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# Title

# The potential of *Lespedeza cuneata* as bio-active forage in the management of gastrointestinal nematodes in sheep

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**Thesis submitted in fulfilment of the Degree Magister Scientiae (Master of Science) at the Department of Paraclinical Sciences, Faculty of Veterinary Sciences University of Pretoria,**

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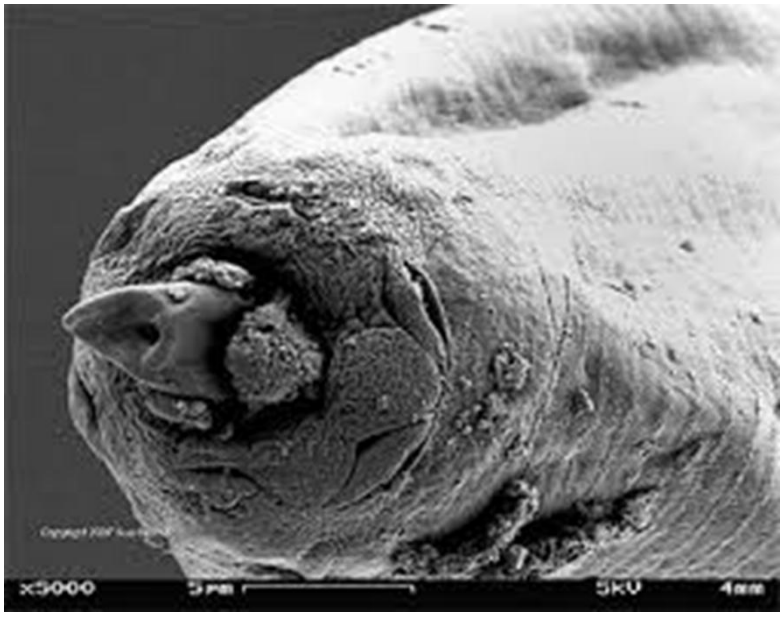
**Merino ewes grazing on *Lespedeza cuneata*.**

***Haemonchus contortus* adult worms parasitizing the abomasum of a sheep.**




***Haemonchus contortus* (5000x magnification image).**

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- Most of all, my Creator. I humbly thank You, My Lord.

Bible Rev. 22:2 "..and the leaves of the tree were for the healing of the nations"

# DECLARATION

I, Erika A van Zyl, declare that this thesis entitled, **The potential of *Lespedeza cuneata* as bio-active forage in the management of gastrointestinal nematodes in sheep,** which I herewith submit to the University of Pretoria in fulfilment of the requirements for the degree Magister Scientiae (Master of Science) is my own original work, and has never been submitted for any academic award to any other institution of higher learning.

_____ _____

**DATE**

## CONFERENCE ABSTRACTS and PUBLICATIONS

Van Zyl E A (2013) Response of *Lespedeza cuneata* (*Sericea*) to different cutting regimes in the Sandy Sourveld of KZN. Grassland Society of Southern Africa. Congress 48. Modimole.15-19 July 2013.

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## PAPERS PREPARED FROM THIS THESIS

Van Zyl EA, Botha FS, Eloff J, Oosthuizen PA and Msuntsha PP (2015) Response of *Lespedeza cuneata* (*sericea*) to different cutting regimes in the Sandy Sourveld of KZN. In final preparation for submission to the African Journal of Range and Forage Science.

Van Zyl EA, Botha FS, Eloff J, Oosthuizen PA and Msuntsha PP (2015) The use of *Lespedeza cuneata* for natural control of gastrointestinal nematodes in merino sheep. In final preparation for submission to the *BMC Veterinary Research*.

# LIST OF ABREVIATIONS

| | | |
|---|---|---|
| a | - | annum |
| ADF | - | Acid detergent fibre |
| AR | - | Anthelmintic resistance |
| ARC | - | Agricultural Research Council |
| °C | - | Degree Celsius |
| COWP | - | Copper oxide wire particles |
| CP | - | Crude Protein |
| CT | - | Condensed tannins |
| DM | - | Dry Matter |
| DRS | - | Dundee Research Station |
| FEC | - | Faecal egg count |
| FECRT | - | Feacal egg count reduction test |
| g | - | gram |
| GIN | - | Gastrointestinal nematodes |
| ha | - | hectare |
| h | - | hour/s |
| kg | - | kilogram |
| mg | - | milligram |
| min | - | minutes |
| ml | - | millilitre |
| mm | - | millimetres |
| n | - | number |
| NDF | - | Neutral detergent fibre |
| PCV | - | Packed cell volume |
| QTL | - | Quantitative trait loci |
| Rpm | - | Revolutions per minute |
| TST | - | Targeted selective treatment |

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# ABSTRACT

The rapid increase in the magnitude of anthelmintic resistance towards commercial chemical anthelmintics, calls for alternative methods to complement treatment or replace anthelmintics. During the last two decades more and more calls have been made for a holistic management solution. Recent studies on breeding for resistant animals and bioactive forages highlight the potential of these to contribute towards parasite control.

For this study, gastrointestinal infection in a merino flock was investigated by means of faecal egg counts (FEC). The level of parasite infection in weaned merino lambs in their first summer could be linked to weather conditions. Anthelmintic resistance in the flock was quantified and compared to the resistance level some years earlier. Within the flock, 18% of the ewes appeared to be resistant with consistently ($P<0.05$) lower FEC than the rest of the flock.

*Lespedeza cuneata,* a tannin-containing legume, is, according to scientific literature, one of the promising bioactive forages. Depending on the level of condensed tannin (CT), ruminant digestion can be complemented or compromised by the tannins. Small plot trials were conducted to establish production criteria for *L. cuneata,* currently lacking in South Africa. The grand mean dry mass (DM) yield for the first growing season, (characterized by above normal rainfall), was 8.3 t $ha^{-1}$, compared to the 2.56 t $ha^{-1}$ for the second growing season, (characterized by below normal rainfall). Highly significant differences ($P<0.001$) were measured between the yields produced under different cutting frequencies (6, 8 and 12 weeks) and cutting heights (5 cm and 15 cm). Except for crude protein levels, ADF (acid detergent fibre) and NDF (neutral detergent fibre) analysis of the complete plant indicated a less acceptable nutritional quality. Since sheep selected leaves during grazing, separated leaf samples were analysed. Chemical feed analyses of leaves were more favourable, compared to feed analysis of the whole plant. The condensed tannin (CT) content of leaves increased significantly ($P<0.05$) with increased moisture stress and varied between 24.5 and 122 g $kg^{-1}$ DM over the growing seasons.

Different dried herbage diets were offered to Merino ewes in a follow-up trial to evaluate the anthelmintic properties of the forage. The leaf portion of *Lespedeza cuneata* hay and *Medicago sativa hay* was offered *ad libitum* to confined sheep. *Lespedeza cuneata* is a tannin rich legume, while *Medicago sativa*, known for its very low tannin content, was used as control. FEC were significantly ($P<0.05$) lower in the *Lespedeza* group after 35 days.

To determine the influence of condensed tannin (CT) level on the hatch efficacy of *Haemonchus contortus* eggs, acetone leaf extracts of *L. cuneata* plants with different condensed tannin contents (73.5, 88, 102 and 122 g $kg^{-1}$ DM) were tested in an *in vitro* assay *on H. contortus* eggs. Concentrations of between 0.63 and 20 mg $ml^{-1}$ were used in the assay. Concentrations higher than 10 mg $ml^{-1}$ led to complete inhibition of egg hatching with all the plant extracts regardless of CT content. A typical dose related response of the extracts of plants with very high tannin content was found, but not with plant extracts containing lower tannin concentrations.

The results of the study indicate that *L. cuneata* can be incorporated in a fodder flow programme as an alternative or complementary strategy to other methods, to manage the detrimental effect of gastrointestinal parasites in sheep.

# CHAPTER 1

# Introduction

Infestation with gastrointestinal nematodes (GIN) in small ruminants causes severe economic losses and endangers animal-welfare throughout the world. Today anthelmintic chemotherapy forms the backbone of these GIN control programmes as it has been for many years. Increased public awareness towards chemical drug residues in agricultural products, together with the increasing development of resistant strains of parasites, has enforced the search for sustainable alternatives (Taylor *et al.,* 2002; Min and Hart, 2003; Heckendorn, 2007; Rahmann and Seip, 2007; Morgan *et al.,* 2013).

