs more than NZ$300M/annum. The effects on the animal include loss of appetite and

reduction in metabolic efficiency which ultimately decreases the nutrients available for body maintenance and growth.

- The chemical composition of forage determines the nutritive value of that feed and ultimately will determine the properties of that forage in the body of the animal. The chemicals contained in forage can either be primary or secondary compounds. Major primary compounds are carbohydrates and proteins. The presence of microorganisms in the reticulo-rumen make it possible for extraction of energy from high fibre containing forages through the rumen fermentation process and make it usable by the ruminant. Most extracted proteins are converted to amino acids and ammonia, some of which is incorporated into microbial protein which is later digested by the animal intestinal enzymes into AA. Some of the forage proteins escape microbial digestion (UDP) and are therefore digested in the abomasum by animal enzymes. Good quality proteins help boost immunity against internal parasites.

- The secondary compounds CT and HT are mainly found in cell vacuoles of some forage legumes, trees and shrubs. The two compounds differ in chemical structure. CT are the most widely distributed and their reactivity depends on the concentration, molecular weight and chemical structure. They play a major role in the digestion of primary compounds and also in animal production related issues. CT protect proteins from microbial digestion in the rumen. A CT concentration between 10 – 40 g/kg DM is enough to protect the protein. Proteins are bound by CT at pH 6.0 -7.5 and released in the abomasum at pH<3.5. At moderate CT concentration (25-35 g/kg DM), carbohydrate digestion is not affected but at higher concentration (95-106 g/kg DM), readily fermentable carbohydrate digestion is depressed. Voluntary feed intake is not affected at CT concentration less than 50 g/kg DM. CT at moderate concentrations promote body growth, reproduction, wool growth and animal health. Frothy bloat is effectively controlled with inclusion of 5 g/kg DM of CT in a diet.

- Antiparasitic effects of CT are believed to be mediated via direct anthelmintic and/or indirect nutritional mechanisms. CT react directly with organism protein, since CT have high affinity for proteins, thereby disturbing normal physiological functions of the nematodes. On the other hand, the indirect action of CT is through bound proteins which increase protein availability in the abomasum, correcting a balance between protein loss through nematodes action and that which is absorbed and as such resilience is increased. A CT concentration threshold of 30-40 g/kg DM is necessary if antiparasitic effects are to be seen.

- Legumes have higher FV than grasses. However, grass is the main source of ruminant feed in NZ. During the vegetative stage of grass, the CP content is very high and exceeds the rate of microbial protein synthesis, leading to high rumen ammonia release. This leads to inefficient use of proteins. Legumes remain in the vegetative state for a longer time than grass and because legumes breakdown quicker with higher outflow rate, the VFI is also higher. Furthermore, legumes tend to have higher, ME, CP, soluble sugars and less fibre content than grass. Tree forages on the other hand are less used in NZ but slowly gaining popularity because of their CT content and the FV is often high. The commonly used tree forages are poplar and willow. These have been successfully used as emergency sources of feed during summer/autumn feed shortage and droughts.

- Willow tree forage is gaining popularity as animal feed especially in drier regions of NZ because of its low toxicity and its high FV. The edible parts are mainly leaves and fine stems (less than 5 mm diameter). The N content ranges from 16-24 g/kg DM and ME 9.5-10.5 MJ/kg DM and CT is as much as 35-50 g/kg DM. Its NDF is far less than drought pasture (355 g/kg DM vs 603 g/kg DM willow and drought pasture, respectively).

- All sheep nematodes have a basic life cycle comprising adult, eggs and four larval stages. The L_3 is the most infective stage and most resistant to adverse environmental conditions. No nematodes are beneficial to animal productivity and welfare. Their effects include: reductions in LWG, wool growth or quality,

fertility, and milk production; increased mortality; predisposing animals to other diseases; and reducing carcass quality.

- The common control method of internal parasites is through use of oral anthelmintic drenches. Three major classes of anthelmintics are used: macrocyclic lactones, benzimidazoles, and imidothiazoles. These chemicals have been very effective until nematodes started developing anthelmintic resistance against these action groups. Combinations of these action groups have in the recent past been developed to combat the resistance. The recent advance of these broad spectrum anthelmintic groups on the market is AAD's with active monepantel. Resistance against this latest class of anthelmintic will eventually appear.

- The development of resistance to anthelmintics and consumer concerns about the presence of these chemicals in animal products has led to studies into alternative control strategies in NZ sheep production system. Several methods are being practiced and the use of tanniferous plants is on an increase. Studies with lambs grazing willow fodder blocks have shown reduction in parasite burdens and at the same time, promoted increased body growth. It has also been shown that CT may disrupt egg hatching, larval migration and development into the next stage. The exact mechanisms of action remain unclear but the effects of this action can be measured through FEC, faecal egg output, eggs/adult female worm, faecal cultures and counting of adult worms at necropsy.

- Several CT-containing forages have been studied and satisfactory results obtained in the areas of parasite burden reduction, LWG, wool growth and milk production. There is now need to study the mechanism of how feeding willow fodder might affect established internal parasites, including any effects on parasite mortality and on female parasite egg laying abilities (i.e., fecundity).

1.9
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CHAPTER 2

THE EFFECT OF FEEDING WILLOW UPON THE DEATH OF ESTABLISHED PARASITES AND UPON PARASITE FECUNDITY

2.1 INTRODUCTION

New Zealand (NZ) has relied mainly on the use of anthelmintics to control internal parasites in grazing sheep. While these control measures have initially been effective, continued regular use of anthelmintics has led to the development of resistance by these parasites (Ketzis et al., 2006; Leathwick, 2004; Leathwick, D M. et al., 2001), coupled with consumer concerns about chemical residues in animal meat products (Niezen et al., 1993). These concerns, plus the cost of anthelmintic control in NZ (more than NZ$300M per annum; Rattray, 2003), with more than 50% of animal remedy expenditures on a world wide scale being spent on anthelmintics (Coles, 2002), have led to research into alternative control methods against these parasitic nematode worms (Diaz-Lira, 2008; West et al., 2002).

Nutritional control of parasites (Kyriazakis & Houdijk, 2006) is one area of interest and novel plants are being studied for this application (Ketzis et al. 2006). These include plants containing condensed tannins (CT; Barry et al., 2001; Diaz-Lira, 2008; Min et al., 2003; Musonda et al., 2009; Niezen et al., 1995, 1998b; Ramirez-Restrepo & Barry, 2005b) that are believed to have some antiparasitic action (Ketzis et al., 2006; Waghorn, 2008), and also to increase the absorption of essential amino acids (EAA) from the small intestines (Barry & McNabb, 1999; Min et al., 2003) leading to enhanced immuno-responses (Kyriazakis & Houdijk 2006).

A number of studies have been done to define the anthelmintic effects of CT; these include the indirect mode of action (Min et al. 2003) and the direct mode of action (Athanassiadou et al., 2001; Molan et al., 2002). In the indirect mode of action, the CT form complexes with proteins and protect these from ruminal degradation. These complexes dissociate in the abomasum and release protein, thus increasing protein absorption. Since nematode parasitism leads to a loss of protein and decreased protein absorption, the intake of tanniferous forages may counteract the protein loss and thereby increase resilience. In the direct mode of action, CT directly react with the proteins on the surface of the parasites and disturb the normal physiological functions of the nematodes such as mobility, food absorption or reproduction. For CT to be effective in these reactions, the CT contents in forages must range between 35 – 55 g/kg DM (Athanasiadou et al., 2005; Min & Hart, 2003). Higher concentrations of CT (>55g/kg

DM) are known to lead to reduced feed digestibility, reduced feed intake and consequently lower productivity (Aerts et al., 1999; Barry & McNabb, 1999; Dawson et al., 1999). Coop & Kyriazakis (2001) concluded that the intake of tanniferous plants would only be beneficial in grazing small ruminants if the negative consequences were offset by the positive effects attributed to their anti-parasitic properties.

Willow trees have been used as a supplementary feed during drought conditions in the drier regions of NZ (Wilkinson, 1999) and the leaves plus small stems contain 30-50 g/kg DM of CT (McWilliam et al., 2005a). CT have the potential to help control parasite nematodes infections (Diaz-Lira et al. 2008) and since they also increase protein supply by protection of protein from microbial degradation (Barry & McNabb, 1999), the increased protein supply could enhance immunocompetence (Bown et al., 1991; Coop & Holmes, 1996). These actions together could potentially lead to reduced use of anthelmintics (Niezen et al., 1995; 1998b). The aim of this study was to examine the mechanism of how willow feeding might control internal parasites in young sheep through studying effects upon the death of established parasites and upon parasite fecundity.

2.2 MATERIALS AND METHODS

Two indoor experiments were conducted at the Animal Physiology Unit (APU) of Massey University, both involved young sheep and both involved studying effects of feeding willow and lucerne diets upon aspects of parasitology. In both experiments, the weaned lambs were males and were shorn.

2.2.1 EXPERIMENT 1

Experimental Design

A 10 week feeding experiment was conducted from 20/01/09 to 01/04/09 to investigate the effect of willow feeding upon the death of established worms and upon worm fecundity. This involved 24 weaned lambs housed indoors in individual pens and randomly allocated to 2 experimental groups (both groups had similar average weights) comprising 12 animals in each group. Chaffed lucerne feed was used as a control. An adaption period of 5 weeks was allowed for the animals to get used to pen handling, pen feeding and worm establishment with the final 5 weeks as experimental weeks comparing the two diets. Chaffed lucerne was fed *ad libitum* to all animals during the adaption period; during the experimental period one group was fed chopped willow while the other group was fed lucerne chaff. Both of these feeds were fed as a sole diet. The infection with L_3 larvae comprising *Teladorsagia*, *Trichostrongylus*, *Cooperia* and smaller numbers of *Haemonchus* was given 3 weeks after the start of the adaption period (2 weeks before willow feeding commenced). Throughout the experimental feeding period, daily sampling of feed offered and refused, weekly faecal sampling and liveweight measurements and in the last two weeks total faecal output collection, were undertaken. The lambs were slaughtered at the end of Week 10. Factors investigated included FEC (eggs/gram and total faecal egg excretion), larval and adult worm counts (representing establishment of these worms), fecundity per female nematode (from eggs in uterus of female worms and from eggs excreted per established female worm), liveweight change, voluntary feed intake (VFI), apparent digestibility, haematology and serum biochemistry, carcass weight and GR measurements.

2.2.1.1 Animals and housing

Experimental lambs were sourced from Tuapaka Farm (Massey University, NZ). Initially, 30 weaned lambs were selected and brought to the APU and upon arrival, they were effectively treated with a combination anthelmintic containing 22.7 g/L oxfendazole + 40 g/L levamisole + 1 g/L abamectin (Matrix, Ancare New Zealand Ltd, Auckland, NZ). The lambs were also treated with Zapp pour-on at 20 ml/sheep (Bayer New Zealand Ltd) to remove external parasites and this was done only once. They were then identified with numbers from 1 – 30. The initial weight was also taken and recorded (27.9 kg ± 0.24). The placement of lambs into the labelled pens (1 – 24) was done at random and the extra 6 lambs were placed in mobile cages in the animal house. On the 5^{th} day of Week 1, the non responsive lambs, based on non consumption of feed and bad temperament, were removed. The remaining 24 animals were fitted into the pens. All lambs were restrained by means of collar chains fixed to the pen rails.

Weight monitoring of the animals was done once a week at the same time of the day using electronic scale (Tru-test, Auckland, New Zealand). Rectal faecal samples for FEC were taken at the same time. During the final two weeks of the experiment, total faecal collection to measure total daily faecal output was undertaken over a 24-hour period on 3 consecutive days of each week. Harnesses and bags were placed on each animal to trap the faeces and the emptying of the bags was done every 12 hours. The collection for the day (24-hour period) was pooled per animal.

2.2.1.2 Feeds

Lucerne chaff was purchased from Premier Stock Feeds Limited, Fielding, New Zealand, while willow fodder was supplied by Akura Nursery, Greater Wellington Regional Council, Masterton, New Zealand. On three occasions per week, willow was cut in the morning and transported in the afternoon from Masterton to Palmerston North. Upon arrival at the project site, the willow was kept at 4^{o}C before feeding. The chopping of willow was done daily just before the morning feeding, offering approximately 5 cm lengths with a diameter of less than 5 mm.

Plate 1: Lamb eating chaffed lucerne

Plate 2: **Lamb eating chopped willow**

During the first 5 weeks (adaption period), all lambs were fed *ad libitum* with lucerne. In the second 5 week period half the lambs were fed willow. The willow treatment was also given *ad libitum* but the lambs were quite selective of the parts they consumed, mainly selecting leaves and very soft twigs. From Week 2 in the experimental period (Week 7 overall), the amount of lucerne chaff was restricted so that kg feed DM eaten was similar for both the willow and lucerne groups during weeks 9 & 10. Fresh feeds were presented at 1000 hours and 1600 hours with free access to water each day, while the residuals were removed at 0900 hours each day.

2.2.1.3 Voluntary feed intake and apparent digestibility

Voluntary feed intake was measured each day by weighing the amount of feed offered to each lamb and the amount refused, on a daily basis and correcting each to a DM-basis (Van Soest, 1994) by taking sub-samples and drying over a 24-hour period at 70^{o}C (Contherm Digital Series Oven, NZ). The difference between the feed on offer (DM-basis) and the refusals (DM-basis) was the VFI of that feed.

Duplicate samples of each feed offered and individual animal refusals (100g lucerne; 150g willow) were taken daily for chemical analyses. Of the duplicate samples, one set (100g for lucerne and 150g for willow) was immediately put into the oven for DM analysis (see above) and the other set was put in the cold room at -20^{o}C each day and pooled weekly for later analysis of diet chemical composition. In the last two weeks of the experiment, total faeces produced by each lamb were collected over a 3 day periods in each week (refer to section 2.2.1.1) used for the calculation of apparent digestibility.

The faeces samples were oven dried over a 36 hour period at 70^{o}C and ground before sending them to the laboratory for ashing.

2.2.1.4 Parasitology

Infective Dose

Lambs were infected with a mixed culture of nematodes; *Teladorsagia*, *Trichostrongylus*, *Cooperia* and smaller numbers of *Haemonchus* using the method described in Wood et al. (1995). This was undertaken on 12/02/09, i.e., 23 days after the start of the adaption period and 12 days before willow feeding commenced. Each lamb received an estimated 20,650 *Teladorsagia*, 1,320 *Trichostrongylus*, 330 *Cooperia* and trace *Haemonchus* L_3 larvae via the oral route using a stomach tube (Plates 3 & 4; see Appendix 1 for details). The recovery of eggs and culture of larvae (Appendix 8) and also their (L_3 larvae) recovery after incubation (Appendix 4), was done using the methods described by MAFF (1986).

Plate 3: Pushing the stomach tube into the rumen in readiness for worm infection

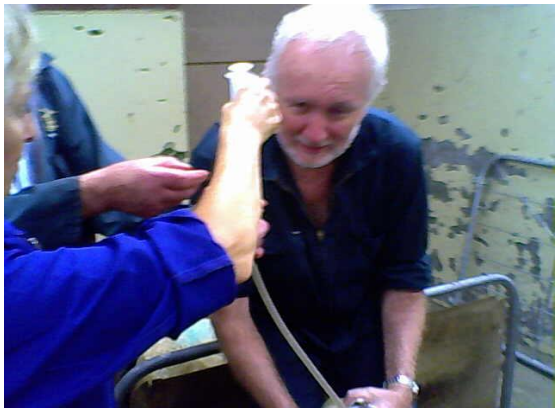


Plate 4: Administering the infective larvae

Total Egg Production per day

Faecal sampling for egg counts was done weekly by rectal palpation or by taking fresh faeces from the floor. Individual animal faeces were analysed in the laboratory for the number of eggs contained per gram of faeces (FEC). Part of the faeces collected during the 3 day bagging periods were for the purpose of determining total eggs produced by each lamb per day. Here, two faecal sub-samples were collected from each sheep over 24 h periods at the same time (one at 0800 hours and other at 1600 hours) and pooled

before subjecting to FEC. The number of eggs counted in each gram of faeces was multiplied by total faeces collected per animal per day.