Kaplan and Vidyashankar (2012) warned the farming community of the rapid increase in both the prevalence and magnitude of anthelmintic resistance towards commercial anthelmintics in their publication, "*An Inconvenient truth: Global worming and anthelmintic resistance*". The lack of development of new chemical anthelmintic drug classes and the high costs involved in the development of these drugs emphasizes the seriousness of the situation. There is an urgent need for livestock producers to develop a new vision for managing endo-parasites and for parasitologists to improve methods used for diagnostic anthelmintic resistance (Rahmann and Seip, 2007; Kaplan and Vidyashankar, 2012; Morgan *et al.,* 2013).

These facts motivated researchers during the last decade to have a renewed interest in the development of alternative methods of controlling GIN. One of the areas of interest is in the field of Phytomedicine. The use of plant remedies to cure or control diseases has been used for centuries (Hutchings, 1996). Many recent scientific studies, not only abroad (Khurshid and Mudasir, 2012), but also locally (Bizimenyera, *et al.*, 2006; Kandu-Lelo, 2009; Mpohosa *et al.,* 2010; Ademola and Eloff, 2011; Vatta *et al.,* 2011; Ahmed, 2012; Ahmed *et al.,* 2013) were done to verify the anthelmintic value of plants.

Rahmann and Seip (2007) did an extensive review on the current scientific knowledge of alternative strategies to prevent and control GIN in organic farming

systems. It was concluded that a major potential exists within the field of bioactive forages, phytotherapy and copper-oxide particles. Investigations into several well-known pasture species such as chicory (*Cichorium intybus)* (Athanasiadou *et al*., 2007, Heckendorn, 2007; Foster *et al*., 2011; Sainfoin (*Onobrychis viciifolia*) (Heckendorn 2007), sulla (*Hedysarum coronarium*) (Niezen *et al.*, 1998; Niezen *et al*., 2002) and some other species, showed potential in anthelmintic control. However, according to the literature search, none of the mentioned species were researched sufficiently in on-farm experiments to recommend any strategies for implementation. Therefore, Rahmann and Seip (2007) strongly recommended further research on these pasture species, for use as bioactive pastures.

*Lespedeza cuneata* is one of the planted pasture species that is mentioned in scientific literature to have anthelmintic properties. Significant outcomes were measured regarding a reduced feacal egg count and increased packed cell volume, but again insufficient on-farm implementation strategies were recommended (Shaik *et al.,* 2004; Shaik *et al*., 2006; Terril *et al.,* 2007; Burke *et al*., 2011; Gujja *et al*., 2013; Komururu, 2014).

Despite the overwhelming amount of scientific proven anthelmintic properties found in different plants and pastures, a need exists to find verified strategies to exploit these anthelmintic properties for on-farm implementation. Bioactive pastures, incorporated in the fodder flow programme of small stock, could play an invaluable role in a solo or in an integrated GIN control programme.

# CHAPTER 2

# General aim and objectives

## 2.1 Aim

The aim of this study was to investigate the management of gastrointestinal infestation in the sheep flock at Dundee Research Station and the potential of *Lespedeza cuneata* as bio-active forage for sheep.

## 2.2 Objectives

The objectives of this study were to:

I. Investigate the occurrence of gastrointestinal nematodes in the sheep flock at Dundee Research Station and determine gastrointestinal nematode resistance to standard anthelmintics in this flock.
II. Determine the yield, nutritional value and tannin level of *L. cuneata* under different defoliation frequencies and intensities.
III. Investigate the anthelmintic potential of *L. cuneata* hay in sheep.
*IV*. Determine the efficacy of acetone leaf extracts of *L. cuneata,* containing different levels of condensed tannins, on egg hatch efficacy of *Haemonchus contortus.*

## 2.3 Hypothesis

*L. cuneata* has anthelmintic properties and can be used in a management strategy of GIN in sheep, with special reference to *H. contortus* infestation.

## 2.4 Benefits arising from the study

- To develop grazing and management strategies for *L. cuneata* with the aim of deceasing GIN infestation in sheep.
- Publication of articles in peer reviewed journals

# CHAPTER 3

# Literature review

## 3.1 Gastrointestinal nematodes of sheep

Gastrointestinal nematodes (GIN) are responsible for the most prevalent and most important group of diseases in small stock. GIN can cause dramatic production losses and contribute heavily to the veterinary bill of livestock farming (Taylor *et al.,* 2002; Min and Hart, 2003; Heckendorn, 2007; Rahmann and Seip, 2007; Morgan *et al.,* 2013). Helminth parasites belong to different genera within the class of Nematodes. The families, examples and predilection sites of more common nematodes in small stock are summarized in Table 3.1.

When the host's responses to GIN in goats and sheep were compared, besides that the same nematode species occur in both small ruminant species, key differences were established. Feeding behaviour between sheep and goats differs; goats are primarily or intermediate browsers of bush and tree foliages, whereas sheep are primarily grazers of grass and forages. This difference affects the risk of infection greatly. Goats do not show the same level of immune response against the installation of a larval challenge as sheep do. Goats, under imposed grazing conditions, will be more severely challenged by GIN infection compared to sheep (Hoste *et al.,* 2008). Therefore, for this study, only sheep will be referred to and not small stock in general.

**Table 3.1: Families, predilection sites, examples and effects caused by the important nematode parasites of sheep and goats common in the tropics.**

| Family | Parasites | Predilection sites | Action |
|---|---|---|---|
| Trichostrongylidae | *Trichostrongylus axei* (Stomach Bankrupt worm) | Abomasum | Mucosal damage |
| | *Haemonchus contortus* (Wire worm; Barber's pole worm)) | Abomasum | Blood sucking |
| | *Ostertagia ostertagi* (Brown stomach worm) | Abomasum | Mucosal damage |
| | *Trichostrongylus vitrinus* (Bankrupt worm) | Small intestine | Mucosal damage |
| | *Trichostrongylus colubriformis* (Bankrupt worm) | Small intestine | Mucosal damage |
| | *Cooperia curticei*/ *Nematodirus spathiger* (Long-necked Bankrupt worm) | Small intestine<br>Small intestine<br>Small intestine | Mucosal damage<br>Mucosal damage<br>Mucosal damage |
| Cyathostomidae | *Oesophagostomum venulosum* (Nodular worm) | Large intestine | Mucosal damage |
| | *Oesophagostomum columbianum* (Nodular worm) | Large intestine | Mucosal damage |
| Ancylostomatidae | *Bunostomum trigonocephalum* (Grassveld Hookworm) | Small intestine | Mucosal damage |

**Sources:** Thienpont *et al.,* 1979; Hansen and Perry, 1994; Bath and De Wet, 2000.

*H. contortus* is amongst the most important strongylid nematodes in the tropics and subtropics that cause severe economic losses in sheep farming (Vatta and Lindberg, 2006; Adamu, 2012; Adamu *et al*., 2013). They are voracious bloodsucking endo-parasites living in the digestive tract of their host, creating extensive damage to the gastrointestinal mucosa, resulting in increased plasma leakage and losses of endogenous protein and interference with retention of nitrogen, vitamins and minerals (Kimambo *et al.,* 1998; Villalba *et al.,* 2014). Clinical signs are anaemia (paleness in mucous membranes due to blood loss), bottle jaw and oedema (loss of blood protein, manifested as a soft watery swelling under the jaw), digestive disturbances (poor digestion or uptake of nutrients), dark-coloured faeces and a loss of appetite, all resulting in production losses or animal deaths (Bath and De Wet, 2000; Taylor *et al*., 2007). *H. contortus*, unlike other GIN species, is not a primary cause of diarrhoea (Roeber *et al*., 2013).

The life cycles of these nematodes follow a similar pattern (with a few exceptions, e.g. *Nematodirus*). Sexually dimorphic adults live in the digestive tract of the host. They are short-lived, surviving only a few months in their host (Roeber *et al*., 2013). Adult female nematodes produce an estimated 5000 to 15000 eggs per day. The eggs are passed out in the faeces of the host and will, under suitable environmental conditions, hatch. The hatched larvae undergo five stages of development, separated by four moults. The $L_1$ and $L_2$ stages feed on micro-organisms.

Suitable conditions are, e.g. when moisture, such as rain or dew, disintegrate the crust of the faecal material and allow the larvae to migrate out of the faeces. Depending on adequate moisture conditions, the infective $L_3$ larvae can migrate ± 5-10 cm (15 cm, according to Krecek *et al*., 1995) up and down the grass lamina. During lower moisture conditions the larvae hide in the base layer of the herbage and even in the soil (Krecek *et al*., 1995). Therefore, wet soil types, or for that matter leaking troughs, create supportive conditions for nematode development. During heavy rain, the larvae may even contaminate drinking water and infection can occur in this manner (Hansen and Perry, 1994).