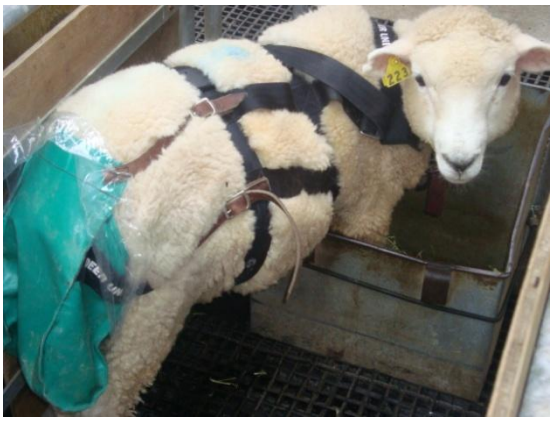


Plate 5: Bag attached for faecal collection

2.2.1.5 Haematology and Immunology

Blood samples for haematological and immunological analyses were collected on four occasions. The first and second collections were done two weeks (11/02/09) and 2 days (23/02/09), respectively, before the start of the willow feeding, and the last two were done two weeks into willow feeding (12/03/09) and the last day of the project (31/03/09). The collection was through the jugular vein using the BD Vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA) containing tetra-acetic acid (Plate 6 below). Two vacutainers were collected per animal at each sampling bled at similar time of the day (mornings at 0800 hours). The collected blood samples were mixed thoroughly immediately after collection to avoid blood clotting. After blood was collected from each animal, it was taken to the laboratory for processing and analysis.

Plate 6: **Blood sampling**

2.2.1.6 Slaughter sampling and measurements

All lambs were slaughtered in a humane manner by stunning with an airgun on the head followed by incising the jugular vein. This was undertaken at the end of Week 10. Immediately after slaughter, the gastrointestinal tract (GIT) was removed, sorted out into abomasum, small and large intestines (plate 7). All fat attached to the GIT was carefully removed. The sections of the GIT were labelled corresponding to the lamb they came from and then stored at -20^{o}C for later adult worm counts and speciation.

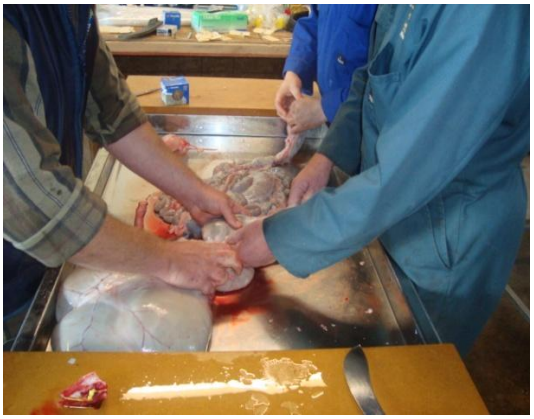


Plate 7: Sorting out GIT sections

The carcasses were put in the cold room for chilling. Cold dressed weight was then taken and a subcutaneous fat depth was measured at 11 cm from the dorsal midline between the 11th and 12th ribs (GR) according to Kirton (1989).

2.2.1.7 Laboratory analyses

2.2.1.7.1 Diet chemical methods

Lucerne and willow feed samples stored at -20^{o}C were freeze-dried (W.G.G. Cuddon Ltd., Blenheim, New Zealand), and ground to pass a 1mm diameter sieve (Apex mill, Soho Square, London). The faecal samples were oven dried and ground to the same size as the feeds. The following standard (AOAC, 2000) procedures were used for analysis: dry matter (convection oven, 105 °C, AOAC 930.15, 925.10), total nitrogen (Leco, total combustion method, AOAC 968.06) and ash (furnace, AOAC 942.05). The ashing was done to obtain the organic matter (OM) from which the organic matter digestibility

(OMD) was calculated (Equation 1). The digestible organic matter/100g DM (DOMD) was also calculated (Equation 2).

$$OMD = \left[\frac{(\text{DM offered} \times \text{OM\%}/100)-(\text{DM refused} \times \text{OM\%}/100)}{(\text{DM offered} \times \text{OM\%}/100)-(\text{DM refused} \times \text{OM\%}/100)}\right]-\text{Faeces DM} \times \text{OM\%}/100 \quad \text{…….[1]}$$

$$DOMD = \left[\frac{(\text{DM offered} \times \text{OM\%}/100)-(\text{DM refused} \times \text{OM\%}/100)}{(\text{DM feed offered}-\text{DM feed refused})}\right]-\text{Faeces DM} \times \text{OM\%}/100 \quad \text{…..[2]}$$

$$ME\ (\text{MJ ME/kg DM}) = DOMD \times 16.3 \quad \text{……..[3]}$$

The metabolisable energy (ME) content was then calculated from the DOMD (Equation 3; Ulyatt et al., 1980).

Willow was analysed for extractable, protein-bound and fibre-bound CT fractions, using the butanol-HCL colorimetric procedure (Terrill et al., 1992a) and the total concentration was calculated by adding the three fractions. The willow feed offered and the individual animal feed refusals in the last two weeks of the experiment were analysed for CT. Lucerne was not analysed for CT because it is known to contain only trace levels of CT (about 0.5 g/kgDM; Jackson et al., 1996).

2.2.1.7.2 Parasitology methods

Egg Counts

The FEC (eggs/g) of the wet faeces was determined using the McMaster technique (MAFF, 1986; Stafford et al., 1994) where each egg counted was equivalent to 50 eggs/g (see Appendix 2).

Larval Culture

Larval cultures to determine the genera present and their relative proportion were prepared for each animal in each of the 3 consecutive days in the second faecal collection period (in the final week of the experiment). Twenty-five grams of faeces from each animal was mixed with vermiculite and water, and cultured at 25^{o}C for 10 days (see Appendix 3 for details). The larvae from the cultures were recovered using a Baermann technique (see Appendix 4; MAFF, 1986). They were then counted (Appendix 5) and identified (Appendix 6) on a slide under a microscope.

Worm Counts

The worm burden was determined from 5% aliquots as described by Wood et al. (1995) from the abomasum and the small intestines (see Appendix 7). The total worm burden per sheep was obtained by multiplying the number found in the 5% aliquot by 20. While counting the worms, up to 50 males and 25 females of each genus were extracted per animal for speciation and *in utero* egg counts respectively. If less than this number were in the counted aliquot then all were extracted and retained.

2.2.1.7.3 Haematology and Immunology

Blood samples were analysed using standard procedures as described by Ramírez-Restrepo et al. (2010b). Haematology analyses were done by New Zealand Veterinary Pathology Ltd, Palmerston North, NZ, using an ADVIA 120 Hematology System (Siemens, Deerfield, IL, USA). Composition of red and white blood cells, haematocrit, haemoglobin, red blood cell indices and differential leukocyte counts were all measured. The variation in lymphocyte subpopulations grouped in clusters of differentiation $CD21^{+}$ and Gamma Delta (γδ) T Cell Receptor (TCR^{+}) cells was determined on a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

2.2.2 EXPERIMENT 2

Experimental Design

A 2 x 2 changeover experiment was conducted from 10/11/2009 to 15/12/2009, involving two time periods (Period 1 and Period 2) each of 2 weeks duration and two diets (chopped willow and lucerne chaff) fed to parasite-free young sheep. A known number of *Teladorsagia* eggs was added to faecal samples from these sheep and FEC then determined (see Appendix 9), to see if egg recovery was affected by these diets. The objective of Experiment 2 was to determine the effect of diet on egg recovery and larval development, leading to two specific objectives: 1) to obtain a correction factor for Experiment 1 following the discrepancies noticed between the FEC and the larval culture results of willow-fed sheep since more larvae were hatched than eggs were counted; 2) to determine if feeding willow reduced the number of eggs counted while maintaining their viability.

2.2.2.1 Animals and feeds

Nine parasite free weaned male hoggets were housed indoors and randomly allocated to individual pens after drenching them with 12.0 mls per animal of a combination anthelmintic containing 22.7 g/L oxfendazole + 40 g/L levamisole + 1 g/L abamectin (Matrix, Ancare New Zealand Ltd, Auckland, NZ). Placement of plastic ear tags and taking of initial weight were done 3 days into Week 1. A second weight monitoring was done on the first day of Week 3. The animals were numbered from 1 – 9 and the initial average weight was 47.3 kg ± 1.92.

The first week of the experiment was for acclimatization in which all lambs were given the same type of feed (half lucerne chaff and half chopped willow). The introduction of willow at this stage was done to minimize low initial willow intake experienced in Experiment 1.

The assignment of animals into the treatment diets was done on the first day of Week 2 and this was the start of Period 1 of the experiment. The lucerne treatment had 4 animals while the willow treatment had 5 animals, both fed as the sole diet. The diets were changed between animals for Period 2. Representative faecal samples for both periods were taken over the last 24 hours of each period from all sheep by bagging them. During Period 1 (2 weeks duration) and Period 2 (2 weeks duration), the willow treatment group was fed *ad libitum* while the lucerne treatment group was restricted in order to have similar overall DMI to the willow treatment (approximately 1.1 kg DM/day).

2.2.2.2 Parasitology

Parasite-free faecal samples collected during Periods 1 and 2 were taken to the laboratory to be artificially mixed with nematode eggs. Eggs from a sheep infected with *Teladorsagia* spp. were recovered using the method described by Hubert & Kerboeuf (1992; see Appendix 8) and then added to the experimental faeces to give 500 egg/g.

Fifteen grams of faeces were manually shaped into a ball and then a depression was made at the top. The 25 ml containing the eggs was transferred into this depression and the faeces were then left for 2 hours for the fluid to soak into the faeces. The faeces

were still well formed at this stage. They were then thoroughly mixed manually. This process was repeated for each of the 5 faecal samples taken per animal per period (for detailed faecal mixture, see Appendix 9). The faecal mixture was then allowed to stand (at 4^{o}C) for 24 hours (to allow bonding of eggs with chemical substances, if any, in the faeces) after which a normal FEC was conducted using the procedure described in Experiment 1. From each faecal mixture, 5 FECs were estimated.

A sample from each animal in Period 2 was mixed with *Teladorsagia* eggs and processed for L_3 larval culture (for detailed procedure, see Appendix 3) using the same concentrations of eggs as used in FEC for this period. This was to estimate how many L_3 larvae would develop from samples of each of the diets.

2.2.3 Statistical analyses

All data were analysed using the Statistical Analysis System (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA 2003) version 9.1. Data on chemical composition of the diet offered to each of the animals were analysed using the MIXED procedure with a linear model that included the effect of diet. Repeated measures on the same animal for feed intake and live weight were analysed with the MIXED procedure. The linear model included the fixed effects of diet, week and the diet-week interaction plus the random effect of animal. A compound symmetry error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals. Least square means and their standard errors were obtained for each diet and week of measurement. The CW and GR measurements were analysed using the MIXED procedure with a model that included the effect of diet. CW was included as a covariate in the model to analyse GR.

Data on egg recovery and L_3 larval development collected in Experiment 2 was analysed after a logarithmic transformation ($\log_{10}x+1$) to normalise their distribution. All FEC data in Experiment 1 were adjusted for lack of egg recovery using the correction factor determined in Experiment 2. The log-transformed FEC data were then analysed using the MIXED procedure for repeated measures with a linear model that included the fixed effects of diet, week and animal, the diet-week interaction and the random effect of animal.

Both unadjusted and adjusted total egg output data and the individual worm species egg output data were analysed using the MIXED procedure with a linear model that included the fixed effect of diet. No transformation of data was undertaken here as data followed a normal distribution with the exception of *Haemonchus* egg output, was transformed due to lack of normal distribution. Worm fecundity data were analysed at two levels; the per capita fecundity and the *in utero* fecundity. The variable per capita fecundity for each individual worm spp. was log-transformed ($\log_{10}x+1$) and the variable *in utero* fecundity for *Teladorsagia* and *Trichostrongylus* spp. was not transformed because the data followed a normal distribution. Analysis of variance was performed using the MIXED procedure with a linear model that included the fixed effect of diet.

The L_3 larval development data from Experiment 1 was analysed considering repeated measurements taken on the same animal after a logarithmic transformation ($\log_{10}x+1$) using the MIXED procedure. The samples for hatched larvae were collected over three days from the same animal and incubated according to sampling date. The model included the fixed effect of diet and the random effect of animal with a compound symmetry error structure.

Worm burden data were log-transformed ($\log_{10}x+1$) to normalise their distribution and were analysed using the MIXED procedure with a linear model that included fixed effects of diet, sex of worm species, diet-sex interaction and the random effect of animal. Multiple comparisons between means for diet and sex of worm species were performed.

Immunological data were square root transformed to normalise their distribution. Individual components of WBC and lymphocytes were analysed by repeated measurement, taking days 20 and 34 as a covariate for days 51 and 70, using the MIXED procedure. Days 20 and 34 were periods before introduction of willow feed. The linear model had fixed effects of diet, day, the diet-day interaction and random effect of animal with a compound symmetry error structure.

2.3 RESULTS

2.3.1 Diet composition

The chopped willow feed offered was of lower DM and CP content than lucerne chaff (Table 2.1), but had a significantly higher OM content. The CT was not analysed in lucerne, which was assumed to contain only traces of CT. The CT level in willow was 27 g/kg DM.

Table 2. 1: Chemical composition of the forages offered to penned sheep fed either lucerne chaff or chopped willow.

	Lucerne Chaff (n = 5)	Willow (n = 5)	SEM	Significance of Difference
Dry matter (g/kg)	888.4	345.8	4.60	***
Organic matter (g/kg DM)	918.4	928.8	1.21	**
Crude protein (g/kg DM)	146.6	128.8	3.11	*
Condensed tannins (g/kg DM)[1]	-	26.8	-	-

[1] Extractable CT + Protein-bound CT + Fibre-bound CT.
* P<0.05; ** P<0.01; *** P<0.001

Table 2. 2: Apparent digestibility of dry matter, organic matter and calculated metabolisable energy concentration in penned sheep fed either lucerne chaff or chopped willow.

	Lucerne Chaff (n = 6)	Willow (n = 6)	SEM	Significance of difference
Feed offered (kg DM/head/day)	1.29	1.53	0.005	***
Feed intake (kg DM/head/day)	1.11	1.13	0.017	NS
Apparent digestibility:				
Dry matter (DMD)	0.60	0.62	0.007	*
Organic matter (OMD)	0.60	0.65	0.008	***
DOMD[1]	0.55	0.58	0.007	**
Metabolisable Energy:				
MJ ME/kg DM (M/D)	8.96	9.48	0.109	**

[1] Digestible organic matter/100g DM
* P<0.05; ** P<0.01; *** P<0.001

The DM feed offered to willow-fed sheep was more than the lucerne-fed sheep (Table 2.2; P<0.001) to take care of selectivity of leaves more than stems by the willow-fed,

however the DM intakes in the two treatments were similar (P>0.05). Apparent digestibility for DMD (P<0.05), OMD (P<0.001), DOMD (P<0.01) and calculated ME (P<0.01) were higher for the willow diet.

2.3.2 Voluntary feed intake

The VFI for the two groups of animals were similar (P>0.05) from week 1 to week 5 (adaption period), but after the introduction of the willow diet VFI for this group declined in week 6 (P<0.001) and then progressively increased up to week 10 (Fig 2.1). The reduction in feed offered to the lucerne group from week 7 resulted in no difference in DMI between the two groups in week 9 and 10 (P>0.05).

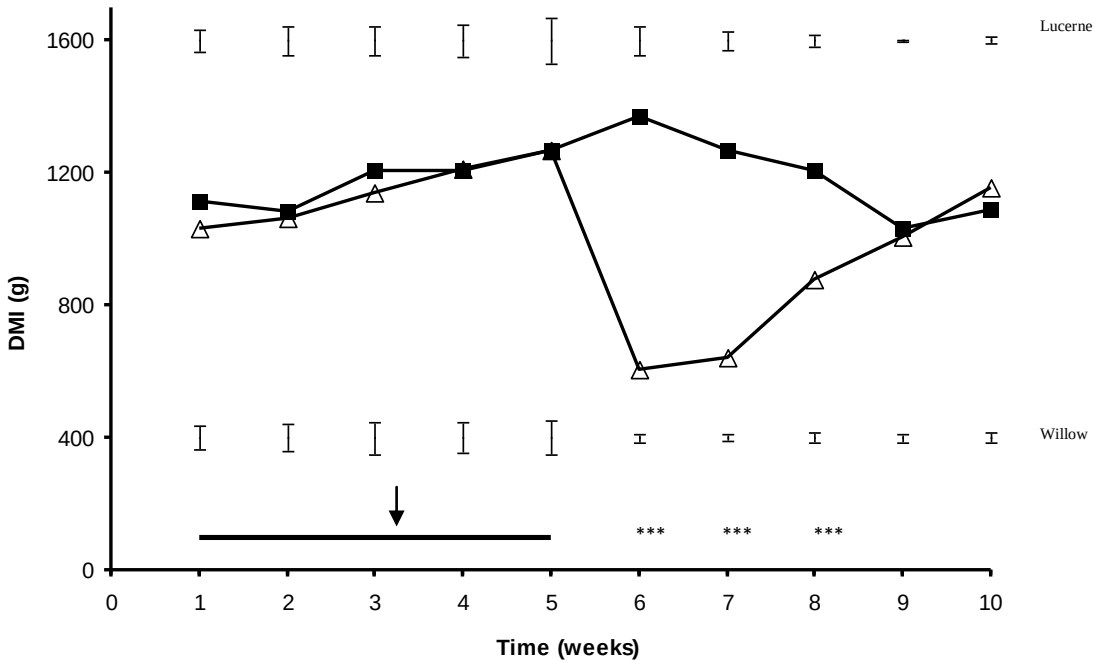


Figure 2. 1: Dry matter intake for penned sheep offered either lucerne chaff (■) or chopped willow (∆) diets. Solid line (—) is the period of adaption on lucerne chaff. (***) DMI during these weeks differ significantly (P<0.001). (I) Standard error of the means. ↓ larval infection (22, 300 larvae) given.