When temperatures drop below 10°C, development of the larvae stops. When unfavourable conditions develop, such as during autumn, larvae undergo arrested development, called hypobiosis. The hypobiotic larvae will resume activity in the following spring, often in synchronisation with the peri-parturient decreased immunity phenomenon in ewes (increased GIN egg shedding), causing enhanced infection, together with an increase in the survival and egg production of existing parasites (Roeber *et al*., 2013). The life cycle of *H. contortus*, the most important Strongylid nematode, is displayed in Figure 3.1.

The sheath, which represents the cuticular layer shed during the transition from the $L_2$ to $L_3$ larval stages, protects the $L_3$ larval stage against unfavourable environmental conditions and prevents feeding until ingested by the new host. The sheath is then cast off in the abomasum and the $L_3$ undergoes a mould to the $L_4$ stage, which moulds into the reproductive adult stage (Reinecke, 1983; Bowmans, 1995; Krecek *et al*., 1995; Coffey *et al*., 2007; Taylor *et al*., 2007; Roeber *et al*., 2013).

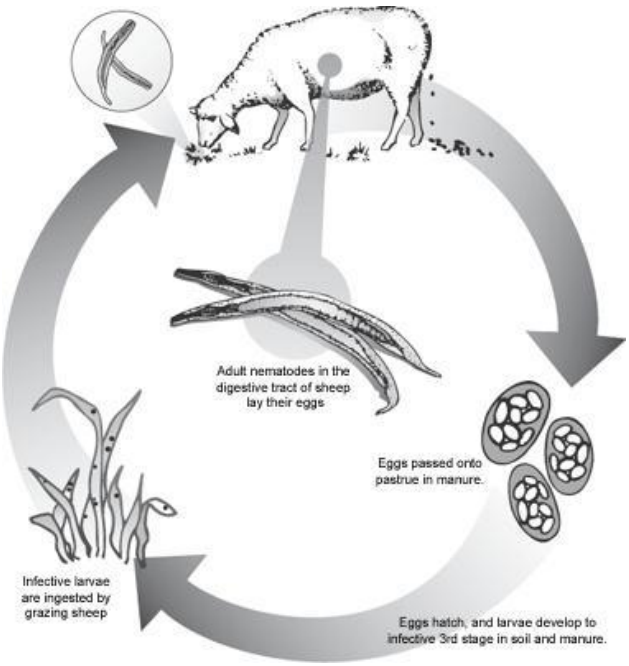


**Figure 3.1: Life cycle of *H. contortus*.**

**Source:** *Haemonchus contortus. Google search*

Under ideal conditions, such as sufficient moisture and heat, the development from eggs to infective larvae can be as short as seven to ten days. The time from ingestion of infective larvae till adults, laying eggs, called the prepatent period, varies amongst nematodes. For *H. contortus*, this period is two weeks (Reinecke, 1983; Bowman, 1995; Duval, 1994; Hansen and Perry, 1994; Coffey *et al*., 2007).

## 3.2 GIN control methods and strategies

### *3.2.1 Anthelmintics*

Anthelmintics are chemotherapeutics or chemical drugs developed to control internal parasites and currently form the backbone for health care in livestock production. However, the extensive use of chemical anthelmintics for the control of helminth infections in grazing livestock has resulted in the development of parasite resistance towards these drugs (Taylor *et al.*, 2002; Adamu *et al*., 2013).

A rapid increase in both the prevalence and magnitude of anthelmintic resistance (AR) towards chemical drugs is experienced globally (Min and Hart, 2003; Kaplan

and Vidyashankar, 2012; Morgan *et al.,* 2013). The levels of resistance in South Africa are among the highest in the world (Van Wyk *et al*., 1999; Bath, 2006; Vatta and Lindberg, 2006). Resistant *H. contortus* infestations in the benzimidazole group were reported for the first time in South Africa in 1975 (Berger, 1975). This was followed by successive reports of resistance covering other generic classes of anthelmintics (Van Wyk and Gerber, 1980; Van Wyk and Malan, 1988; Malan *et al*., 1988). The main groups of chemical anthelmintics commercially available are displayed in Table 3.2.

**Table 3.2: The main groups of chemical anthelmintics commercially on the market for sheep and goats.**

| Group | Generic class | Example |
|---|---|---|
| 1 | Macrocyclic lactones | Ivermectin |
| 2 | Benzimidazoles and probenzimidazoles | Albendazole |
| 3 | Imidazoles | Levamisole |
| 4 | Halogenated salicylanilides | Rafoxinide, Closantel |
| 5 | Nitrophenols | Nitroxyl |
| 6* | Sulphonamides | Chlorsulon |
| 7 | Organophosphores | Trichlorphon |
| 8* | Isoquinoline | |
| 9 | Others | Praziquantel |
| 10 | Combinations | Abectin/Closantel |

*Not mentioned in IVS Desk Reference Vol 12.

**Sources**: IVS Desk Reference Vol 12. IDR 2013/14. MIMS Media. P.O. Box 1741, Saxonworld 2132; Bath and De Wet, 2000.

### *3.2.2. Host resistance and resilience*

The term resistance, in regard to GIN infection, is used to describe the ability of the host to prevent or suppress the establishment and subsequent development of the parasite infection, while the term resilience refers to the ability of the host to maintain relatively unrepressed production when subjected to parasitic challenges (Bisset and Morris, 1996; Van Houtert and Sykes, 1996).

Resistance also refers to the immune response of the host against GIN, either by preventing or limiting the establishment or subsequent development of parasites (Duval, 1994). Genetics, physiological status and level of production may modulate the host's response to GIN (Hoste *et al.,* 2008).

### *3.2.2.1 Resistance and resilience breeds*

Substantial evidence that certain sheep breeds are more resistant to internal parasites than others, is well established. Regarding South Africa's own indigenous sheep breeds, claims of both resistance and resilience to internal parasites are made in popular and semi-scientific literature. The Blinkhaar Ronderib Afrikaner sheep is described as "tolerant of diseases and parasites" and the Nguni (Zulu) sheep as "tolerant of internal and external parasites". The Damara breed is mentioned for a quality described as a "high resistance level to parasites" (Ramsey *et al.,* 1998). Du Toit (2008) also described the Damara as a breed with "high resistance characteristics to internal parasites".

The indigenous (African) sheep breeds, e.g. the Zulu sheep, are viewed as a valuable source of genetic material due to their adaptation to harsh environments, nutritional fluctuations and "resistance to diseases and parasites". However, in a survey conducted where 76 farmers were asked to identify livestock production constraints, 58 mentioned parasites and predators as a constraint (Kunene and Fossey, 2006; Kunene *et al*., 2007). It seemed that even resilient breeds, to a lesser or greater degree, fall victim to parasite infections. Many other scientific evidences of superiority of some breeds regarding GIN resistance are found in the literature. Some of these are summarized in Table 3.3.

The variation in GIN resistance in sheep creates an opportunity for selection and crossbreeding for resistance to internal parasites (Kosgey and Okeya, 2007). Complete resistance, generally ignored as a commercial reality, can be the ultimate solution (Saddiq *et al*., 2011). However, a major drawback of this innate resistance is that none of the breeds named for resistance, are of the recognised higher producing meat and wool breeds (Adamu, 2012).

**Table 3.3: Summary of the results of nine research projects where researchers compared different breeds to internal parasite resistance.**

| Resistant Breed | Comparison Breed | Parasite Species* | Reference |
|---|---|---|---|
| Targhee | Rambouillet | Osp | Scrivner (1964) |
| Florida Native | Rambouillet | Hc | Jilck and Bradley (1969) |
| Florida Native | Suffolk | Hc | Ruvuna and Stephens (1997) |
| Florida Native | Dorset xRambouillet | Hc | Zajac *et al.* (1988) |
| Florida Native | Barbados | Hc | Courtney *et al*. (1985a) |
| Red Maasai | Merino, Corriedale Hampshire | Hc | Preston and Allonby (1978) |
| Red Maasai | Dorper | Hc | Baker *et al.* (1994) |
| Red Maasai | Blackheaded Somali, Dorper and Romney Marsh | Hc | Mugambi *et al.* (1997) |

* Hc: *Haemonchus contortus*; Tsp: *Trichostrongylus* species; Osp: *Ostertagia*; (*Teladorsagia*) species
**Source**: Correa *et al*., 2012; Mugambi *et al.*, 1997.