Calculated DM intake per head/day during the last two weeks of the experiment were similar for the two groups (P>0.05; Table 2.3). However, the willow group had slightly higher ME intake (P<0.01) and higher CP (P<0.001) intake per animal/day (Table 2.3). The CT consumption was calculated to be 28g CT/animal/day.

Table 2. 3: Calculated intakes of dry matter, crude protein and condensed tannins during the last two weeks of experiment in penned sheep fed either lucerne chaff or chopped willow.

	Lucerne Chaff (n = 12)	Willow (n = 12)	SEM	Significance of Difference
Dry matter (kg/head/day)	1.07	1.05	0.098	NS
ME (MJ/day)	9.59	10.04	0.093	**
Crude protein (g/day)	156.6	196.0	0.99	***
Condensed tannins (g/day)	-	28.0	-	-

NS → Not Significant, P>0.05; ** P<0.01; *** P<0.001

2.3.3 Liveweight and carcass weight

Liveweight progressively increased with time (Fig 2.2) during the adaption period (P>0.05; week 1-5) and was similar for the two groups. Liveweight declined after the willow diet was introduced in week 6 (P<0.001) and then increased with time and was not significantly different from the lucerne group by week 10 (P>0.05).

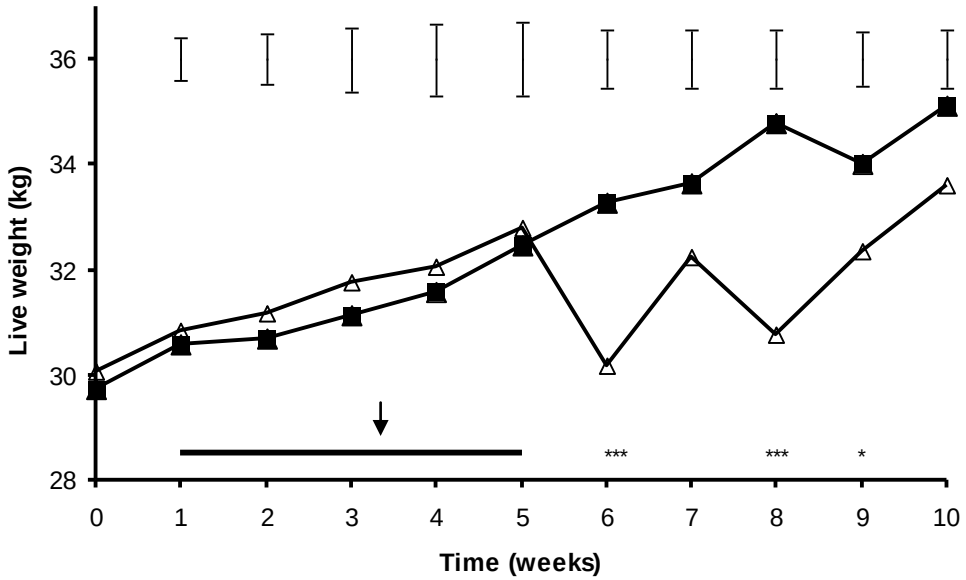


Figure 2. 2: Liveweight in penned sheep offered either lucerne chaff (■) or chopped willow (∆) diets. Solid line (—) is the period of adaption on lucerne chaff. (***) and (*) Liveweight during these weeks differ significantly (P<0.001 and P<0.05, respectively). (I) Standard error of the means. ↓ larval infection (22, 300 larvae) given.

Table 2. 4: The effect of feeding either lucerne chaff or chopped willow on final liveweight, carcass weight and carcass fatness of sheep.

	Lucerne Chaff (n = 12)	Willow (n = 12)	SEM	Significance of Difference
Final liveweight (kg)	35.1	33.6	0.55	NS
Carcass weight (kg)	13.9	13.4	0.33	NS
GR [1]	5.0	3.7	0.28	**

NS → Not Significant, P>0.05; ** P<0.01
1. with cold carcass weight as covariate

The final liveweight of the two treatments and also the carcass weights were similar for sheep fed both lucerne chaff and willow diets (P>0.05; Table 2.4). After adjusting GR using carcass weight as a covariate, willow-fed lambs had lower GR measurements compared to lucerne-fed lambs (P<0.01).

2.3.4 Nematode counts at slaughter from GI sections

Feeding willow reduced the burden of *Haemonchus* worms in the abomasum (P<0.01; Table 2.5a), with both male and female worms being affected. There was a significant forage x sex interaction on the burden of *Teladorsagia* worms in the abomasum (P<0.05; Table 2.5a). This can be explained through reduced proportion of female worms by more than half (P<0.05) in sheep feeding on willow diet, while there was no difference in proportion of females and males in sheep fed on chaffed lucerne diet (P>0.05). No differences were seen in the burden of *Trichostrongylus* spp from both willow and lucerne diets in the abomasum (P=0.2166; Table 2.5a).

The small intestines contained only small numbers of *Trichostrongylus* spp., *Cooperia* spp. and *Nematodirus* spp. (Table 2.5a). *Trichostrongylus* and *Nematodirus* worm burdens were not affected by feeding chopped willow but the burdens of *Cooperia* spp. were greatly reduced by chopped willow (P<0.01; Table 2.5a), with the effect being evident for both male and female worms.

Table 2. 5a: The effect of feeding lucerne chaff or chopped willow upon the burdens of adult worms in the abomasum and small intestines of young sheep. Mean data for both transformed $log_{10}(x)$ and back-transformed to geometric means.

Nematode	Lucerne Chaff (n = 12)		Willow (n = 12)			Significance of forage difference
	M	F	M	F	SEM	
Log transformed data						
ABOMASAL SPECIES						
Haemonchus spp.	3.1^{a}	3.1^{a}	1.6^{b}	1.4^{b}	0.47	**
Teladorsagia spp.	7.2^{a}	7.2^{a}	7.4^{a}	6.6^{b}	0.24	NS
Trichostrongylus spp.	1.3^{a}	2.3^{ab}	1.8^{ac}	3.2^{bd}	0.50	NS
SMALL INTESTINES SPECIES						
Trichostrongylus spp.	2.6^{a}	5.6^{b}	3.0^{a}	5.3^{b}	0.33	NS
Cooperia spp.	1.0^{a}	3.4^{b}	0.0^{ac}	1.9^{ad}	0.40	**
Nematodirus spp.	3.0^{a}	3.2^{a}	2.6^{a}	3.0^{a}	0.47	NS
Back transformed data						
ABOMASAL SPECIES						
	M	**F**	**Total**	**M**	**F**	**Total**
Haemonchus spp.	20.5	21.6	**42.1**	4.1	3.2	**7.3**
Teladorsagia spp.	1347.4	1389.8	**2737.2**	1708.6	733.5	**2442.1**
Trichostrongylus spp.	2.8	8.8	**11.6**	4.9	24.3	**29.2**
SMALL INTESTINES SPECIES						
Trichostrongylus spp.	12.2	275.3	**287.5**	18.3	194.0	**212.3**
Cooperia spp.	1.7	27.7	**29.4**	0.0	5.6	**5.6**
Nematodirus spp.	18.3	22.9	**41.2**	12.4	18.5	**40.9**

NS → Not Significant, P>0.05; ** P<0.01
Values with different superscripts in the row (^{abcd}) are significantly different at P<0.05

Table 2. 5b: Nematode speciation data showing the mean proportion of worm species and estimated burden of each species found in the abomasum and small intestines of lambs fed either lucerne chaff (n=12) or chopped willow (n=12).

Nematode species	Lucerne Chaff		Willow	
	Proportion	*Worm burden*	*Proportion*	*Worm burden*
Abomasum				
Haemonchus contortus	1.00	42.1	1.00	7.3
Teladorsagia spp.				
T. circumcincta	0.98	2682.5	0.96	2344.4
T. trifurcata	0.02	54.7	0.04	97.7
Trichostrongylus spp				
T. colubriformis	0.32	3.7	0.11	3.2
T. axei	0.68	7.9	0.89	26.0
Small intestines				
Trichostrongylus spp.				
T. colubriformis	0.43	123.6	0.68	144.4
T. vitrinus	0.49	140.9	0.32	67.9
T. axei	0.08	23.0	0.00	nil
Cooperia curticei	1.00	29.4	1.00	5.6
Nematodirus spp.				
N. spathiger	1.00	41.2	0.94	38.4
N. filicollis	0.00	nil	0.06	2.5

It was not feasible to allocate genera identified down to individual species (Table 2.5b) for each animal especially for *Trichostrongylus* spp. as there was considerable variation between animals. For some animals only *T. colubriformis* were found and for others only *T. vitrinus* hence a statistical comparison at the species level was not possible for this genus in the small intestines. For *Teladorsagia* the two 'types' identified are actually the major (*T. circumcincta*) and the minor (*T. trifurcata*) morph types of the one species. For *Haemonchus* and *Cooperia* only one species of each was identified. Similarly in the abomasum, for *Trichostrongylus* spp., there were too few worms to be able to allocate to species level for either species found. For *Nematodirus*, as for *Trichostrongylus* in the abomasum, there were too few male nematodes recovered to allow a meaningful allocation down to the species level.

2.3.5 Faecal egg, total egg production, L_3 larval count and fecundity

2.3.5.1 Faecal egg counts

Experiment 2

Table 2. 6: Recovery of *Teladorsagia* eggs added to the faeces (500 eggs/g wet faeces) of parasite-free young sheep fed either chaffed lucerne or chopped willow (log_{10} transformed data with back transformed data in parenthesis).

	Lucerne chaff	Willow	SEM	Significance of Difference
FEC (eggs/g wet faeces)	n = 9 6.05 (423)	n = 9 5.58 (264)	0.031	***
FEC as a fraction of eggs added	0.85	0.53	0.018	***
Larvae/g wet faeces (Period 2)	n = 5 6.04 (417)	n = 4 5.88 (355)	0.056	P = 0.08

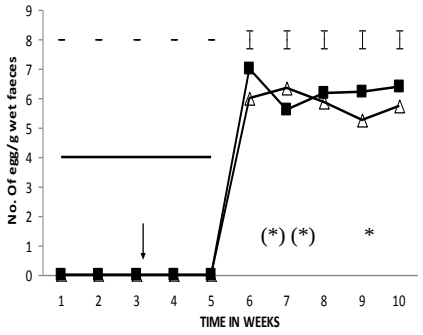
*** P<0.001

More eggs were recovered from the faeces of sheep fed the lucerne diet than the willow diet, either as total egg count or the proportion of eggs added (P<0.0001; Table 2.6); the two feeding periods due to the effect of the cross-over design did not have any influence on the outcome of the egg recovery (P>0.05). The proportion of eggs recovered with each diet (0.85 and 0.53 for lucerne-fed and willow-fed sheep, respectively) was subsequently used as a correction factor for FEC determined in Experiment 1. The number of larvae that developed per gram of wet faeces tended to be lower for sheep fed willow than lucerne chaff (P=0.08; 71% vs 83% of eggs added).

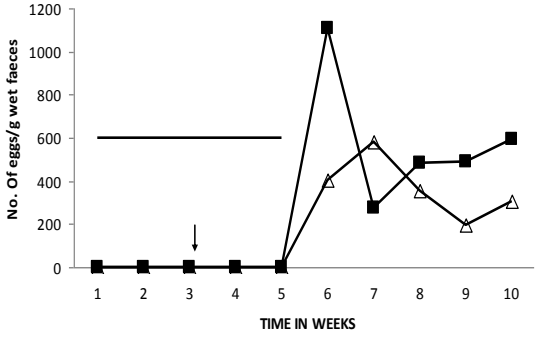
Experiment 1

Unadjusted log transformed FEC data (Fig 2.3a) showed a significant time x nutritional treatment interaction (P<0.01), explained by willow feeding reducing FEC and the effect increasing with time (Fig 2.3a & b). However, when FEC was divided by the correction factor derived in Experiment 2, to correct for loss of egg recovery, it resulted in the elimination of this willow effect (Fig 2.3c & d).

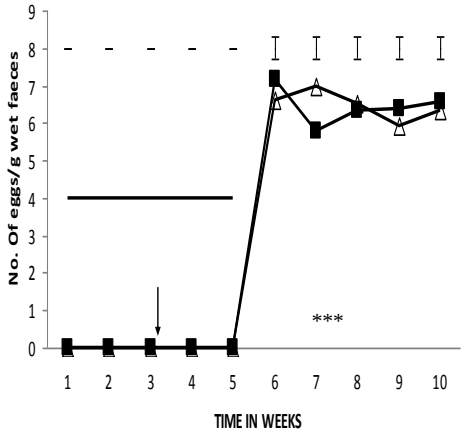
(a) Uncorrected transformed FEC



(b) Uncorrected back-transformed FEC



(c) Corrected transformed FEC



(d) Corrected back-transformed FEC

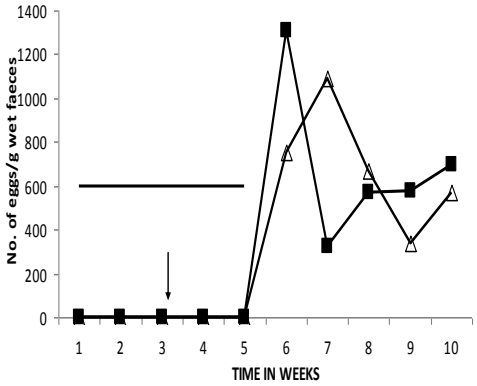


Figure 2. 3(a), (b), (c) and (d): FEC for penned sheep offered either lucerne chaff (■) or chopped willow (Δ) diets. Solid line (—) is the period of adaption on lucerne chaff. (*), * and * FEC during these weeks differ significantly (P<0.10; P<0.05 and P<0.001 respectively). (I) Standard error of the means. ↓ larval infection given, with willow feeding started 12 days later. Figures (c) and (d) have been corrected with correction factors of 0.85 and 0.53 for lucerne and willow-fed sheep, respectively.**

2.3.5.2 Total egg output for the nematodes

Unadjusted total egg output (Table 2.7) was much lower for sheep fed willow than lucerne chaff (P<0.01). Dividing total egg output by the correction factor obtained in

Experiment 2, to correct for eggs not recovered, reduced this effect but it was still evident (P<0.1).

Table 2. 7: The daily faecal egg production by genus in young sheep fed chaffed lucerne or chopped willow. Adjusted data have been corrected for loss of egg recovery determined in Experiment 2 (0.85 and 0.53 for lucerne and willow-fed sheep, respectively). This was calculated pre-slaughter by estimating the total number of eggs per day and allocating these to genera based on results of a worm culture in Table 2.9.