### *3.2.2.2 Resistance and resilience within a breed*

Individual sheep within a breed differ regarding their resistance and/or resilience to internal parasites and these traits offer the possibility of curbing production losses caused by nematode parasitism. Studies are underway to identify the quantitative trait loci (QTLs) responsible for resistance to *H. contortus* (Marshall *et al*., 2013). Different approaches are described, namely: i) breed for resistance (reduced parasite numbers, as determined by FEC), ii) breed for resilience (production despite parasitism) or iii) number of treatments required during parasitism (Bisset and Morris, 1996; Woolaston and Baker, 1996; Sayers and Sweeney, 2005; Bishop, 2012).

The intensity of parasitism in a flock, as represented by FEC, presented a positively skewed distribution, which indicates that parasites aggregate within a few individuals, while some others develop some degree of GIN resistance (Hoste *et al*., 2008). Systems like the "The five point check© system of targeted selective treatment"(TST) and the FAMACHA© system, were developed to identify less GIN resistant animals in a flock for treatment and possible culling (breeding for resistance), while excluding blanket treatment of a flock and the treatment of healthy animals (Bath and Van Wyk, 2001; Bath 2006; Bath and Van Wyk, 2009; Morgan *et al.*, 2013). A major threat of resilient individuals in a flock is, while coping

production-wise with GIN, they contribute to pasture contamination, therefore compromising the rest of the flock.

The age, condition and reproductive stages of the host influence susceptibility to parasitic infections within a flock. Animals in good condition seem to tolerate GIN better, most likely due to their ability to better manage the protein and iron losses induced by the parasites, opposed to poorly fed animal especially on low protein diets (Van Houtert and Sykes, 1996). Lambs under the age of six months are known to be more prone to infection.

Targeted nutritional supplementation with protein, energy, or a combination of both, during critical stages, particularly with additional dietary protein, appears to be effective in enhancing specific immune responses to withstand parasitism (Brown *et al., 1991;* Coop and Kyriazakis, 2001; Hoste *et al.*, 2008). Protein supplementation in the diet supports parasitic protein losses and appears to be effective in enhancing specific immune responses (Brown *et al., 1991.)* Protein, energy or a combination of both were found to consistently decrease GIN infestation (Hoste *et al.*, 2008).

### *3.2.3 Copper oxide wire particles (COWP)*

This treatment is based on the lethal effects of the COWP on the parasite. COWP, administered in the form of boluses, have shown promising results as an alternative for chemical anthelmintics (Vatta *et al*., 2009). However, Burke *et al.,* (2007) found that COWP did not control the $L_4$ larval stage.

### *3.2.4 The use of fungus and bacteria*

Attempts to control GIN with fungus and bacteria have been researched (FAO 1997). The nematophagus micro fungus, *Duddingtonia flagans,* forms sticky traps that capture infectious larvae in faeces, preventing them from migrating onto the pasture and infecting hosts. Although the system gave promising results, it did provide some serious practical challenges (Larsen, 2006).

The toxins from certain strains of the bacteria, *Bacillus thuringiensis,* could harm the free living stages of multiple nematode species and can be considered as an alternative strategy for controlling parasites (Wei *et al*., 2003 as cited by Ahmed, 2010).

### *3.2.5 Grazing management*

Over the years, many strategies have been advocated to minimize the risk of parasite infection by implementing certain grazing strategies (Duval, 1994; Hansen and Perry, 1994; Bath, 2006; Adamu 2012). These strategies are mainly based on the survival success of the free living stages of the parasites in certain environmental conditions, plus the susceptibility of hosts. Some strategies are:

- Animal density – heavily stocked areas increase parasite infection risk.
- Grazing time/height – The drier the herbage, the closer the larvae will stay to the base of plants. Up to 80% of larvae are concentrated in the bottom five cm of the herbage layer. Therefore, graze higher than five cm. On the other hand, severe grazing (short grazing) will expose the bottom herbage layer to sunlight and high temperatures, resulting in the dry conditions that will theoretically kill larvae.
- Multispecies grazing – many parasites are host specific, i.e., cattle can be used to "clean up" a pasture by digesting a lot of larvae, before sheep are allowed onto the pasture.
- Grazing by age group – Graze heavily infected pastures with the more resistant animals, i.e., older animals.
- Resting – Duval (1994) stated that a rest of at least three years is necessary to clean out pastures from parasites completely, which is in the majority of cases impractical, but Hansen and Perry (1994) declared that pastures are parasitologically safe after ten weeks or more of a prolonged period of dry weather. Bath (2006) viewed 12 weeks as an optimal time of absence of susceptible animals.

The level of nutrition affects the ability of animals to tolerate or succumb to infection with internal parasites (Coop and Kyriazakis, 2001). As mentioned in Section 3.3.6, animals in good condition seem to tolerate GIN better. Especially around the time of

weaning and parturition, sheep are most susceptible to parasitism. During the last trimester of pregnancy, ewes showed an increased shedding of parasite eggs, likely due to hormone changes induced by peri-parturient relaxation of resistance. This phenomenon unfortunately enhances the contamination of pastures for the young lambs (Jeffcoate *et al*., 1990; Knox *et al*., 2006; Adamu, 2012).

Many of these strategies, supported by the administration of chemical anthelmintics, are in use by farmers worldwide with varying levels of success, but grazing management alone, is not adequate as a solution to helminth control (Adamu, 2012).

### 3.2.6 *Use of bio-active forages in ethnoveterinary medicine with special reference to tanniferous plants*

Bio-active plants are plants that contain secondary plant substances and metabolites which are considered to be beneficial for animal health, rather than only contributing to, or optimizing animal nutrition. In this context, tannin rich forages, like big trefoil (*Lotus corniculatus*), sulla (*Hydysarium coronarium*), sanfoin (*Onobrychis viciifolia*), and *Lespedeza cuneata,* with levels less than 50 mg condensed tannin $kg^{-1}$ bodyweight in feed, can play a role in the reduction of bloating and increased milk production, a reduction in internal parasite numbers, egg output, and hatchability (Gilboa, 1995; Athanassiadou *et al*., 2001; Mehanho, 1987 as cited by Min and Hart, 2003; Hoste *et al*., 2006;). However, feeding tanniferous plants is not only associated with positive effects, but also with negative consequences (Rahmann and Seip, 2007).

Tannins are defined as any phenolic compound of sufficiently high molecular weight, containing sufficient hydroxyls and other phenolic groups which effectively form strong complexes with proteins and other macromolecules in a particular environment. The ancient technology of preserving animal hides through a process called tanning, (meaning to "preserve and to waterproof") depends on the special characteristics of tannins (Hovarth, 1981; Reed *et al*., 1982; Van Soest *et al*., 1987).

Tannins are mainly found in the surface wax or vacuoles of plants, either in the buds, leaves, roots and seeds or stem tissues where they do not interfere with the metabolism of the plant. Tannins can be present in neutral detergent fibre (NDF) and Acid detergent fibre (ADF) in significant amounts, which are tightly bound to the cell wall and cell protein (Reed *et al*., 1982; Makkar, 2003). Effects of tannins vary, depending on the type of tannin, concentration, and on the animal consuming the tannins.

Based on their molecular structure, tannins can be subdivided into two groups.

- Hydrolysable tannins (HT) contain a carbohydrate as central core and can, depending on which compounds they are metabolized to (e.g., pyrogallol), potentially be toxic to animals.
- Condensed tannins (CT) are polymers of flavonoid compounds and are also called pro-anthocyanidins (PA), because of their property of forming red pigments when treated with acid. CT are more widely distributed than HT. CT are the most common type of tannin found in forage legumes (including *L. cuneata*), trees and shrubs (Reed *et al*., 1982; Van Soest *et al*., 1987; Min and Hart, 2003; Mueller-Harvey, 2006).

CT can form complexes with many other types of molecules such as proteins, minerals, polysaccharides and nucleic acids. The formation of these complexes is influenced by factors such as pH and the molecular weight of both the tannin and the compound with which it can form a complex. CT seemed to have a much lower affinity for carbohydrates, particularly starches, than for proteins (Min and Hart, 2003).