	Lucerne chaff (n = 12)	Willow (n = 12)	SEM	Significance of Difference
Total egg output (x 10^5)				
Unadjusted	11.2	4.4	1.49	**
Adjusted	14.1	8.2	2.11	(*)
***Haemonchus* egg output**				
Unadjusted (x 10^5)	1.7	0.6	0.29	**
Adjusted transformed [1]	11.7	10.6	0.34	*
Adj. back transformed (x 10^5)	1.2	0.4		
***Teladorsagia* egg output (x 10^5)**				
Unadjusted	5.7	1.8	0.83	**
Adjusted	7.5	3.3	1.35	*
***Trichostrongylus* egg output (x 10^5)**				
Unadjusted	3.1	1.7	0.53	(*)
Adjusted	3.7	3.3	0.74	NS
***Cooperia* egg output (x 10^5)**				
Unadjusted	0.12	0.06	0.037	(*)
Adjusted	0.14	0.12	0.047	NS
***Nematodirus* egg output (x 10^5)**				
Unadjusted	0.5	0.2	0.12	(*)
Adjusted	0.6	0.4	0.15	NS

NS – Not significant (P>0.1); (*) P<0.1; * P<0.05; ** P<0.01.
1. Log-transformed data to minimise variations

In unadjusted data, feeding willow reduced egg production for *Haemonchus* and *Teladorsagia* to less than half that of lucerne-fed sheep (P<0.01) and slightly reduced egg production from *Trichostrongylus*, *Cooperia* and *Nematodirus* at P<0.1. After adjusting for eggs which were not recovered using the correction factor obtained in Experiment 2, willow feeding still significantly reduced egg production from *Teladorsagia* and *Haemonchus* (P<0.05), but corrected egg production did not differ between diets for *Trichostrongylus*, *Cooperia* and *Nematodirus* (P>0.1). On the whole,

it appears willow feeding markedly reduced egg production, especially for the two principal abomasal species, *Teladorsagia circumcincta* and *Haemonchus contortus*.

2.3.5.3 Larvae hatching from incubated faeces

Table 2. 8: The total number larvae per sheep developing to ensheathed L3 from incubated faeces of young sheep fed chaffed lucerne (n=12) or chopped willow (n=12). ($Log_{10}(x)$ transformed data with back transformed means for a range of nematode species). This was calculated pre-slaughter by culturing 25 g of faeces per sheep, recovering the larvae, identifying them to genus, and multiplying by the faecal output per day.

	Lucerne chaff (n = 12)	Willow (n = 12)	Significance of Difference SEM	
Total larvae output				
Transformed	13.63	13.16	0.165	(*)
Back-transformed (x 10^5)	8.31	5.21		
Haemonchus contortus				
Transformed	1.15	1.04	0.003	*
Back-tranformed (x 10^5)	0.99	0.33		
Teladorsagia circumcincta				
Transformed	1.28	1.22	0.019	*
Back-transformed (x 10^5)	3.58	2.01		
***Trichostrongylus* spp.**				
Transformed	1.23	1.21	0.019	NS
Back-transformed (x 10^5)	2.27	1.75		
Cooperia curticei				
Transformed	0.55	0.54	0.073	NS
Back-transformed (x 10^5)	0.002	0.002		
Nematodirus spathiger				
Transformed	0.78	0.74	0.056	NS
Back-transformed (x 10^5)	0.03	0.02		

NS – Not significant (P>0.1); (*) P<0.1; * P<0.05

Larvae produced from incubated faeces are a function of egg production, egg hatchability and larvae development from L_1 to L_3. Transformed data of the number of L_3 larval hatching after 10 days of incubation showed that willow feeding reduced the total L_3 larval production (P=0.0588) and also reduced *Haemonchus contortus* and *Teladorsagia circumcincta* larvae (P<0.05; Table 2.8), while there was no effect on the production of *Trichostrongylus, Cooperia,* and *Nematodirus* larvae between the lucerne-fed or chopped willow diets (P>0.1).

2.3.5.4 Worm fecundity

Worm fecundity was derived in two ways, both ways giving the same outcome but with different sensitivity of tests used: the *per capita* fecundity (based on total daily faecal egg production allocated to genera after larval culture, divided by total female nematodes in each genus counted); and the *in utero* fecundity (based on eggs counted in a female nematode at necropsy). Using the corrected data to calculate daily egg production per worm (*per capita* fecundity; Table 2.9), willow feeding resulted in over a 7 fold reduction in the number of *Haemonchus* eggs produced per worm per day ($P<0.05$) but did not show any statistical differences from those of lucerne-fed sheep for *Teladorsagia*, *Trichostrongylus*, *Cooperia* and *Nematodirus* species. However, there is a common trend for reduced egg production in all these species in willow-fed sheep compared to lucerne-fed sheep even though the differences were not large enough to be statistically different. On the other hand, the actual eggs counted at necropsy (*in utero* fecundity) has shown that willow feeding reduced the eggs per worm in both abomasal *Teladorsagia circumcincta* and small intestinal *Trichostrongylus* spp. (Table 2.9; $P<0.001$).

Table 2. 9: The calculated daily faecal egg production per female worm[1] and eggs/female worm recovered at necropsy[2] in young sheep fed chaffed lucerne or chopped willow. Daily egg production was calculated by dividing the data in Table 2.7 by the number of female worms per genus.

	Lucerne chaff (n = 12)	Willow (n = 12)	SEM	Significance of Difference
Calculated egg production per female worm per day [1] (log_{10} transformed and back-transformed means)				
Haemonchus contortus				
Transformed	8.3	6.3	0.66	*
Back-tranformed	4159.0	558.0		
Teladorsagia circumcincta				
Transformed	5.6	5.5	0.19	NS
Back-transformed	267.0	254.0		
***Trichostrongylus* spp.** †				
Transformed	6.83	6.81	0.276	NS
Back-transformed	930.3	904.1		
Cooperia curticei				
Transformed	2.9	2.6	0.82	NS
Back-transformed	17.0	13.0		
Nematodirus spathiger				
Transformed	4.4	3.4	0.69	NS
Back-transformed	77.6	29.1		
Eggs/female worm at necropsy [2]				
Abomasal *Teladorsagia circumcincta*	38.0	29.5	0.80	***
Small intestinal *Trichostrongylus* spp.	22.6	20.3	0.38	***

NS – Not significant (P>0.1); * P<0.05; *** P<0.001

1. Calculated from corrected faecal egg output/day and worms present at necropsy.
2. Counting from microscopic examination of female worms recovered after necropsy.

† For this calculation it was not possible to differentiate between eggs from 3 species of *Trichostrongylus* recovered.

2.3.6 Immunological results

Table 2.10: Square root transformed white blood cell (WBC) data for young sheep fed lucerne and willow using the mean of pre-treatment Day 20 and Day 34 as a covariate. Values in parenthesis are back-transformed.

	Lucerne chaff (n = 12)	Willow (n = 12)	SEM	Significance of Difference
WBC (IU*10^9/L)				
Day 51	3.31 (9.97)	3.26 (9.61)	0.046	NS
Day 70	3.13 (8.79)	3.03 (8.21)	0.046	NS
Lymphocytes (IU*10^9/L)				
Day 51	2.69 (6.25)	2.69 (6.22)	0.036	NS
Day 70	2.59 (5.70)	2.54 (5.47)	0.036	NS
Neutrophils (IU*10^9/L)				
Day 51	1.94 (2.77)	1.91 (2.64)	0.040	NS
Day 70	1.81 (2.29)	1.78 (2.18)	0.040	NS
Monocytes (IU*10^9/L)				
Day 51	1.21 (0.47)	1.21 (0.46)	0.002	NS
Day 70	1.21 (0.46)	1.21 (0.47)	0.002	NS
Eosinophils				
Day 51	1.07 (0.15)	1.06 (0.13)	0.013	NS
Day 70	1.06 (0.11)	1.06 (0.12)	0.013	NS
Basophils (IU*10^9/L)				
Day 51	1.04 (0.07)	1.04 (0.07)	0.00004	NS
Day 70	1.04 (0.07)	1.04 (0.07)	0.00004	NS
Gama Deltas lymphocytes (IU*10^9/L)				
Day 51	1.45 (1.11)	1.44 (1.08)	0.016	NS
Day 70	1.47 (1.16)	1.42 (1.02)	0.016	NS
CD21 lymphocytes (IU*10^9/L)				
Day 51	1.51 (1.28)	1.53 (1.36)	0.041	NS
Day 70	1.41 (0.99)	1.40 (0.96)	0.041	NS

NS – Not significant (P>0.1). Back-transformed formula used: $x = Z^2-1$, x is normalised value; Z is transformed value of original data.

Total WBC, total lymphocytes, subsets of lymphocytes and other white-cell groups (Table 2.10) were not affected by willow feeding ($P>0.1$). No treatment x day interactions were detected in all components analysed.

2.4 DISCUSSION

The objective of this study was to examine the mechanism of how feeding forage willow may control internal parasites in young sheep, through studying effects upon the death of established parasites and upon parasite fecundity. Willow feeding reduced the burden of *Haemonchus* male and female worms, reduced the burden of *Teladorsagia* female worms, and reduced daily egg output of both species. In the small intestines, willow feeding greatly reduced the burden of *Cooperia* worm species only, but had no effect upon faecal egg output of any of the other small intestine species. Willow feeding reduced the fecundity of female *Haemonchus, Teladorsagia* and *Trichostrongylus* worms. These changes were associated with a greater intake of crude protein and with a considerable intake of CT in willow-fed sheep compared with only traces of CT in sheep fed lucerne.

Experiment 2 showed that the recovery of *Teladorsagia* eggs added to the faeces of parasite free willow-fed sheep was considerably less than for eggs added to the faeces of parasite free lucerne-fed sheep. All FEC and faecal egg output data in Experiment 1 were therefore corrected for this effect. The correction factor derived here based on *Teladorsagia* eggs was assumed applicable to all other species as well.

Another important finding from this experiment was that willow feeding did not appear to induce immunity against internal parasites, as found by Ramírez-Restrepo et al. (2010b). Instead, it seems that control of internal parasites was achieved through effects in the GIT. Each of these aspects will now be discussed in detail.

2.4.1 Worm burdens

An important design aspect of Experiment 1 was to allow a period for nematode establishment in the GIT of young sheep before willow feeding commenced. It essentially takes about a week from infection for L_3 to develop into 4^{th} stage larvae (Athanasiadou et al., 2005; Vlassoff, et al., 2001) and 15-21 days from infection to establish and develop into mature adult worms (Vlassoff et al., 2001). The 12 day interval between infection and the start of willow feeding in Experiment 1, during

which all sheep were fed chaffed lucerne, allowed sufficient time for larvae to develop into immature adult worms, which is assumed to be the same for both groups of animals. It appears that our experiment met these conditions since the FEC conducted at weekly intervals indicated it had taken 19 days after infection for eggs to appear in the faeces – a sure sign of presence of adult worms. Therefore, decreases in worm burdens between lambs fed the two experimental diets following willow feeding can be attributed to differences in the death of established parasites.

Willow feeding reduced the burdens of some parasites but not others. *Haemonchus contortus* in the abomasum and *Cooperia curticei* in the small intestines from sheep fed willow were less than half that of those fed lucerne ($P<0.01$). Furthermore, it appears willow feeding reduced female worms of *Teladorsagia circumcincta* but had no apparent effect on numbers of males ($P<0.05$). This is in agreement with the finding of Niezen et al. (1998a) who used *Lotus pedunculatus* without PEG addition and found a 41% reduction in established *Teladorsagia* female worms. The reasons for these differences are unknown, but in general abomasal worm species appear to be more affected by willow feeding than small intestinal worm species.

Niezen et al. (1998b) suggested that as dietary protein intake increased, the establishment of worms tended to reduce. In our case the reduced worm burdens from willow feeding were associated with higher intake of CP per day from willow than from lucerne (196 g/day vs 156 g/day) and the fact that there was presence of CT in willow (27 g CT/kg DM) vs trace in lucerne. Increased protein intake is likely to affect parasite establishment through effects upon the immune system (Barry & McNabb, 1999; Hoste et al., 2006; Kyriazakis & Houdijk, 2006; Min et al., 2004; Ramirez-Restrepo & Barry, 2005b; Ramírez-Restrepo et al., 2010b). For those parameters measured there is no indications that willow feeding in Experiment 1 influenced immunological aspects, so it seems most likely that willow feeding in the present work influenced parasitology through interactions in the gut between established worms and willow CT.

Studies conducted on Massey University farms in NZ, both indoors and under grazing, have shown that feeding temperate CT-containing forages was associated with reduced burdens of abomasal parasites *Haemonchus* and *Teladorsagia*, for this study with willow, for *Lotus corniculatus* fed to sheep (Ramirez-Restrepo et al., 2005) and for sulla

fed to young deer (Hoskin et al., 2000). Reduced burdens of *Nematodirus* in the small intestines were also observed by Ramirez-Restrepo et al. (2005) and by Diaz-Lira et al. (2008) for sheep grazing *L. corniculatus* and willow fodder blocks respectively. Reduced burdens of C*ooperia* in this study and by Ramirez-Restrepo et al. (2005) were found for sheep fed chopped willow and *L. corniculatus* respectively. Moreover, the burdens of *Cooperia* found in the present experiment were very small and care should be taken interpreting the differences seen. It appears that small intestine *Trichostrongylus* spp. are not affected by CT-containing forages, as this study and other studies (Athanasiadou et al., 2005; Niezen et al., 1998a, 2002) showed no effect on these species. Oral drenches of CT extracted from tropical *Acacia molissima* in sheep had no effect upon the burden of *Trichostrongylus* spp. but reduced the burden of abomasum dwelling *Haemonchus* spp. (Minho et al., 2008). These studies show that different parasites may be affected by CT from different plants (Hoste et al., 2006), highlighting that CT structure may be important for controlling parasites (Barry et al., 2001) as it is for effects upon amino acid absorption (Bermingham et al., 2001; Min et al., 2003; Waghorn et al., 1987b, 1994). Dose rate of CT given and site of the parasite in the GIT may also influence which parasites are affected. A threshold of 30-40 g CT/kg DM has been suggested for antiparasitic activity to be observed (Hoste et al., 2006), with the value of 28 g/kg DM in the present study being close to the bottom of this range.

The observation of reduced *Teladorsagia* females in the abomasum of willow fed young sheep is particularly interesting. This finding if exploited further and found to be repeatable, may have significance in the control of nematodes since it is the female that lays eggs that will eventually become worms. Any attempts to reduce the females will certainly reduce the worm population. The question that remains unanswered is; why there appears to be a forage x sex interaction for *Teladorsagia* spp. but not for other parasites. It should also be considered that whilst the burden of *Teladorsagia* females was reduced it was still half that of lucerne-fed sheep suggesting the effect of willow feeding is not sufficient on its own to control their numbers.

2.4.2 Fecundity

Two methods were used in Experiment 1 to determine the fecundity of female nematodes. Method 1 involved calculating the corrected daily egg production per female worm (using the correction factor obtained in Experiment 2) and total faecal egg samples (per capita fecundity). Method 2 involved counting actual eggs in uterus (*in utero*) of each female worm recovered at necropsy (refer to Table 2.9, section 2.3.5.4). Per capita fecundity of *Haemonchus* spp. was reduced by willow feeding ($P<0.05$), but there were also tendencies for reduced egg production per female worm in the other worm species studied, despite the differences not attaining statistical significance (Table 2.9). However, fecundity calculated from *in utero* eggs was lower for both *Teladorsagia* and *Trichostrongylus* in sheep fed willow ($P<0.001$). To help explain these differences, the coefficient of variation (CV) for worm fecundity was calculated for each method for *Teladorsagia* and *Trichostrongylus* species. Lower CV values were found for fecundity calculated from *in utero* eggs than for per capita fecundity, for both *Teladorsagia* (8% vs 11%) and for *Trichostrongylus* spp (6% vs 14%). The *in utero* egg method was thus more sensitive than the per capita method for calculating worm fecundity, thus explaining why it detected difference for these two species as significant. A realistic conclusion is therefore that willow feeding reduced the fecundity of *Haemonchus, Teladorsagia* and *Trichostrongylus* female worms and may have reduced the fecundity of other species.

Athanasiadou et al. (2000, 2001) found a reduction in *Trichostrongylus colubriformis* per capita fecundity in sheep when drenched with 16% Quebracho CT extracts, but Min & Hart (2003) argue that this can not be conclusively attributed to CT actions. Paolini et al. (2003a, 2003b) showed in goats that the decrease in egg excretion after administration of tanniferous extracts was due to a reduction in female worm fecundity. In the present study, the reduction in total faecal egg output in sheep fed willow can be attributed to both reductions in female worm burdens (especially those of *Teladorsagia* spp.) and in female fecundity, especially for *Teladorsagia* and *Haemonchus* spp. ($P<0.01$). Other studies, however, showed no effects of grazing CT-containing forages on fecundity of female worms (Athanasiadou et al., 2005; Niezen, et al., 1998a; Tzamaloukas et al., 2005).