Tannins play a role as a defence mechanism in the plant and protect the plant against pathogens and defoliation. Generally, tannins will defend against utilization of the plant by means of:

- *Lowering the palatability (bitter, unpleasant taste or dry feeling in the mouth).* This happens due to the formation of complexes between tannins and salivary glycoproteins, with the effect of lowering acceptability of feed, lower intake and a negative effect on animal production (Shimada, 2006). In some animals, the salivary proline-rich proteins are thought to be the first defence against

tannins (Mueller-Harvey, 2006). When field dried, the tannins in tannin-rich forages become more polarized, resulting in a lower number of free hydroxyls available for binding proteins, which in practice implies that, in hay form, safe intake levels could be higher, compared to green grazing (Hovarth, 1981; Terrill *et al*.,1989; Barry and McNabb, 1999; Reed *et al*., 2003). However, animals normally consuming tannin-rich feed appear to develop defensive mechanisms against tannins (Makkar, 2003).

- *Negative effects on digestion*

  Tannins decrease digestion of fibre, by reducing cell-wall digestibility by binding bacterial enzymes and forming indigestible complexes with cell-wall carbohydrates. Dietary and metabolic proteins are complexed by tannins and these bounded proteins are generally resistant to breakdown by proteases and hence may be unavailable for animal's nutrition, leading to a decline in animal productivity. (The use of tannins in leather tanning is an excellent example of how tannins can bind proteins). The slowdown in digestion caused by tannins can, in its turn, also lower intake due to gut fill (Hovarth, 1981; Barry and McNabb, 1999; Reed, *et al*., 2003). Goats and sheep differ in their level of tolerance to the effects of CT. Goats produce more protein–rich saliva during eating than sheep and are generally more tolerant of CT, hence their browsing habits (Min and Hart, 2003.)

Apart from the negative effects, there are also positive effects. According to Min and Hart (2003) and Hoste *et al*., (2006), moderate concentrations of CT (20-40 g CT $kg^{-1}$ DM) are helpful to animals, while high-forage CT concentrations (>55 g CT $kg^{-1}$ DM) may have negative effects. Positive effects include an increase in bypass protein and cause the animals to use protein more efficiently. The mechanism behind this is that CT will bind to protein by hydrogen bonding to form CT-protein complexes in the rumen where the pH level is normally near neutral (pH 6.0 to 7.0). These complexes cannot be degraded by rumen micro-organisms. When rumen content progresses to the abomasum, where the pH level is lower than 3.5, the complexes dissociate and release the protein and in such a way increase the amino acid supply to the abomasum and small intestines, enhancing the nutritional status of the animals.

When ruminants are fed on high quality forages, containing high amounts of nitrogen, e.g. ryegrass, carbohydrate digestion in the rumen is optimal, but the degradation of the forage nitrogen can lead to a surplus of ammonia in the rumen, which will then be excreted as urea in urine. Tannin complexing can therefore increase protein assimilation by safeguarding some proteins safely through the rumen to the rest of the intestines, instead of being lost through secretion. However, the chemical structures of compounds and CT concentration will affect this process either negatively or positively (Min and Hart, 2003; Hoste *et al.,* 2006).

CT may thus have an indirect effect on GIN by replenishing the extensive endogenous protein losses encountered in the abomasum and small intestine during GIN parasitism, thus enhancing the immunity or improving host homeostasis (Brown *et al.,* 1991; Niezen *et al*., 2002; Min and Hart, 2003; Hoste *et al*., 2006).

Opposed to the indirect effects of tannins on parasites, are the direct effects of tannins on the parasites, which mechanisms are yet more a subject of speculation, than proven facts. A hypothesis was put forward that the binding ability of CT can play a role in the direct effect of tannins on nematodes, by CT bonding with the proline and hydroxyproline-rich cuticle that covers the body and lines certain parts of the digestive tract and reproductive system of the nematode (Hoste *et al*., 2008). Repressed female nematode reproductive activity may explain the decrease in FEC output measured in several trials where feeding experiments were conducted with tannin-rich forages (Terrill *et al*., 1989; Niezen *et al.*, 1998; Min and Hart, 2003, Min *et al*., 2005; Niezen *et al*., 2002; Shaik *et al*., 2004, Shaik *et al*., 2006; *Lange et al*., 2006; Heckendorn, 2007; Ahmed, 2010). However, when the animals were removed from the forage, FEC increased again, indicating that worms were inhibited and not killed (Coffey *et al.,* 2007). Furthermore, electron microscopic investigations showed cuticular changes after contact with tannins that may inhibit or delay the exsheatment of the $L_3$ after indigestion by the host (Hoste *et al*., 2006).

*L. cuneata* is a condensed tannin (CT)-containing pasture and has scientifically proven anthelmintic properties (Terrill *et al.,* 1989; Min and Hart; 2003, Min *et al*., 2005; Shaik *et al*., 2004, Shaik *et al*., 2006; Bath, 2006; Lange *et al*., 2006 and Ahmed, 2010). *L. cuneata* is a perennial, erect, summer growing, deep rooted, drought tolerant,

non-bloating leguminous pasture. Being a legume, the plant's nitrogen fixing abilities, together with its adaptability to low fertility soils, characterized it as a low cost pasture on poor soils. The pasture has been known for many years, but it had a stigma of poor animal production due to the low digestibility of high tannin-containing cultivars that were available on the market. The more recent cultivars, AU Lotan and AU Donelly, are improved lower tannin cultivars with higher digestibility and are associated with better animal performance (Dannhauser, 2002).

The plant is endemic to the eastern parts of Asia. Originally it was exported to the USA to be used in soil rehabilitation works. It started to gain popularity around 1940 when it was "discovered" by poor farmers as a low cost pasture, able to grow on acidic and low phosphorus soils, and acquired the name "poor man's lucerne". The pasture, especially the newer cultivars, is slowly gaining popularity in South Africa. Locally, only a few scientific publications exist, but several popular articles were published.

# CHAPTER 4

# Investigation into the gastrointestinal nematode resistance to standard anthelmintics in the sheep flock at Dundee Research Station

## 4.1 Aim

To Investigate:

- Gastrointestinal nematode (GIN) resistance to standard anthelmintics in the sheep flock at Dundee Research Station.
- Resistance to gastrointestinal nematode (GIN) within the sheep flock at Dundee Research Station.

## 4.2 Introduction

Over the last few decades, extensive use of synthetic anthelmintics to control GIN infections in grazing livestock has resulted in the development of widespread nematode resistance to chemical anthelmintics (Taylor *et al*., 2002; Ahmed, 2010; Morgan *et al.,* 2013; Greef *et al.,* 2014). The mechanisms underlying anthelmintic resistance (AR) appear to be through receptor loss or a decrease of the target site's affinity for the drug. Important influential factors in the development of resulted resistant strains are driven by frequency of treatment and under-dosing of animals (Bezier and Love, 2003; Preston *et al*., 2014).

It becomes increasingly difficult to manage GIN infections on farms, leading to a highly increased veterinary bill with which farmers attempt to counteract decreased animal production. Therefore, at farm level it becomes critically important to determine the status of nematode resistance to commercially available anthelmintics in order to identify the continued efficacy of chemical anthelmintics for use in the control programme. The data collected in this study will also provide important background information for use in the *in vivo* feeding trial (Chapter 6).

## 4.3 Literature review

Köhler (2001) stated that parasite resistance is the genetically transmitted loss of sensitivity in populations that were previously susceptible to the same drug.

Anthelmintic chemotherapy forms the backbone of helminth control programmes and will remain so for the foreseeable future. Therefore, it is important to develop strategies where the different anthelmintics currently available on the market, can be utilized as effectively as possible until sustainable and implementable alternative methods have been developed at farm level. The success of these strategies relies heavily on the timely, effective monitoring, and sensitive methods to detect and manage infection and resistance. Views on the current status of GIN diagnostic procedures commented that the worm egg count test (FEC), despite other methods available, is still relatively reliable, and time and cost effective. However, other methods have been developed successfully and will in time be more widely used at farm level, e.g. lectin test, polymerase chain reaction and haemonchus "dipstick test" (Colditz *et al*., 2006; Roeber *et al*., 2013; Preston *et a*l., 2014).

The most widely used method to identify and monitor internal parasite resistance *in vivo*, is the faecal egg count reduction test (FECRT). The FECRT provides an anthelmintic efficacy by comparing FEC pre-and post-treatment (Taylor *et al*., 2002; Coles *et al*., 2006). The test has limitations (Rinaldi *et al.,* 2011; Preston *et al*., 2014), because it is labour intensive and unclear criteria, regarding optimum sampling and counting, exists. However, the test is still a useful indicator of drug efficacy in the field. Results must only be seen as an indication of the level of infection, because the test only measures egg output by mature worms, which does not always correlate well with actual worm numbers (Taylor *et al*., 2002; Morgan *et a*l., 2013; Preston *et al.,* 2014). However, good correlations have been found between faecal egg counts and worm counts for *Haemonchus contortus* but not for *Trichostrongylus colubriformis* (Sangster *et al.,* 1979 as cited by Taylor *et al*., 2002; Roeber, 2013; Preston *et al*., 2014).

Several researchers advised that FEC for the FECRT must be done only after 10 to 14 days post-treatment (Martin *et al.,* 1985; Taylor *et al*., 2002; Hotson *et al.,* 2008). However, when Levamisole resistances was expected, it was found that egg counts must be done after 11 or more days, due to the maturation of immature stages. False results may occur if faecal samples are taken at less than seven days (Grimshaw *et al*., 1996).

Helminthoses involves multiple parasite species. In certain cases key-specie identification of the parasites is needed, e.g. to ensure appropriate treatment. Where treatment decisions are based on increasing FEC, specific identification is not a priority (Morgan *et al.,* 2013).

Broad-spectrum anthelmintics in sheep are divided into two broad types, namely short-acting products (repeat after three weeks) or long-acting products (repeat after 100 days). The World Association for the Advancement of Veterinary Parasitology Guidelines (Coles *et al.,* 1992) set the following standards for anthelmintic effectiveness which needed to be expressed against each genus/specie (larvae/adults): Highly effective (>98% control); effective (90-98% control); moderately effective (80-89% control), or insufficient effective (less than 80% control).

At the Dundee Research Station a FECRT was done on the resident Merino flock in 2003. The FECRT was repeated at a neighbouring farm to investigate the level of anthelmintic resistance in both flocks and to develop parasite control strategies for both farms. The results from these FECRT's (Table 4.1) highlighted the differences of parasite resistance on farms, as a result of their history of anthelmintic use. For the Research Station, only Levamisole and Nitroxinyl were effective in GIN control. Doramectin and Closantel gave insufficient control and in the case of Ivermectin, zero control. On the neighbouring farm Closantel was highly effective and Doramectin gave effective control. The control by Ivermectin and Levamisole was ineffective. At Dundee Research Station parasite resistance could be established for Doramectin, Ivermectin and Closantel, and Ivermectin and Levamisole at the neighbouring farm (Van Zyl and Sadie, 2003).

**Table 4.1: Internal nematode control (%) with administering different anthelmintics in a FECRT at Dundee Research Station and neighbouring farm.**

| | **Anthelmintic Active** | | | | |
|---|---|---|---|---|---|
| **Active** | Doramectin 1% m/v* | Ivermectin 1% m/v* | Levamisole HCL,2.56% m/v* | Closantel 5% m/v* | Nitroxinyl 34% m/v* |
| **Anthelmintic group** | 1 | 1 | 3 | 4 | 5 |
| **Trade name** | Dectomax injection | Ivermectin Injection | Prodose red oral solution | Prodose Yellow | Trodax |
| **Control at Dundee Research Station (%)** | 47% (Insufficient control) | 0% (Zero control) | 89% (Moderately effective) | 46% (Insufficient control) | 99% (Highly effective) |
| **Control at the neighbouring farm (%)** | 87% (Effective) | 45% (Insufficient control) | 50% (Insufficient control) | 100% (Highly effective) | Not applicable |

*Mass volume$^{-1}$

**Sources:** *Van Zyl and Sadie, 2003: Progress report: The resistance of internal parasites in sheep to various active ingredients contained in commercial anthelmintics. KZN Department of Agriculture and Environmental Affairs.*
*IVS Desk Reference, Vol. 12. IDR 2013/14. MIMS Media. P.O. Box 1741, Saxonworld, 2132.*

Bezier and Love (2003), referring to the high level of anthelmintic resistance experienced in Australia, emphasized that the sheep industry urgently needs to adopt approaches that minimise reliance on chemical control, such as the breeding of worm resistant sheep, the use of specific grazing strategies and the application of flock treatment tactics, e.g. targeted selective treatment (TST), to minimise further resistance development (Bath and Van Wyk, 2009; Morgan *et al.*, 2013; Greef *et al*., 2014; Karrow *et al*., 2014).

## 4.4 Materials and methods

### *4.4.1 Experimental site*

The field trials were conducted at the Dundee Research Station, Department of Agriculture and Environmental Affairs, KwaZulu-Natal. The Research Station is situated about 10 km east of Dundee (latitude 28°10'S and longitude 30°31'E) at an altitude of 1219 m (2830AB). The long-term mean rainfall is 782.8 mm a$^{-1}$ and the

distribution is a typical summer rainfall pattern with convectional rainfall in the months from October to March. The Veld type is Sandy Sourveld.

### 4.4.2 Experimental design and sheep management

#### 4.4.2.1 Experiment 1: Monitoring of GIN infection in merino lambs

Thirty four Merino lambs, three months post-weaning, from the resident flock at Dundee Research Station, were monitored for GIN infection. After the first good rains of the season in November 2013, feacal egg counts (FEC) counts were done on the lambs individually and were repeated over time. The lambs grazed on grazing maize (unharvested maize) since birth in May 2013, weaned in September and were then moved to veld (Sourveld) for summer grazing. They were allowed to graze freely and *ad-libitum* for the duration of the trial (November until beginning of January). The sheep were supplemented with a protein containing commercial lick (Maxiwol pellets, intake aimed at 400 g $lamb^{-1}day^{-1}$) since the spring and early summer were dry. Clean drinking water was available *ad-lib*.

#### 4.4.2.2 Experiment 2: Monitoring of GIN infection in merino ewes and Feacal egg count reduction test (FECRT)

Thirty-nine dry, but pregnant Merino ewes, initial live weight 53.7 kg ± 9.5 kg, were used in a FECRT, for duration of two weeks, but were monitored in terms of live weight and FEC for a further 49 days post-treatment. The sheep also overwintered on grazing maize and were moved for summer grazing to a rain-fed Nile grass (*Acroceras macrum)* pasture where they stayed for the duration of the trial (January till April 2014 – mid to late summer), and became naturally infected with GIN. The pasture is situated in a low lying area on moist sandy soil and is continuously used for sheep grazing; therefore it represents ideal conditions to support the parasite life cycle and be contaminated with parasites. A rotational grazing system was used in small camps. The sheep were supplemented with only a summer lick (P6) during the trial period with an aimed intake of 30 g $animal^{-1}day^{-1}$. Clean drinking water was available *ad-libitum*.

Weekly FEC were done prior to the experiment for three weeks to monitor the parasite infestation level. Sheep with positive FEC counts exceeding 800 eggs per gram (EPG) were stratified by live weight and their initial FEC. Each group was randomly assigned to one of four different commercial chemotherapeutic treatments, representing the four different active ingredient groups, shown in Table 4.2.

**Table 4.2: Commercial chemotherapeutic products used in the FECRT at Dundee Research Station, 2014.**

| Active ingredient | Concen-tration % | Dosage rate | Group | Group name | Trade name | Persis-tancy | Company |
|---|---|---|---|---|---|---|---|
| **Ivermectin** | 1 m/v* | 1 ml/50 kg | 1 | Macrocy clic lactones | Ivomec injection | None | Merial |
| **Levamisole** | 2.56 m/v* | 15 ml/50kg | 3 | Imida-zoles | Prodose red oral solution | None | Virbac |
| **Rafoxanide** | 3 m/v* | 2.5 ml/10 kg | 4 | Halogen ated Salicyl-analides | Nasalcur | None | MSD Animal Health SA |
| **Trichlorfon** | 20 m/v* | 2.5 ml/10 kg | 7 | Organo-Phos-phate | Unidose | None | MSD Animal Health SA |

*Mass volume$^{-1}$

**Source:** IVS Desk Reference, Vol. 12. IDR 2013/14. MIMS Media. P.O. Box 1741, Saxonworld, 2132.

### *4.4.3 Sample collection and parasitological analysis*

Fresh dung samples were taken during the morning on a weekly basis. The samples were taken directly from the rectum of each individual sheep to determine FEC in the sheep in both Experiment 1 and 2. The FEC was done on roundworms as a group, since individual species are very difficult to distinguish by egg morphology and require further processing into larvae cultures (Thienpont *et al*., 1979; Preston *et al*., 2014).

The faecal samples (10-15 g) were analysed for FEC using the McMaster technique (Hansen and Perry, 1994). Three grams of faeces were diluted into 30 ml of a saturated 40% sugar solution. After mixing the solution a sample was taken with a pipette and dropped into the McMaster slide chambers. Using a microscope,

helminth eggs were counted in both sides of the chamber and multiplied by 50 to estimate the total number of eggs in the sample as eggs per gram (EPG) of faeces.