2.4.3 Action of condensed tannins on nematodes in the gut

The more consistent effect of willow CT in reducing the burden and fecundity of abomasal worms than small intestinal worms might be partly explained by the changing reactivity of CT as it passes through the digestive system (Terrill et al., 1994). These authors found that during the digestion process, CT from *L. corniculatus* declined in extractability and was increasingly bound to the digesta constituents as it continued down the digestive system of sheep. Thus more CT would be free and available to interact with parasite larvae in the abomasum than in the small intestine. This may explain some of the greater effects of willow CT in reducing the burden and fecundity of worms dwelling in the abomasum than those dwelling in the small intestines.

2.4.4 Immunology

The non significance of immunology in all components of the of WBC and total lymphocytes (Table 2.10) in sheep fed willow contradicts the findings of Ramirez-Restrepo et al. (2010b), who found that feeding willow fodder blocks to trigger drenched sheep increased WBC and some components of the total lymphocytes. In *in vitro* studies, Schreurs et al. (2010) found lower ability to prime gamma delta (γδ) TCR^+ cells for CT extracted from willow than CT extracted from other forages and suggested that the responses found by Ramírez-Restrepo et al. (2010a) could have been due to other natural compounds as well as CT. The failure of the present study to show any immune responses compared to Ramírez-Restrepo et al. (2010b) could have been due to differences in the length of time these studies were undertaken (35 vs 131 days). They found that antibody responses were first detected from day 63 of their experiment which is nearly twice as long as the present study. Schreurs et al. (2010) found that lamb gamma delta lymphocytes had poorer responses primed by CT from a range of forages than did gamma delta lymphocytes from calves. This could also have an influence on the results obtained in the present study.

Two main hypotheses of CT reactivity against gastrointestinal nematodes have been proposed. The first being indirect action of CT through improving the host immune response (Min et al., 2003; Waghorn & McNabb, 2003) and the second being the direct anthelmintic properties of CT on nematodes (Athanassiadou et al., 2001; Molan et al., 2002). It appears that responses obtained in the current work can be explained by the

second proposed mode of action and that CT in willow have direct anthelmintic properties on nematodes, especially those dwelling in the abomasum. More work may however be needed with other CT-containing forages to approve or disapprove this conclusion.

2.4.5 Determining FEC in animals fed CT-containing forages

Experiment 1 (Figure 2.3 & Table 2.7) showed that willow feeding reduced the egg production per nematode species but that more larvae hatched (Table 2.8) and developed into L_3 than the eggs that were counted, a situation that is clearly impossible. Experiment 2 was designed to investigate this discrepancy to see if there was masking of eggs by willow feeding, hence making them uncountable while maintaining their viability.

In Experiment 2 (Table 2.6) it can be seen that 85% of the eggs that were added to the faeces of sheep fed lucerne could be counted and that 83% of added eggs hatched and developed into L_3, showing both a visibility and hatchability of close to 100%. On the other hand, only 53 % of the eggs added to the faeces of willow-fed sheep were recovered, yet their hatchability and development into L_3 was greater than this (71%). This shows that there are substances present in the faeces of willow-fed sheep that dramatically reduced eggs counted using standard techniques, with some (but not all) of the masked eggs still hatching. A more likely hypothesis is that binding of proteineous eggs by CT (Barry & McNabb, 1999) occurred, reducing their ability to float and hence be counted or inhibiting them from hatching (Iqbal et al., 2007; Molan et al., 2002, 2003b). As forage CT are indigestible (Terrill et al. 1994), their concentration in faeces will be greater than in feeds; as they are likely to be insoluble and associated with indigestible plant matter in faeces, they could bind nematode eggs to indigestible plant matter, so reducing their ability to float free for microscopic counting. The difference between hatchability and recovery of eggs added to the faeces of willow-fed sheep suggests that binding of eggs in this manner reduced hatchability of some eggs (approximately 12%) but not others (approximately 18%). To the authors' knowledge, this study is the first to show that conventional methods of measuring FEC underestimate eggs present in the faeces of animals fed a CT-containing forage.

The percentage of egg recovery in Experiment 2 for both willow and lucerne-fed sheep was used as a correction factor to adjust for unrecovered eggs in Experiment 1. With the adjusted FEC results, the total egg output per day showed that willow-fed sheep had reduced egg production than lucerne-fed sheep ($P<0.1$; Table 2.8), with the major contributors to the reduction being *Haemonchus* spp. ($P<0.1$) and *Teladorsagia* spp. ($P<0.05$). Daiz-Lira et al. (2008) found an initial increase in FEC which then significantly decreased over time in undrenched sheep on full and restricted access to willow fodder blocks. While the present study found reduced egg production from *Haemonchus* and *Teladorsagia* spp., Diaz-Lira et al. (2008) found increased *Haemonchus* egg production from undrenched sheep on full access fodder blocks. These differences could be due to lower concentrations of CT in the diet of sheep grazing fodder blocks. Reduced FEC have been reported from feeding other CT-containing forages such as: *L. pedunculatus* (Niezen et al., 1998b), *L. corniculatus* (Marley et al., 2003), Sulla (Niezen et al., 1995; 1998a; 2002) and sainfoin (Hoste et al., 2005; Paolini et al., 2003, 2005; Thamsborg et al., 2003). However, none of them have been corrected for loss of egg recovery in animals fed CT-containing forages as was done in this study. The technique used to compare recovery of eggs from faeces is artificial and may not fully reflect what happens as faeces pass through the large intestines where they may have even further opportunity to bind with CT. Thus it is unknown if the correction factor truly reflects the situation in nature.

For both forages, the FEC in this study showed an inverse trend with changes in DMI (Fig. 2.4). From week 7 onwards, lucerne DMI was restricted whereas willow DMI increased with time, such that DMI of both groups was similar over weeks 9 and 10. Corresponding to this, corrected FEC of lucerne-fed lambs increased with time whereas that of willow-fed lambs decreased with time. The most appropriate way of correcting for these effects is to calculate total egg output per day (Diaz-Lira et al., 2008), as the product of corrected FEC and total weight of faeces excreted/day, as was done in this study.

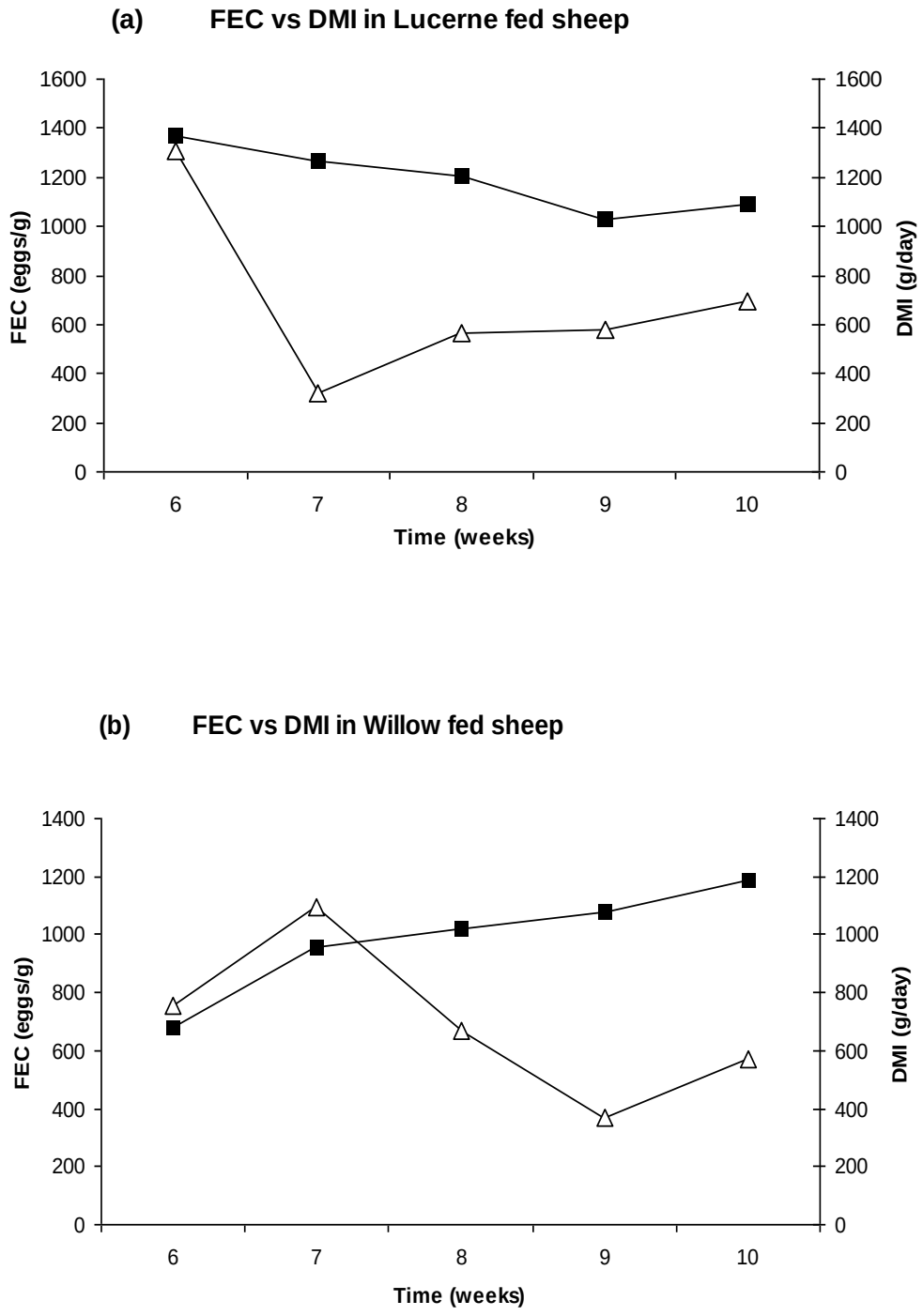


Figure 2. 4a & b: Relationship between corrected faecal egg count (Δ) and dry matter intake (■) in young sheep fed either chaffed lucerne or chopped willow diets in Experiment 1.

2.4.6 Effects of CT on the life cycle of gastrointestinal nematodes

Unlike synthetic anthelmintic drugs which basically are effective against adult and/or larval nematodes (Ketzis et al. 2006) and from which nematodes develop drug resistance (Gopal et al., 1999, 2001; Leathwick, D. M. et al., 2001; Macchi et al., 2001; McKenna, 1994; Pomroy, 2006; Scherrer et al., 1989; Waller, 2006), the present study and other previous studies have shown that the action of CT-containing forages affect several stages of the life cycle of the nematodes. This is an important fact since most of the synthetic broad spectrum anthelmintics have been designed to combat the existing infection rather than to prevent infection (Ketzis et al. 2006). When some nematode eggs are prevented from hatching by the action of CT, fewer worms will be hatched and some degree of infection prevention is achieved, which may not be achieved by synthetic anthelmintics that do not act on nematode eggs.

The action of CT in the present study was seen in three stages of the nematode life cycle, namely; adult, egg and larval stages. There were reduced burdens of adult worms in willow-fed sheep, indicating that increased mortality of some adult worms occurred. Similarly, the worm fecundity in willow fed sheep (the egg stage) and the egg hatching (egg/larval stages) were reduced by action of CT. Other studies have also shown CT to reduce larval motility (Alonso-Diaz et al., 2008; Marley et al., 2003; Min et al., 2005; Molan et al., 2003a, 2003b). Collectively, this indicates that use of CT-containing forages in grazing systems should reduce both animal worm burdens and pasture levels of infective larvae. No cases of anthelmintic resistance have been reported through use of CT-containing forages as, is the case with synthetic worm remedies. Even though CT will not eliminate the nematodes completely, they may reduce worm burdens to a minimum level (Ketzis et al. 2006), allowing increased rates of animal growth. This is indeed good news for sustainable ruminant production in organic farming systems.

2.5 CONCLUSION

Feeding chopped willow to young sheep was found to reduce nematode worm burdens in the abomasum, especially both male and female *Haemonchus contortus* and reduced female worm burdens of *Teladorsagia circumcincta*. Female worm fecundity of both species was also reduced by willow feeding. These reductions have been associated with CT content in the willow feed. The reduced worm burdens have been attributed to the death of the established worms by CT, since there was no evidence of immune priming in willow-fed sheep. The immune priming may be time dependent and therefore the number of days of feeding willow fodder to young sheep may need to be greater than the 35 days in this current study in order to see this effect.

Condensed tannins have been found to mask some of the nematode eggs, making them invisible by microscopic examination while keeping their viability. Eighteen percent (18%) of the masked eggs hatched and 12% did not. In the faeces of willow-fed sheep, some nematode eggs were probably bound to insoluble CT attached to indigestible plant fibre. This shows that conventional techniques for measuring FEC underestimated eggs present in the faeces of animals fed CT-containing willow and this may also be the case for other CT-containing forages.

The action of CT was seen to affect all stages of the nematode life cycle. The reduction in numbers at each stage of the life cycle resulted in a cumulative control effect against nematodes. This can therefore be employed in sustainable animal production systems if forages containing CT can be developed for use in grazing systems. The major problem with CT-containing temperate forages that are currently available is their poor persistence under grazing (Ramirez-Restrepo & Barry 2005b), including tree/forage willow browse blocks (Barry et al., 2006).

While positive results have been found with use of CT-containing forages to control nematode infections, acceptability of these forages by farmers becomes an issue because of their low persistency under grazing. It is therefore proposed that Molecular Plant Breeders should investigate incorporating CT genes into main pasture plants (such as

ryegrass/white clover mixture) to promote sustainable animal production. This can be done through either conventionally breeding the CT-containing plants for greater persistency or through genetically modifying the main pastures to contain this gene (transgenic process). While it is agreed that genetically modifying pastures with CT may have a negative public perception, there are a lot of animal welfare and environmental benefits to be gained through incorporating CT into forages that are widely used, including bloat resistance (Barry & McNabb, 1999; Waghorn, 2008; Waghorn & McNabb, 2003; Waghorn et al., 1987b) and reduced methane formation (Ramirez-Restrepo et al., 2010a; Waghorn & McNabb, 2003), in addition to more sustainable management of internal parasites. The use of chemical control against nematodes or any other parasite/disease is increasingly becoming a concern and poses negative welfare issues to livestock, humans and the environment. Genetically engineered pastures with CT genes will not pose other dangers and therefore becomes an alternative. This is indeed a big challenge to plant breeders; for animal nutritionists have shown CT-containing forages to be of great benefit to sustainable production.

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Appendices for Materials and Methods

Appendix 1: Artificially infecting lambs with *Teladorsagia, Trichostrongylus, Cooperia* and smaller numbers of *Haemonchus*

Materials/equipment/chemicals

L_3 larvae

Syringes

Stomach tube

Storage bottles

Parasite-free lambs

Method

1. L_3 larvae were recovered from infected animals using L_3 larvae recovery method descried in appendix…
2. The concentration of recovered L_3 larvae was made to 100 larvae/ml. and in each litre of the suspension; 50,000 *Teladorsagia,* 40,000 *Trichostrongylus,* 10,000 *Cooperia* and smaller numbers of *Haemonchus* were added.
3. The presentation of the L_3 larvae suspension was done in two solutions: Solution A had pure *Teladorsagia* species and Solution B had a mixture of *Teladorsagia, Trichostrongylus* and *Cooperia*. The source of L_3 larvae for Solution A was Agresearch. Solution B had *Trichostrongylus* coming from Agresearch and pooled sheep faecal samples from Limestone Down and Tuapaka farms.
4. By use of a stomach tube and syringe, 40 ml of Solution A containing 19,000 *Teladorsagia* and 33 ml Solution B containing 1650 *Teladorsagia*, 1320 *Trichostrongylus*, 330 *Cooperia* and trace amounts of *Haemonchus* larvae were introduced into the gut of lambs. A stomach tube was pushed into the rumen and the measured suspension in a syringe was introduced through the stomach tube.
5. Twelve (12) days were then allowed for worm establishment before commencement of willow feeding.

Appendix 2: Modified McMaster Method for counting of eggs

Equipment/chemical

Bowl (100ml capacity)

Coarse sieve (0.85mm)

Universal bottle (28ml capacity)

Electronic balance (accuracy ±0.1g)

McMaster egg counting slide

Counter (Clay Adams)

Pasteur pipette and rubber bulb

Saturated sodium chloride solution (NaCl; specific gravity 1.2)

Spoon

Microscope

Method:

1. A 100 ml bowl with a 0.85 mm sieve and a spoon were zeroed on the electronic scale.
2. Two grams of faeces were then weighed out.
3. 28 ml of saturated NaCl solution (specific gravity 1.2) was added and mixed well to a fine suspension using a spoon through the coarse sieve into the bowl and residuals on the sieve were discarded.
4. A sub-sample was taken using a Pasteur pipette into the McMaster slide while mixing well and a repeat procedure done to fill both chambers of the slide.
5. Counting of eggs seen within the ruled areas of the McMaster slide was done under the microscope and each egg counted represented 50 eggs per g faeces.

Appendix 3: Larvae culture

Equipment/chemical

Bowl

Spoon

Scoop

Deionised water

Nuplex fine grade vermiculite

Plastic culture jars with perforated lids

Incubator (25°C)

Method

1. 25 g of faecal sample were weighed out and crushed in the bowl using a spoon into fine consistency.
2. The vermiculite was then added plus water to a moist and crumbly consistency making sure that the faeces were not too wet.
3. The mixture was then put in previously labelled culture jar to about ¼ to ½ marks and perforated lid place securely on top. Perforations allow for free circulation of air.
4. The jar was then placed in an incubator at 25 ^{o}C for at least 10 days.
5. Little water was sprayed in the culture whenever noticed to be dry.
6. After 10 days, larvae were recovered using Baermann's Technique.

Appendix 4: Baermann's Technique for larvae recovery

Equipment/chemical

Sieve (0.15 mm apperture)

Culture flask (200 ml)

Counter (Clay Adams)

Fine paper tissue

Deionised water

Retort stand

Spring clip

Rubber tubing

Funnel

Method

1. The retort stand was assembled and funnel attached with tubing at the stem was place on the stand. The spring clip was fitted on the rubber tubing to have a water tight seal.
2. The funnel was then filled to 2 cm of the top with deionised water.
3. The faeces from the culture jar were placed on the wire mesh sieve placed on the funnel with the layer of tissues underneath the faeces.
4. More deionised water was added in the funnel so that the faeces were submerged.
5. The suspension was left to stand for 24 hours.
6. The clip was release to collect the sediment containing the larvae into the culture flask filling the flask to about ¾ full in readiness for worm count and identification as described in appendices 5 & 6.

Appendix 5: Counting third stage larvae

Equipment/chemicals

Automatic pipette

Counting slide

Cover slip

Lugol's iodine

Electronic microscope

Method:

1. Larvae were recovered from faecal cultures as described in Appendix 3.
2. The volume of contents of larvae in the culture flask was read off and recorded. The flask was then shaken to mix evenly the larvae in the flask and then 0.5 ml sub-sample was taken out with an automatic pipette.
3. The sub-sample was then placed in a glass counting slide and a drop of Lugol's iodine was added to kill the larvae hence arresting their movements. A cover slip was placed on top.
4. The slide was left to settle for about 30 seconds and then was placed on the electronic microscope.
5. Counting of the 3^{rd} stage larvae in the whole area of the slide was done. The larvae found in 1 mm of the sub-sample was multiplied by the volume of the sample to estimate total worms in 25 g faecal sample. Total number of worms per animal per day was found by knowing amount of faeces collected per day per animal. The total number of larvae in 25 g faeces was multiplied by the total faeces found per animal and answer divided by 25g. Thus:

$$\text{Total larvae} = \frac{\text{number of larvae in 25g x total faeces}}{\text{25 g faeces}}$$

Appendix 6: Identifying third stage larvae

Method:

1. The culture flask containing the larvae was place at an angle to allow larvae settle in one area.
2. Using a pipette, a sub-sample from the settled larvae was drawn and placed on a slide. A drop of Lugol's iodine was place and larvae identified on a microscope to genera using the x40 objective.
3. Where possible, identification of 100 third stage larvae to genus level was performed.
4. The identification of the 3rd stage larvae of common gastro-intestinal nematodes was performed using the Ministry of Agriculture, Fisheries and Food (1986) techniques were:

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.
Nematodirus spp.	Head broad rounded, 8 gut cells. Long tail, notched bilobed or trilobed.

Appendix 7: Worm count

Equipment/chemical

Agee jar (1L)

Aluminum foil

Beakers (50ml, 250ml)

Bucket (10L)

Concentrated Hydrochloride acid (HCL)

Counter (Clay Adams)

Gloves

Gut scissors

Pepsin BDH

Petri dish (grid marked on it)

Sieve (38μm, 106μm)

Scoop (50ml)

Stereo microscope

Stirring rod

Trays

Water bath

Wash bottle

Method

(a) Contents

1. At slaughter, the abomasum and small intestine were tied off into segments and then separated by cutting ends of each section. These gut sections were then frozen at -20°C whilst waiting for further processing.
2. Each section of abomasum or small intestine was placed in a bucket for thawing, and then was cut along its length with scissors.
3. The mucosal surface was washed under a light stream of water with constant manual manipulation to remove any adhered worms and/or mucus.
4. The volume of the contents were made to up 2L or 4L and mixed thoroughly with a to and fro motion with the stirring rod. While stirring, a 5% by volume

mixture was removed and placed in an Agee jar and this was repeated for a spare sample. Formalin was added into the spare sample for preservation.

5. The contents from Agee jar was placed in a 38μm sieve and washed gently under running water until clear. The materials on top of the mesh were transferred into beaker and by using a wash bottle, contents on the sieve were rinsed off into the beaker. The beaker was placed on a tray when filling to make sure no loss due to spillage.
6. The contents in the beaker were transferred in small portions to the counting dish and examined under a stereo microscope under 15-20X magnification. The worms were pulled out with a probe and examined under a stereo microscope.
7. The worms were counted and identified by genus.
8. During counting 50 male and 25 female nematodes of each genus (if available) were recovered into formalin for speciation and *in utero* egg counts, respectively.

(b) Pepsin digest

1. The section of abomasum were cut into small pieces and placed into a beaker containing a pepsin solution (600mls water+2.5g pepsin+10ml concentrated HCL).
2. The beaker was then covered with aluminium foil and incubated for 2 hours at 37°C in a water bath.
3. The digested abomasum was placed in the bucket and was washed under a light stream of running water.
4. The volume of the contents were made up to 2L or 4L and mixed thoroughly with a to and fro motion with the stirring rod while removing a 5% by volume of the mixture onto Agee jar. A repeat procedure was done for the spare sample but formalin added for preservation.
5. The contents from Agee jar was placed in a 38μm sieve and washed gently under running water until clear. The materials on top of the mesh were transferred into beaker and by using a wash bottle, contents on the sieve were rinsed off into the beaker. The beaker was placed on a tray when filling to make sure no loss due to spillage.

6. The contents in the beaker were transferred in small portions onto the counting dish and examined under a stereo microscope under 15-20X magnification. The worms were pulled out with a probe and examined under a stereo microscope.
7. The worm were counted and identified by genus.

Appendix 8: Recovery of nematode eggs from faecal samples

Equipment/chemical

Beakers (50ml)

Bucket

Centrifuge

Cover slips

Deionised water

Electronic balance

Eppendorp pipette

Incubator @25°C

Counter (Clay Adams)

Gloves

Sieve (20µm, 60µm)

Wash bottle

Procedure

(a) Egg recovery

1. FEC were carried out to estimate the total number of eggs required for inoculation before recovering the nematode eggs.
2. The pooled faecal samples were mixed with water by hand to make faecal slurry.
3. The faecal slurry was worked through a 60µm sieve and then the washings in a bucket underneath were collected. The residual in the sieve was discarded.
4. The filtrate was then washed through a 20µm sieve by running a hand underneath the sieve until the water ran clean into the sink. The particulate matter (eggs/debris) was retained on top of the sieve.
5. The eggs were collected in a 50ml Falcon tube by using a wash bottle to rinse the sieve and filled to the 50ml mark. These tubes were centrifuged at 3000g for 7 minutes to concentrate the eggs at the bottom.
6. The supernatant were discarded and the egg residue was thoroughly mixed. The egg residue was then transferred into a 50ml Falcon tube with two layers of sugar gradient (10ml of each; 10% sucrose solution at the top and 25% sucrose

solution at the bottom of the tube) in it with the tube at an angle of approximately 45 degrees. The residue containing the eggs were added on top the sugar gradients using a disposable pipette.

7. The tubes were then placed in the centrifuge buckets ensuring they were accurately balanced and then centrifuged at 3000g for another 7 minutes.
8. The eggs plus residues were collected from the interface of both layers sugar solutions (approximately 9-12ml per tube) into clean 50ml Falcon tube and filled to the 50ml mark with deionised water. Water was used to remove all sucrose. Centrifuged again at 3000g for another 7 minutes.
9. The water and sugar supernatant were discarded leaving approximately 10ml containing the clean eggs at the bottom of the tube.
10. The eggs recovered were concentrated to 800 egg/ml of the suspension and then added to the faecal pies described in appendix 9.

(b) Sugar solution

1. Two sugar solutions were made from saturated solution of 60g sugar+40ml deionised water.
2. 10% sugar solution was dissolved in 10ml saturated solution+90ml deionised water.
3. 25% sugar solution was dissolved in 25ml saturated solution+75ml deionised water.

Appendix 9: Procedure for Faecal Pie Making - SOP

i), **Apparatus**

- 50g pottles (medium sized)
- Spatula
- Electronic scale
- 50 cc syringe
- Spoon

ii) **Procedure of pie mixing**

- weigh 15g of faeces in the pottle
- Determine the number of eggs to put in the mixture (500 eggs/g of faeces/aliquot mixture). The aliquot containing this concentration of eggs should have been determined and measured already after the egg recovery procedure.
- Measure out 25ml of the aliquot containing eggs in a 50cc syringe (ratio of 3 : 5 faeces to volume of aliquot).
- Make a depression in the middle the faeces
- Add the measured volume of aliquot to the faeces in the pottle in the depression
- Allow 2 hours soaking
- After 2 hours crush the faeces using a spatula if in pellet form before the actual mixing
- Then do the mixing with a spatula for 2-3 minutes until a squishy paste is formed. You can determine squishiness by tipping over the pottle and nothing should fall off.
- After thorough mixing allow to stand for 12-24 hours in the fridge at $4^{o}C$
- Then carryout FEC using the normal FEC procedure (Appendix 2).

NB. The procedure described above works well with typical dry faecal pellets of sheep or goats. You can determine the dryness of the faeces by pressing or squeezing a pellet between your thumb and forefinger. When the pellet is crumbles up and no moisture peeps out, then the ratios described above will do. For slightly soft to soft faeces use a ratio of 0.5 – 1.0 : 1.0 faeces to aliquot volume.

Appendices for Raw Data

Appendix 10: Dry matter intake per day

THE EFFECT OF FEEDING WILLOW UPON THE DEATH OF ESTABLISHED PARASITES AND UPON PARASITE FECUNDITY

DRY MATTER INTAKE (g/day)

TRT	PEN #	TAG #	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10
Willow	P2	19		1074.5	1089.4	1148.0	1196.1	1296.2	756.6	974.2	1048.5	1085.7	1154.5
Willow	P4	15		982.8	984.2	987.2	913.8	1046.2	627.2	873.1	952.3	997.8	1103.7
Willow	P7	26		1200.5	1306.1	1334.7	1339.2	1481.8	704.1	1012.4	1064.9	1095.8	1218.4
Willow	P9	24		1136.1	1273.1	1366.2	1357.9	1420.2	638.9	977.7	1081.1	1099.7	1302.4
Willow	P10	18		1083.0	1167.2	1287.8	1321.7	1417.9	674.3	945.3	1043.2	1060.3	1189.5
Willow	P11	14		909.9	916.0	916.2	927.8	860.9	638.4	935.6	956.6	1060.9	1191.0
Willow	P12	21		1171.3	1157.4	1369.8	1398.3	1412.5	659.1	975.6	1076.9	1105.3	1232.0
Willow	P18	6		928.6	968.6	901.1	1362.3	1193.9	681.7	901.7	923.9	992.7	1155.4
Willow	P19	4		827.2	1046.3	1126.0	1207.0	1302.9	701.1	949.5	1043.3	1070.1	1173.6
Willow	P20	1		984.3	834.5	1006.1	1165.4	1309.7	650.2	969.6	984.0	1102.6	1130.5
Willow	P21	7		935.4	933.8	1158.2	1212.6	1230.8	648.4	987.5	1021.4	1161.7	1229.5
Willow	P24	9		1125.5	1069.6	1075.4	1147.1	1235.5	756.6	991.4	1019.5	1068.2	1171.0
	AVERAGE		**0.0**	**1029.9**	**1062.2**	**1139.7**	**1212.4**	**1267.4**	**678.1**	**957.8**	**1018.0**	**1075.1**	**1187.6**
Lucerne	P1	23		938.0	774.6	918.3	971.1	1065.9	1148.5	1151.2	1141.8	1031.3	1076.4
Lucerne	P3	16		1078.9	984.3	1378.5	1390.9	777.1	1378.1	1323.4	1263.9	1032.7	1107.4
Lucerne	P5	29		1020.0	841.2	1061.2	1140.7	1022.1	1213.9	1234.0	1141.6	1032.7	1102.0
Lucerne	P6	20		1218.4	1207.4	1330.2	1332.6	1426.4	1438.7	1326.5	1218.7	1032.7	1107.8
Lucerne	P8	13		1042.0	1040.5	1122.4	1196.7	1419.2	1389.8	1275.0	1234.6	1031.0	1094.3
Lucerne	P13	11		1307.9	1141.9	1108.3	893.9	1102.1	1378.2	1287.2	1234.1	1032.7	1097.1
Lucerne	P14	5		1184.0	1134.9	1247.0	1259.1	1391.3	1373.3	1228.8	1192.8	1030.8	1079.7
Lucerne	P15	12		1242.7	1228.3	1226.3	1290.0	1481.1	1486.7	1341.7	1264.7	1032.7	1106.8
Lucerne	P16	2		1044.5	1076.9	1266.9	1071.6	1467.8	1428.2	1315.7	1260.2	1032.4	1104.8
Lucerne	P17	8		1060.4	1245.3	1421.9	1419.9	1501.1	1586.3	1349.7	1253.8	1032.0	1101.7
Lucerne	P22	10		1176.9	1119.4	1071.9	1099.5	1131.7	1119.4	1047.8	1040.7	1020.4	981.6
Lucerne	P23	28		1053.7	1202.9	1352.9	1400.7	1434.8	1514.6	1333.5	1241.2	1032.1	1100.6
	AVERAGE		**0.0**	**1113.9**	**1083.2**	**1208.8**	**1205.6**	**1268.4**	**1371.3**	**1267.9**	**1207.3**	**1031.1**	**1088.3**

Appendix 11: Weekly Liveweight (kg)

THE EFFECT OF FEEDING WILLOW UPON THE DEATH OF ESTABLISHED PARASITES AND UPON PARASITE FECUNDITY

LIVEWEIGHT (kg)