### *4.4.4 Statistical analysis*

The data on FEC were analysed by using Repeated Measures Analysis of Variance. $Log_e$ transformations were used to stabilise variance in egg counts. Fisher's test of least significant differences (LSD) was conducted at a 5% significance level.

## 4.5 Results and discussion

### *4.5.1 Climate*

Climatic data was sourced from the Agricultural Research Council (ARC) (Agrometeorology, 2014*)* weather station, based at the Dundee Research Station (Comp. 30109). The weather data in the trial season, compared to the long-term average per month over 13.5 years, is displayed in Figures 4.1, 4.2 and 4.3.

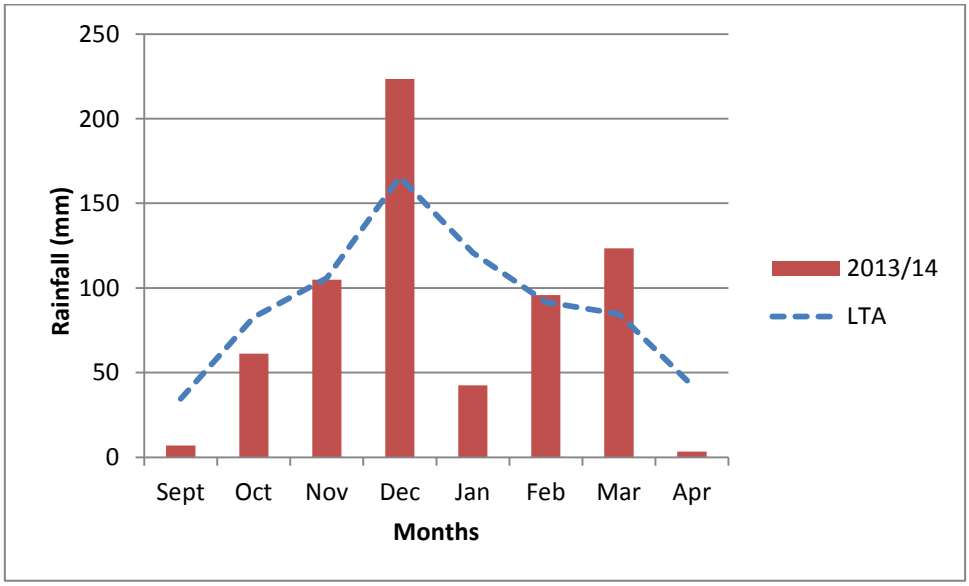


**Figure 4.1: Monthly rainfall (mm month$^{-1}$) for growing season 2013/2014, compared to the long-term rainfall for Dundee Research Station.**

The 2013/14 growing season was characterised by drought, with erratic showers. The total rainfall for the summer months of September until April, during which period the experiments took place, was 661.41 mm, compared to the long-term total of 782.29

mm for this period. The rainfall in September and October were below normal. The November rainfall was close to normal, but was concentrated towards the end of November (70% rain in the last two weeks). The abnormally high rainfall in December was followed by a very dry January; rainfall during February was on average, followed by above normal rainfall for March and a far below average for April (Figure 4.1).

Temperatures, both daily maximum (Tx) and daily minimum (Tn), for this period were normal, except for slightly above average results for September and November. December and March were below average, which corresponds with months with above normal rainfall and are probably related to overcast weather (See Fig 4.2 and how it relates to Fig 4.1).

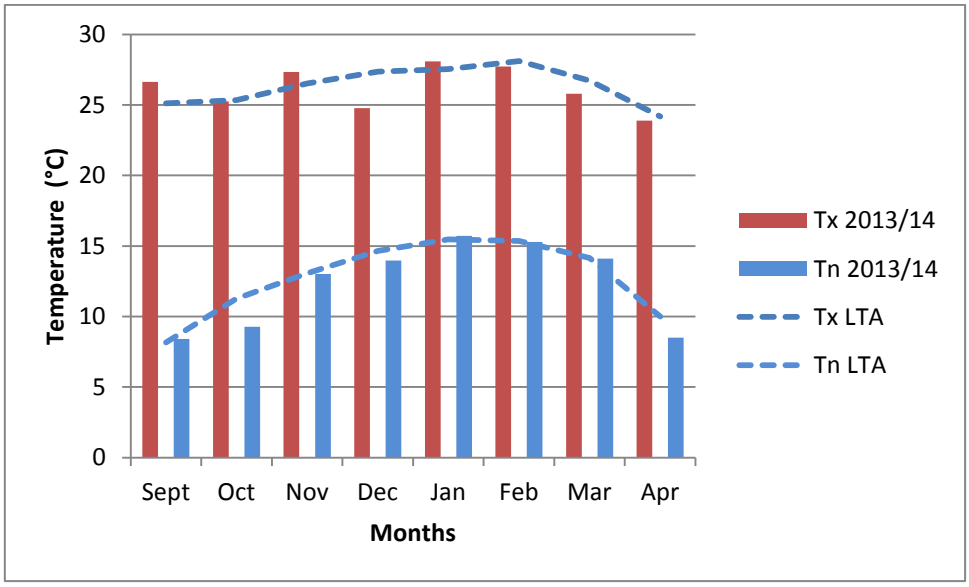


**Figure 4.2: Mean monthly maximum (Tx) and mean monthly minimum (Tn) rainfall for September 2013 until April 2014, for Dundee Research Station, compared to the respective long-term means.**

The relative humidity, more so relative minimum humidity, for the trial period followed the rainfall trend and was also above normal for the wet months, December 2013 and March 2014 (Figure 4.3).

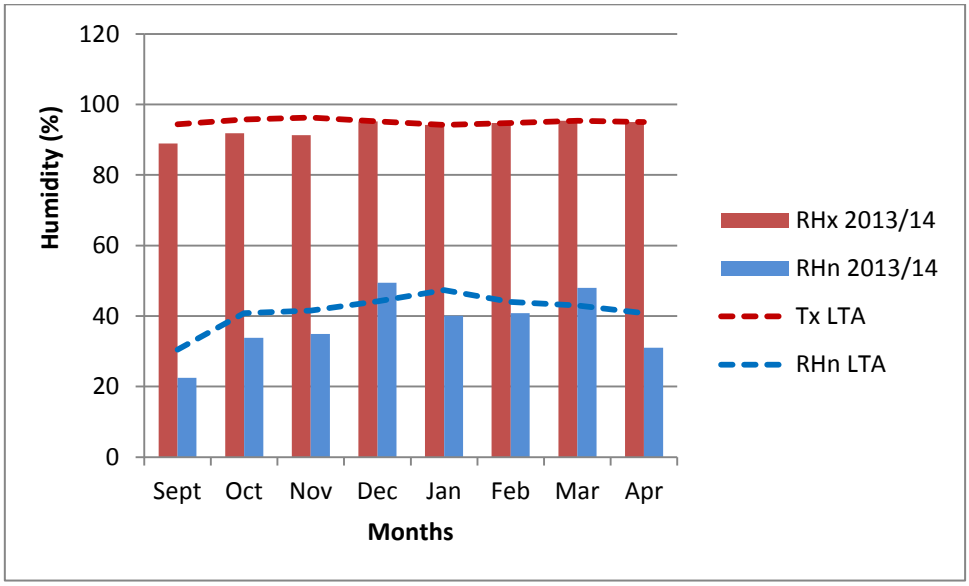


**Figure 4.3: Average Monthly Relative Maximum Humidity (RHx) and Average Monthly Relative Minimum Humidity (RHn) for December 2013 till April 2014, compared to the respective long-term averages for Dundee Research Station.**

### *4.5.2 FEC*

#### *4.5.2.1 Experiment 1: Monitoring of GIN infection in merino lambs*

Thirty four Merino lambs with live weight 34.2 kg ± 6.4 kg were monitored for parasite infection, using FEC as indicator. The initial risk of GIN infection was low due to the low winter temperatures and the dry maize land conditions where lambs spent the winter, followed by the dry climatic conditions in early summer when the animals were moved to the veld camps. The FEC in lambs was done for the first time towards the end of November after good rain fell and GIN infections were expected. The average FEC showed low numbers (Average EPG of 282). This increased to an average of 408 EPG for the FEC count in early December. Due to the holiday season, labour was restricted and no FEC were done until early January, when a sharp increase in FEC's was measured (Figure 4.4) The trial was terminated due to humanitarian reasons and lambs were treated accordingly.