	PEN #	TAG #	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10
Willow	P2	19	30.2	32.3	31.8	31.9	32.5	33.9	30.9	33.2	31.6	32.6	33.6
Willow	P4	15	28.5	29.4	29.4	29.1	28.1	27.8	26.4	27.8	27.1	28.2	29.8
Willow	P7	26	30.2	31.2	32.7	33.5	33	34.4	31.8	33.6	31.9	33.2	34.8
Willow	P9	24	30.9	31.8	32.7	33	34.1	34.4	30.1	32.8	30.9	33.1	33.8
Willow	P10	18	28.8	30.8	31.5	32.6	33.5	34.9	31.7	33.9	32.2	34.5	36.2
Willow	P11	14	28.7	29.1	29.5	30	30.4	30.8	29.2	31.3	29	31.4	32.4
Willow	P12	21	31.7	34.6	34.3	36	36.8	37.3	33.5	35.7	34.9	36.3	37.8
Willow	P18	6	29.3	29.3	30.1	30.3	30.3	30.9	28.8	30.3	29.7	30.5	31.5
Willow	P19	4	31.4	29.5	30.8	31	32	32.4	30	32.6	30.8	32.9	34.1
Willow	P20	1	28.5	30.7	28	29.3	29.3	31.4	28.1	30.8	28.6	30.7	32.3
Willow	P21	7	31.5	29.5	31.7	32.2	32.5	33.6	31.3	32.9	31.4	33.1	34.3
Willow	P24	9	31.2	32.1	31.4	32.1	32.4	31.9	30.4	32.2	30.9	31.6	32.8
	AVERAGE		**30.1**	**30.9**	**31.2**	**31.8**	**32.1**	**32.8**	**30.2**	**32.3**	**30.8**	**32.3**	**33.6**
Lucerne	P1	23	28.5	29.7	29	28.1	28.5	29.8	30.5	30.8	32.2	31.7	33.5
Lucerne	P3	16	31.2	32.9	32.7	34.6	33.7	31.7	33.7	34.2	35.6	35.1	37
Lucerne	P5	29	30.6	31.6	30.9	32.5	33.7	31.6	33.6	34.7	34.7	34.5	36.4
Lucerne	P6	20	29.7	30.5	31.3	31.6	32.4	34.6	35.2	35.7	36.1	35.3	36.9
Lucerne	P8	13	27.4	28.8	28.1	29.8	29.7	31.2	32.3	32.9	33.1	33.1	34
Lucerne	P13	11	29	30.8	28	26.5	26.1	28	30.2	29.8	31.3	31.4	32
Lucerne	P14	5	29.8	30	30.4	31.4	31.5	33.1	33	34.3	35.4	34.3	34.8
Lucerne	P15	12	31	31.8	32.1	32.8	33.1	36.1	35.4	35.6	36.8	35.5	36.1
Lucerne	P16	2	30.7	31.5	32.2	32.5	32.9	34.5	35.2	35	36.3	35.5	36.2
Lucerne	P17	8	29.8	29.9	31.3	32	32	33.2	33.4	33.9	35.4	34.1	34.3
Lucerne	P22	10	29.3	29.8	30.2	29.5	30.9	31.4	31.6	32	33.5	32.7	33.5
Lucerne	P23	28	29.8	29.8	32.2	32.3	34.3	34.4	35.4	35	36.9	35	36.8
	AVERAGE		**29.7**	**30.6**	**30.7**	**31.1**	**31.6**	**32.5**	**33.3**	**33.7**	**34.8**	**34.0**	**35.1**

Appendix 12: GR Measurements

RAW DATA FOR GR MEASUREMENTS DONE ON COLD DRESSED CARCASSES ON 2/04/09

Treatment	Sheep no.	First side(mm)	Second side (mm)	Average (mm)	Cold dressed weight (kg)
Wilow	6	3	3.5	3.25	13.5
Willow	26	5	5	5	14
Willow	24	2	2.5	2.25	13.2
Willow	21	5	5	5	16
Willow	19	4	4	4	13.3
Willow	18	5	4.5	4.75	14
Willow	15	2	2	2	11
Willow	14	3	3	3	12.5
Willow	9	2.5	3.5	3	13
Willow	7	2	2.5	2.25	13
Willow	4	7	8	7.5	15
Willow	1	2	3	2.5	12
Lucerne	29	4	4	4	15
Lucerne	28	5	5	5	13
Lucerne	23	3.5	3	3.25	13
Lucerne	20	5	6	5.5	15
Lucerne	16	5	5	5	14.5
Lucerne	13	5	4.5	4.75	13
Lucerne	12	6	6	6	14
Lucerne	11	3	3.5	3.25	12
Lucerne	10	8	10	9	14
Lucerne	8	7	7	7	13.8
Lucerne	5	3.5	5	4.25	14.5
Lucerne	2	3	2	2.5	14.5
Average - Willow				4.9	13.8
- Lucerne				3.8	13.4

Appendix 13: Weekly FEC (eggs/g faeces)

THE EFFECT OF FEEDING WILLOW UPON THE DEATH OF ESTABLISHED PARASITES AND UPON PARASITE FECUNDITY

FEC COUNTS (egg/g of faeces)

	PEN #	TAG #	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10
Willow	P2	19	0	0	0	0	0	50	400	550	150	200
Willow	P4	15	0	0	0	0	0	300	1200	300	50	50
Willow	P7	26	0	0	0	0	0	1150	300	100	300	50
Willow	P9	24	0	0	0	0	0	200	700	200	100	350
Willow	P10	18	0	0	0	0	0	150	700	650	500	600
Willow	P11	14	0	0	0	0	0	2100	550	950	300	800
Willow	P12	21	0	0	0	0	0	2950	1050	1000	50	350
Willow	P18	6	0	0	0	0	0	800	550	500	300	300
Willow	P19	4	0	0	0	0	0	250	450	50	200	400
Willow	P20	1	0	0	0	0	0	800	500	550	400	800
Willow	P21	7	0	0	0	0	0	700	600	500	300	350
Willow	P24	9	0	0	0	0	0	50	500	300	250	600
	AVERAGE		**0**	**0**	**0**	**0**	**0**	**792**	**625**	**471**	**242**	**404**
Lucerne	P1	23	0	0	0	0	0	1300	550	1500	550	1000
Lucerne	P3	16	0	0	0	0	0	1600	450	1550	800	300
Lucerne	P5	29	0	0	0	0	0	2200	600	550	1950	1050
Lucerne	P6	20	0	0	0	0	0	300	100	100	100	350
Lucerne	P8	13	0	0	0	0	0	2700	0	350	500	150
Lucerne	P13	11	0	0	0	0	0	1400	350	750	700	800
Lucerne	P14	5	0	0	0	0	0	400	800	400	400	1050
Lucerne	P15	12	0	0	0	0	0	2000	750	900	350	1350
Lucerne	P16	2	0	0	0	0	0	1650	1800	700	1250	1050
Lucerne	P17	8	0	0	0	0	0	350	600	300	1000	1850
Lucerne	P22	10	0	0	0	0	0	600	600	400	250	500
Lucerne	P23	28	0	0	0	0	0	2450	100	150	150	100
	AVERAGE		**0**	**0**	**0**	**0**	**0**	**1413**	**558**	**638**	**667**	**796**

Appendix 14: Egg output during the first 3 days of the last week of willow feeding

Forage	Animal	Week	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	FEC 1	Totl eggs	FEC 2	Totl eggs	FEC 3	Totl eggs	Av/animal
Willow	26	1	926	1085	1245	1086	1057	1112	400	**434400**	300	**317100**	450	**500400**	417300
Willow	24	1	1161	1126	1294	1092	1085	1133	650	**709800**	500	**542500**	300	**339900**	530733
Willow	21	2	1104	1077	1163	1398	1193	1306	150	**209700**	50	**59650**	100	**130600**	133317
Willow	19	1	936	969	1161	1099	1072	1198	750	**824250**	50	**53600**	650	**778700**	552183
Willow	18	1	941	1108	1176	967	938	961	950	**918650**	500	**469000**	700	**672700**	686783
Willow	15	1	999	844	1020	1055	1105	993	200	**211000**	100	**110500**	250	**248250**	189917
Willow	14	1	1073	998	1184	1043	989	1066	1300	**1355900**	950	**939550**	600	**639600**	978350
Willow	9	2	1082	1023	1071	1570	1372	1378	300	**471000**	250	**343000**	550	**757900**	523967
Willow	7	2	1101	1028	1180	1331	1043	1109	500	**665500**	300	**312900**	250	**277250**	418550
Willow	6	2	1087	968	1041	923	851	942	150	**138450**	300	**255300**	400	**376800**	256850
Willow	4	2	936	971	1224	1199	867	1066	250	**299750**	200	**173400**	250	**266500**	246550
Willow	1	2	1112	943	1117	1301	1096	1140	250	**325250**	400	**438400**	200	**228000**	330550
Lucerne	29	1	1057	1105	1142	1926	1567	1222	1550	**2985300**	150	**235050**	1200	**1466400**	1562250
Lucerne	28	2	1057	1096	1138	1364	1138	1687	100	**136400**	150	**170700**	350	**590450**	299183
Lucerne	23	1	1057	1105	1142	1552	1328	1309	650	**1008800**	300	**398400**	550	**719950**	709050
Lucerne	20	1	1057	1105	1142	1613	1359	1213	650	**1048450**	700	**951300**	700	**849100**	949617
Lucerne	16	1	1057	1105	1142	1947	1680	1618	550	**1070850**	900	**1512000**	3200	**5177600**	2586817
Lucerne	13	1	1057	1105	1137	1562	1440	1376	450	**702900**	400	**576000**	350	**481600**	586833
Lucerne	12	2	1057	1105	1142	1426	1203	1421	500	**713000**	350	**421050**	1350	**1918350**	1017467
Lucerne	11	1	1057	1105	1142	1863	2002	1929	1300	**2421900**	350	**700700**	1200	**2314800**	1812467
Lucerne	10	2	1042	1086	1055	1406	1205	1324	250	**351500**	250	**301250**	400	**529600**	394117
Lucerne	8	2	1057	1098	1134	1905	1727	2071	650	**1238250**	1000	**1727000**	450	**931950**	1299067
Lucerne	5	2	1057	1096	1139	1228	1172	1262	450	**552600**	400	**468800**	400	**504800**	508733
Lucerne	2	2	1057	1105	1132	1581	1057	1432	1500	**2371500**	1250	**1321250**	1000	**1432000**	1708250

Appendix 15: Egg output per Nematode species (uncorrected)

EGG OUTPUT PER SPECIES

Forage	Animal	Haem1	Haem2	Haem3	Tela1	Tela2	Tela3	Trics1	Trics2	Trics3	Coop1	Coop2	Coop3	Nem1	Nem2	Nem3
Willow	26	13010	26304	25080	195150	135904	79800	100828	276192	77520	3253	0	0	13010	0	45600
Willow	24	0	66063	85920	1138320	607775	358000	972315	647413	844880	166005	0	0	94860	0	114560
Willow	21	14988	26010	29315	227810	72828	143910	53955	69360	71955	0	3468	5330	2998	1734	18655
Willow	19	22104	46880	166584	320508	145328	121152	187884	276592	161536	5526	0	0	16578	0	50480
Willow	18	12461	25530	79128	58149	81696	124344	60918	148074	169560	1385	0	0	5538	0	3768
Willow	15	6655	90741	58223	552365	134547	69313	86515	87612	80403	6655	0	0	13310	0	52678
Willow	14	12383	259050	186390	544830	587180	214349	668655	777150	512573	0	51810	27959	12383	51810	18639
Willow	9	37680	24010	121264	277890	157780	121264	103620	154350	515372	4710	6860	15158	47100	0	0
Willow	7	59755	12050	90032	221445	126525	127104	63270	156650	137696	0	6025	10592	7030	0	174768
Willow	6	0	182182	925920	1622673	343343	300924	653913	147147	694440	145314	14014	46296	0	14014	347220
Willow	4	156860	147368	383670	270940	105263	901625	249550	117894	613872	14260	8421	38367	21390	42105	19184
Willow	1	288189	230400	72240	288189	195840	231168	91377	115200	120400	28116	17280	14448	7029	17280	57792
Lucerne	29	13559	18791	95940	1220310	460380	415740	94913	460380	19188	13559	0	0	13559	0	70356
Lucerne	28	8440	29835	67028	71740	38675	64545	120270	40885	71993	4220	0	0	6330	1105	42203
Lucerne	23	53543	241920	1397952	738887	574560	1760384	278421	665280	1708608	0	15120	51776	0	15120	258880
Lucerne	20	45933	117250	127813	606309	182910	195083	248036	164150	248899	0	0	0	18373	4690	87451
Lucerne	16	24728	5896	210249	527520	20904	295906	255518	26800	218036	0	0	0	16485	0	54509
Lucerne	13	73392	285390	195293	387927	342468	195293	524225	275877	331149	62907	47565	42455	0	0	93401
Lucerne	12	27261	14316	23508	62910	16702	57464	113238	28036	44404	4194	0	0	2097	597	1306
Lucerne	11	232024	55776	129591	685984	135456	280781	80704	207168	230384	0	0	0	10088	0	14399
Lucerne	10	262626	103075	30591	149058	200725	163152	177450	206150	101970	7098	16275	10197	113568	16275	27192
Lucerne	8	26064	47565	35028	325800	145866	140112	65160	117327	315252	0	6342	10008	17376	0	10008
Lucerne	5	20460	22191	177135	96844	105834	342461	15004	42675	53141	2728	0	0	1364	0	17714
Lucerne	2	179118	21155	117312	2209122	131628	469248	328383	75216	351936	149265	4701	29328	119412	2351	527904

Appendix 16: Egg output per Nematode species (corrected)

EGG OUTPUT PER SPECIES - CORRECTED

Forage	Animal	Haem1	Haem2	Haem3	Tela1	Tela2	Tela3	Trics1	Trics2	Trics3	Coop1	Coop2	Coop3	Nem1	Nem2	Nem3
Willow	26	24409	49351	47054	366135	254979	149719	189170	518184	145441	6102	0	0	24409	0	85553
Willow	24	0	77812	101201	1340777	715872	421673	1145247	762559	995147	195530	0	0	111731	0	134935
Willow	21	28119	48799	55000	427411	136638	270000	101229	130131	135000	0	6507	10000	5624	3253	35000
Willow	19	26035	55218	196212	377512	171176	142700	221300	325786	190266	6509	0	0	19527	0	59458
Willow	18	23378	47899	148458	109098	153276	233291	114293	277812	318124	2598	0	0	10390	0	7069
Willow	15	12486	170246	109235	1036332	252433	130042	162317	164375	150849	12486	0	0	24972	0	98832
Willow	14	14585	305124	219541	641731	691614	252472	787580	915371	603737	0	61025	32931	14585	61025	21954
Willow	9	70694	45047	227512	521370	296023	227512	194409	289587	966927	8837	12871	28439	88368	0	0
Willow	7	70383	14193	106045	260830	149028	149710	74523	184511	162186	0	7097	12476	8280	0	205852
Willow	6	0	214584	1090601	1911276	404409	354445	770216	173318	817951	171159	16506	54530	0	16506	408975
Willow	4	184759	173578	451908	319128	123984	1061984	293934	138862	723053	16796	9919	45191	25194	49594	22595
Willow	1	339445	271378	85088	339445	230671	272283	107629	135689	141814	33117	20353	17018	8279	20353	68071
Lucerne	29	25439	35255	180000	2289512	863751	780000	178073	863751	36000	25439	0	0	25439	0	132000
Lucerne	28	15835	55976	125755	134597	72561	121098	225647	76707	135070	7917	0	0	11876	2073	79179
Lucerne	23	63065	284947	1646587	870302	676749	2073479	327940	783604	2012495	0	17809	60985	0	17809	304923
Lucerne	20	86177	219981	239799	1137540	343171	366009	465357	307974	466977	0	0	0	34471	8799	164073
Lucerne	16	46393	11062	394463	989719	39220	555171	479395	50281	409073	0	0	0	30929	0	102268
Lucerne	13	86445	336148	230027	456922	403378	230027	617462	324943	390046	74095	56025	50006	0	0	110013
Lucerne	12	51146	26859	44105	118030	31336	107812	212454	52599	83310	7869	0	0	3934	1119	2450
Lucerne	11	273291	65696	152640	807991	159548	330719	95058	244014	271359	0	0	0	11882	0	16960
Lucerne	10	492732	193386	57394	279659	376595	306101	332927	386773	191313	13317	30535	19131	213073	30535	51017
Lucerne	8	48901	89240	65719	611257	273670	262874	122251	220126	591467	0	11899	18777	32600	0	18777
Lucerne	5	24099	26138	208640	114068	124657	403370	17673	50265	62592	3213	0	0	1607	0	20864
Lucerne	2	336056	39689	220098	4144694	246957	880390	616103	141118	660293	280047	8820	55024	224038	4410	990439

Appendix 17: Production of L_3 larvae through egg incubation

Treatment	Lamb ID #	1st Incubation						2nd Incubation						3rd Incubation						Average
		Haem	Tela	Trichs	Coop	Nem	Totl Larvae	Haem	Tela	Trichs	Coop	Nem	Totl Larvae	Haem	Tela	Trichs	Coop	Nem	Totl Larvae	
Willow	26	32580	488700	252495	8145	32580	**814500**	23744	122680	249317	0	0	**395741**	34347	109287	106165	0	62450	**312250**	507497
Willow	24	0	413625	353305	60320	34469	**861719**	40405	371730	395973	0	0	**808108**	41359	172329	406697	13786	55145	**689317**	786381
Willow	21	49389	750706	177799	0	9878	**987771**	34358	96204	91622	4581	2291	**229056**	30893	151655	75827	2808	19659	**280842**	499223
Willow	19	34605	501777	294145	8651	25954	**865133**	84559	262134	498900	0	0	**845594**	211270	153651	204868	6402	64021	**640211**	783646
Willow	18	76586	357403	374422	8510	34038	**850960**	38901	124482	225624	0	0	**389007**	119342	187538	255734	0	5683	**568297**	602755
Willow	15	5191	430820	67478	5191	10381	**519060**	100237	148627	96780	0	0	**345644**	64060	76262	88464	18303	57959	**305050**	389918
Willow	14	12282	540424	663248	0	12282	**1228237**	57477	130281	172430	11495	11495	**383178**	141906	163192	390241	0	14191	**709530**	773648
Willow	9	24517	180814	67422	3065	30646	**306464**	45984	302180	295611	13138	0	**656914**	91014	91014	386810	0	0	**568838**	510739
Willow	7	76877	284899	81400	0	9044	**452221**	24351	255687	316565	12176	0	**608778**	55805	78783	85349	0	108327	**328264**	463088
Willow	6	0	212238	85529	19006	0	**316774**	198249	373623	160124	15250	15250	**762496**	122083	39677	91562	6104	45781	**305208**	461493
Willow	4	248164	428647	394807	22560	33841	**1128019**	145316	103797	116253	8304	41519	**415189**	107078	251632	171324	0	5354	**535388**	692865
Willow	1	176836	176836	56070	17252	4313	**431308**	68741	58430	34371	5156	5156	**171853**	139700	447041	232834	0	111760	**931334**	511498
Lucerne	29	42711	3843988	298977	42711	42711	**4271098**	70327	1723011	1723011	0	0	**3516348**	97662	423203	19532	39065	71619	**651082**	2812842
Lucerne	28	5238	44521	74638	2619	3928	**130944**	26326	34126	36076	0	975	**97504**	35419	34107	38043	1312	22301	**131181**	119876
Lucerne	23	33772	466047	175612	0	0	**675430**	147614	350584	405939	9226	9226	**922588**	302819	381327	370112	11216	56078	**1121551**	906523
Lucerne	20	51564	680650	278448	0	20626	**1031288**	187624	292693	262673	0	7505	**750494**	87763	133954	170907	9238	60048	**461910**	747897
Lucerne	16	50373	1074619	520519	0	33582	**1679093**	221006	783567	1004573	0	0	**2009146**	145387	204619	150772	0	37693	**538470**	1408903
Lucerne	13	48285	255218	344890	41387	0	**689779**	237773	285327	229847	39629	0	**792576**	124060	124060	210363	21576	59333	**539392**	673916
Lucerne	12	249151	574963	1034934	38331	19165	**1916544**	272090	317438	532842	0	11337	**1133707**	349907	855328	660936	58318	19439	**1943928**	1664726
Lucerne	11	213080	629974	74115	0	9264	**926433**	146463	355696	544006	0	0	**1046165**	115110	249406	204641	57555	12790	**639502**	870700
Lucerne	10	104876	59524	70862	2834	45352	**283450**	132974	258950	265948	20996	20996	**699864**	57197	305050	190656	31776	50842	**635520**	539611
Lucerne	8	40252	503149	100630	0	26835	**670865**	132779	407188	327521	17704	0	**885191**	68890	275559	620008	0	19683	**984139**	846732
Lucerne	5	109046	516153	79967	14540	7270	**726976**	59286	282750	114012	0	0	**456049**	312724	604599	93817	0	31272	**1042412**	741812
Lucerne	2	120085	1481050	220156	100071	80057	**2001420**	214157	1332530	761446	47590	23795	**2379518**	146179	584714	438536	0	657804	**1827232**	2069390

Appendix 18: Adult worm counts – Abomasum

ABOMASUM WORMS														**SPECIATION**			
Treatment	Lamb ID #	HAEMONCHUS			TELADORSAGIA			TRICHOSTRONGYLUS			TL WORMS	**Totl females**	**Totl males**	T. trifurcata	T. circumcincta	T. colubriformis	T. axei
		Male	Female	Subtotal	Male	Female	Subtotal	Male	Female	Subtotal							
Willow	26	20	20	40	1560	880	2440	0	40	40	2520	940	1580	0	2440	0	40
Willow	24	40	0	40	2760	1360	4120	0	20	20	4180	1380	2800	247	3873	0	20
Willow	21	0	0	0	1120	280	1400	0	0	0	1400	280	1120	28	1372	0	0
Willow	19	0	0	0	1360	620	1980	0	0	0	1980	620	1360	40	1940	0	0
Willow	18	0	0	0	2260	880	3140	60	60	120	3260	940	2320	126	3014	40	80
Willow	15	20	0	20	1520	640	2160	20	160	180	2360	800	1560	0	2160	0	180
Willow	14	0	0	0	3420	2180	5600	40	40	80	5680	2220	3460	112	5488	0	80
Willow	9	40	40	80	1700	1320	3020	40	120	160	3260	1480	1780	181	2839	0	160
Willow	7	0	40	40	1360	720	2080	40	60	100	2220	820	1400	125	1955	50	50
Willow	6	20	40	60	3160	660	3820	0	20	20	3900	720	3180	153	3667	0	20
Willow	4	20	0	20	820	420	1240	20	60	80	1340	480	860	60	1180	0	80
Willow	1	0	20	20	1340	360	1700	0	20	20	1740	400	1340	102	1598	0	20
Lucerne	29	20	0	20	2560	3040	5600	40	140	180	5800	3180	2620	0	5600	0	180
Lucerne	28	0	0	0	80	40	120	20	20	40	160	60	100	0	120	40	0
Lucerne	23	40	20	60	1840	1880	3720	20	0	20	3800	1900	1900	149	3571	0	20
Lucerne	20	40	40	80	1240	1200	2440	20	0	20	2540	1240	1300	49	2391	0	20
Lucerne	16	60	140	200	2720	3080	5800	0	20	20	6020	3240	2780	116	5684	20	0
Lucerne	13	40	40	80	1500	1480	2980	0	20	20	3080	1540	1540	0	2980	0	20
Lucerne	12	0	40	40	2060	2440	4500	0	20	20	4560	2500	2060	180	4320	20	0
Lucerne	11	60	40	100	1580	1600	3180	0	20	20	3300	1660	1640	64	3116	10	10
Lucerne	10	20	40	60	880	1540	2420	0	60	60	2540	1640	900	55	2365	30	30
Lucerne	8	40	60	100	1840	1920	3760	0	20	20	3880	2000	1880	0	3760	0	20
Lucerne	5	100	20	120	1200	1120	2320	0	0	0	2440	1140	1300	93	2227	0	0
Lucerne	2	20	40	60	2940	3140	6080	20	0	20	6160	3180	2980	122	5958	20	0

Appendix 19: Adult worm counts – Small intestines

ADULT WORM COUNT (SMALL INTESTINES)											SPECIATION					
Treatment	Lamb ID #	Male Trichs	Female Trichs	Male Coop	Female Coop	Male Nem	Female Nem	Total	Female	Male	T. colubriformis	T. vitrinus	T. axei	C. curticei	N. spathinger	N. filicollis
Willow	26	20	120	0	0	20	40	200	160	40	0	140	0	0	0	60
Willow	24	20	160	0	40	20	60	300	260	40	180	0	0	0	0	80
Willow	21	0	320	0	20	0	60	400	400	0	0	0	0	0	0	0
Willow	19	20	200	0	0	20	60	300	260	40	0	220	0	0	0	80
Willow	18	20	200	0	0	80	60	360	260	100	220	0	0	0	0	140
Willow	15	20	200	0	40	20	0	280	240	40	220	0	0	0	0	20
Willow	14	40	240	0	0	0	0	280	240	40	280	0	0	0	0	0
Willow	9	20	160	0	20	0	100	300	280	20	180	0	0	0	0	0
Willow	7	20	240	0	20	60	60	400	320	80	260	0	0	0	0	120
Willow	6	20	260	0	20	20	20	340	300	40	280	0	0	0	40	0
Willow	4	40	200	0	0	20	0	260	200	60	0	240	0	0	0	20
Willow	1	40	120	0	20	80	40	300	180	120	0	160	0	0	0	120
Lucerne	29	100	780	180	60	20	40	1180	880	300	880	0	0	240	0	60
Lucerne	28	20	240	0	40	40	60	400	340	60	0	260	0	0	0	100
Lucerne	23	20	280	0	20	20	0	340	300	40	0	300	0	0	0	20
Lucerne	20	0	260	0	100	20	20	400	380	20	0	0	0	0	0	40
Lucerne	16	0	240	0	80	80	60	460	380	80	0	0	0	0	0	140
Lucerne	13	0	420	0	20	20	80	540	520	20	0	0	0	0	0	100
Lucerne	12	60	200	0	60	80	0	400	260	140	0	174	86	0	0	80
Lucerne	11	40	160	0	20	40	20	280	200	80	0	200	0	0	0	60
Lucerne	10	0	300	40	20	20	40	420	360	60	0	0	0	60	0	60
Lucerne	8	100	280	0	20	0	60	460	360	100	0	228	152	0	0	0
Lucerne	5	120	320	0	0	0	40	480	360	120	367	73	0	0	0	0
Lucerne	2	20	160	20	60	60	60	380	280	100	0	180	0	80	0	120

Appendix 20: Female worm fecundity (per capita & *in utero*)

PER CAPITA FEMALE FECUNDITY

Treatment	**Lamb ID #**	**Ttl Faecal eggs**	**Ttl Abo females**	**Ttl SI females**	**Total females**	**Av eggs/female**
Willow	26	417300	940	160	1100	379
Willow	24	530733	1380	260	1640	324
Willow	21	133317	280	400	680	196
Willow	19	552183	620	260	880	627
Willow	18	686783	940	260	1200	572
Willow	15	189917	800	240	1040	183
Willow	14	978350	2220	240	2460	398
Willow	9	523967	1480	280	1760	298
Willow	7	418550	820	320	1140	367
Willow	6	256850	720	300	1020	252
Willow	4	246550	480	200	680	363
Willow	1	330550	400	180	580	570
Lucerne	29	1562250	3180	880	4060	385
Lucerne	28	299183	60	340	400	748
Lucerne	23	709050	1900	300	2200	322
Lucerne	20	949617	1240	380	1620	586
Lucerne	16	2586817	3240	380	3620	715
Lucerne	13	586833	1540	520	2060	285
Lucerne	12	1017467	2500	260	2760	369
Lucerne	11	1812467	1660	200	1860	974
Lucerne	10	394117	1640	360	2000	197
Lucerne	8	1299067	2000	360	2360	550
Lucerne	5	508733	1140	360	1500	339
Lucerne	2	1708250	3180	280	3460	494

***IN UTERO* WORM FECUNDITY**

Treatment	Lamb ID #	ABOMASUM TELADORSAGIA Average	SMALL INTESTINES TRICHOSTRONGYLUS Average
Willow	26	27.7	18.3
Willow	24	30.4	19.9
Willow	21	31.8	21.2
Willow	19	29.8	19.1
Willow	18	28.2	19.9
Willow	15	26.1	19.6
Willow	14	27.3	21.7
Willow	9	30	20.1
Willow	7	36	19.1
Willow	6	26.9	21.6
Willow	4	29.2	19.9
Willow	1	30.5	23.2
Lucerne	29	43.5	21.3
Lucerne	28	37	22.4
Lucerne	23	35.6	24.3
Lucerne	20	42.6	22.3
Lucerne	16	38.4	23.8
Lucerne	13	34.1	22.3
Lucerne	12	38.6	21.1
Lucerne	11	36	22.1
Lucerne	10	34.4	24.0
Lucerne	8	38.4	21.7
Lucerne	5	38.8	21.3
Lucerne	2	38.9	24.5

Appendix 21: Experiment 2 egg recovery data

EGG RECOVERY AND FEC FROM FAECAL PIES — 1 egg = 50 eggs/g faeces
PERIOD 1 24/11/2009

Anim no.		SUB-SAMPLE 1						SUB-SAMPLE 2						SUB-SAMPLE 3						SUB-SAMPLE 4						SUB-SAMPLE 5						**SAMPLE**	**X 50**	
	Diet	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**AV**		
8	Willow	6	6	4	8	5	5.8	7	9	8	4	6	6.8	7	11	5	7	10	8	6	5	6	3	3	4.6	7	2	4	4	2	3.8	**5.8**	**290**	
6	Willow	7	8	3	9	5	6.4	7	5	3	6	4	5	7	11	10	3	5	7.2	9	4	3	6	7	5.8	3	10	9	6	5	6.6	**6.2**	**310**	
5	Willow	6	2	1	5	6	4	6	3	3	5	4	4.2	5	8	5	2	5	5	5	4	7	6	3	5	5	4	5	1	3	3.6	**4.36**	**218**	
2	Willow	7	3	11	4	9	6.8	7	4	3	3	3	4	4	6	5	6	5	5.2	4	6	5	2	7	4.8	5	5	6	5	4	5	**5.16**	**258**	
1	Willow	5	8	6	4	5	5.6	4	6	6	6	8	6	7	3	3	4	6	4.6	2	7	7	7	3	5.2	7	8	7	7	7	7.2	**5.72**	**286**	
9	Lucerne	9	9	7	9	8	8.4	10	9	7	13	10	9.8	7	10	7	8	9	8.2	13	6	10	8	7	8.8	8	9	11	9	8	9	**8.84**	**442**	
7	Lucerne	8	7	6	7	8	7.2	6	7	6	5	7	6.2	8	8	8	10	8	8.4	7	8	9	8	8	8	9	8	8	10	8	8.6	**7.68**	**384**	
4	Lucerne	10	9	11	8	7	9	9	9	12	9	7	9.2	7	8	10	9	8	8.4	8	13	8	8	9	9.2	11	8	11	8	7	9	**8.96**	**448**	
3	Lucerne	10	8	7	8	14	9.4	9	9	8	7	8	8.2	10	9	7	7	8	8.2	8	9	8	10	7	8.4	9	12	11	7	9	9.6	**8.76**	**438**	
Average	Lucerne																															**8.56**	**428**	**Percentage recovery: 85.6%**
	Willow																															**5.448**	**272**	**Percentage recovery: 54.5%**

EGG RECOVERY AND FEC FROM FAECAL PIES — 1 egg = 50 eggs/g faeces
PERIOD 2 09/12/2009

Anim no.		SUB-SAMPLE 1						SUB-SAMPLE 2						SUB-SAMPLE 3						SUB-SAMPLE 4						SUB-SAMPLE 5						**SAMPLE**	**X 50**	
	Diet	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**A**	**B**	**C**	**D**	**E**	**AVER**	**AV**		
9	Willow	5	6	3	6	6	5.2	6	8	7	7	5	6.6	3	7	5	6	4	5	5	5	4	6	8	5.6	2	3	6	4	7	4.4	**5.36**	**268**	
7	Willow	7	10	9	4	4	6.8	5	5	6	6	5	5.4	4	7	4	7	8	6	6	4	5	5	6	5.2	5	7	4	6	7	5.8	**5.84**	**292**	
4	Willow	3	9	5	7	3	5.4	7	6	5	4	2	4.8	5	5	3	6	5	4.8	6	4	6	2	4	4.4	4	2	4	5	6	4.2	**4.72**	**236**	
3	Willow	10	4	7	2	11	6.8	7	1	2	6	3	3.8	5	6	1	6	4	4.4	3	7	5	3	5	4.6	6	8	2	4	6	5.2	**4.96**	**248**	
8	Lucerne	8	7	9	8	6	7.6	8	11	10	7	8	8.8	7	10	11	8	7	8.6	8	8	9	7	11	8.6	10	7	9	8	10	8.8	**8.48**	**424**	
6	Lucerne	7	5	8	8	9	7.4	9	8	8	7	6	7.6	7	8	8	9	10	8.4	8	9	7	7	9	8	9	9	8	7	10	8.6	**8**	**400**	
5	Lucerne	8	7	9	9	10	8.6	7	10	11	8	7	8.6	5	7	8	9	8	7.4	10	8	9	8	11	9.2	9	7	10	8	8	8.4	**8.44**	**422**	
2	Lucerne	6	9	7	6	13	8.2	10	8	10	7	10	9	8	8	7	9	9	8.2	8	10	9	8	7	8.4	8	8	10	10	7	8.6	**8.48**	**424**	
1	Lucerne	5	8	9	9	8	7.8	8	11	12	8	11	10	11	7	9	6	9	8.4	12	7	13	5	9	9.2	8	7	8	10	8	8.2	**8.72**	**436**	
Average	Willow																															**5.22**	**261**	**Percentage recovery: 52.2%**
	Lucerne																															**8.424**	**421**	**Percentage recovery: 84. 24%**