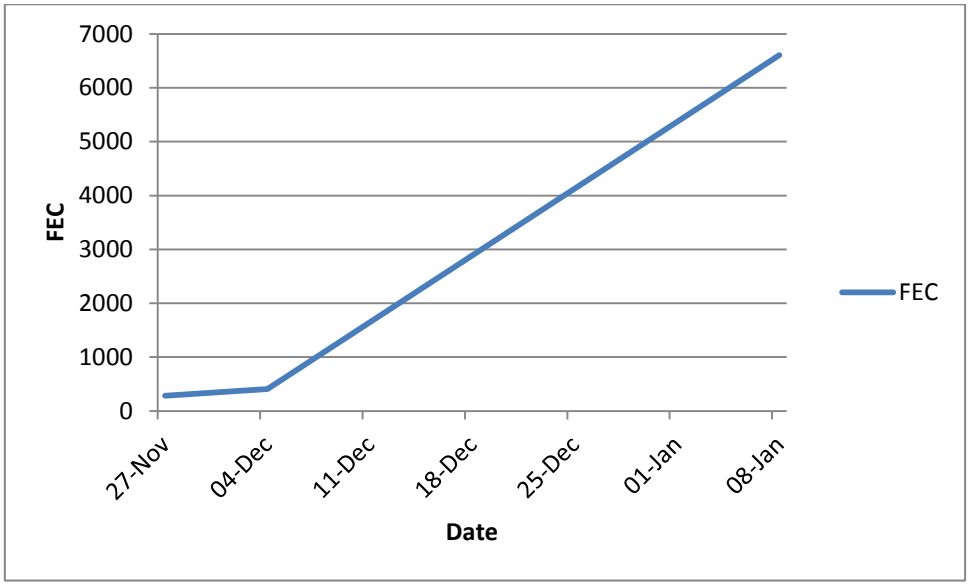


**Figure 4.4: FEC of lambs over time.**

According to Hansen and Perry (1994) EPG counts in dung can be indicative of light infection when mixed EPG counts are between 50 and 800, moderate when between 800 and 1200 and heavy when above 1200. However, Colditz *et al.* (2006) stated that up to a level of 1600 EPG, a mature host is not compromised.

The sharp increase in FEC can be explained, taking climatic conditions into consideration. Krecek *et al.* (1995) showed that air temperature and soil moisture have an important increasing effect on the numbers of $L_3$ larvae, but relative humidity has a lower effect, especially in lower vegetation layers. However, the climatic data, as reported above for this study, may therefore differ from the real conditions in herbage layers, but serves as an indication. According to Hansen and Perry (1994), temperatures between 22°C and 26°C, plus sufficient moisture, provide ideal conditions for larval development. Under favourable climatic conditions eggs can hatch and develop into the infectious $L_3$ stage within seven to ten days and after ingestion by the host, adult parasites can shed eggs within another 14 days. During dry spells, larvae can survive for up to six weeks or even longer, if sheltered e.g. in manure patches (Bath and De Wet, 2000).

Naïve lambs, in order to express an immune response to infection with parasites, need to be challenged to develop immunity. Following the winter period, the maize land conditions and dry veld conditions, the infection risks were low and immune challenge can be regarded as marginal. After the first good rains in November (Figure 4.1), climatic conditions turned favourable for parasite development and an increase in FEC resulted within three to four weeks and escalated thereafter. This observation can be explained based on the findings of previous research. Lambs were in a growing phase and Coop and Kyriazakis (1999), proposed that "the function of growth is prioritized over the expression of immunity". Nutrition levels on the veld were marginal due to the weather conditions. Should short term improved nutrition be provided in such cases, the capability of the hosts to cope with the consequences of parasite infection, should be enhanced. In case of better nutrition, i.e., grazing higher protein-containing forage, it is expected that the lambs should manage the infection better (Hoste *et al.,* 2006).

#### *4.5.2.2 Experiment 2: Monitoring of GIN infection in merino ewes*

- ***Feacal egg count reduction test (FECRT): Day 0 to Day 21***

On the 14th of January 2014 the initial FEC for the ewe group showed an average of 1179 EPG. Comparing the last average FEC results of lambs, exceeding 6000 EPG, taken on (8th January 2014) and the first one taken in the ewe group (14th January), which is one week apart, it was observed that the ewes, notwithstanding the more favourable parasite conditions on their grazing, showed a lower average FEC compared to the lambs. This demonstrated better parasite tolerance by the ewes compared to the lambs.

Amongst the sheep selected for the trial, seven ewes were identified who constantly showed FEC below 200 EPG during the three week pre-treatment phase. The results eliminated them from the Feacal egg count reduction test (FECRT) (Taylor *et al*., 2002). These seven sheep were allocated to their own group (called the not-treated group or NT group), but stayed in the experimental flock to be monitored for the duration of the trial for possible resistance to parasite infection within the flock. The

rest of the sheep, which showed an average FEC of 1179 EPG, were subjected to the four treatments (n=8) on Day 0.

On Day 14 statistically significant differences (P<0.05) were measured between the FEC in the Trichlorfon and Levamisole treatments, as well as in the Trichlorfon and Ivermectin treatments, but not between the FEC in the Trichlorfon and Rafoxinide treatments. On Day 21 only the Levamisole treatment differed significantly (P<0.05) from the other treatments and showed a significantly higher FEC (Figure 4.5).

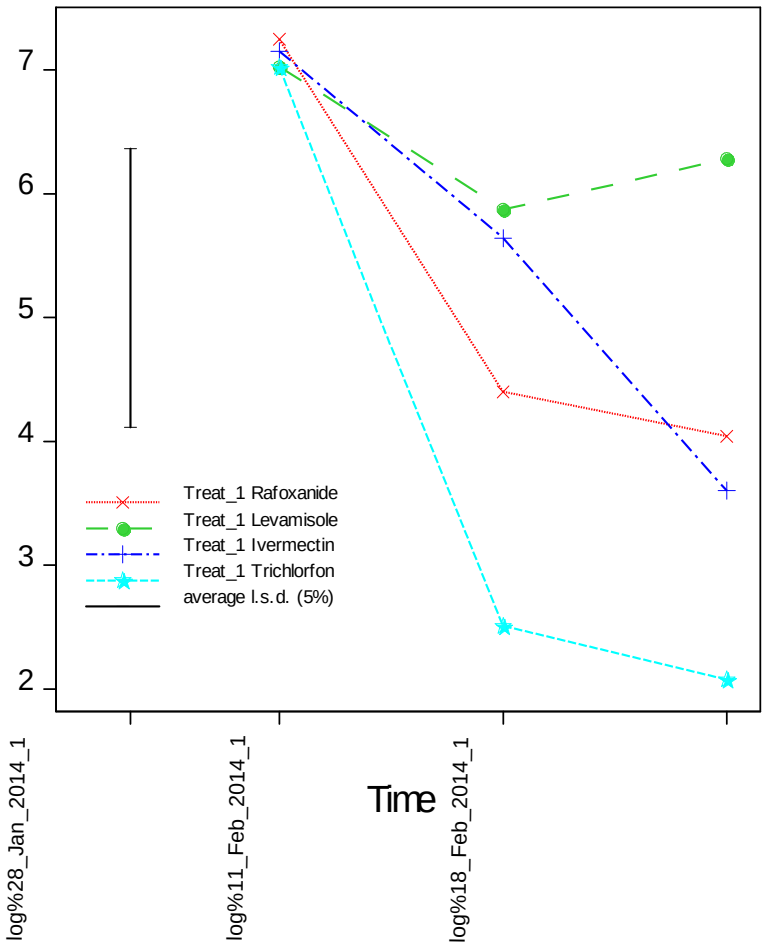


**Figure 4.5: The Log$_e$ transformed data showing the effect of different anthelmintic treatments on FEC for the FECRT: Day 0 (28 January), Day 14 (11 February) and Day 21 (18 February).**

According to the standards set for anthelmintic effectiveness by the World Association for the Advancement of Veterinary Parasitology Guidelines (Coles *et al.*, 1992) [See Paragraph 4.2 earlier] none of the tested chemotherapeutic products

gave highly effective control. The best GIN control measured was in the Trichlorfon treatment with 92% control, according to the reduction in FEC samples. Both the Ivermectin and Rafoxinide treatments showed moderately effective control, 88% and 85% respectively. In the Levamisole treatment only a 50% control was reached on Day 21 and it can be regarded as insufficient and be ascribed to parasite resistance towards this product.

**Table 4.3: FECRT results, showing percentage (%) control between different selected chemotherapeutic products.**

| **Active Ingredient** | **Ivermectin 1% m/v*** | **Levamisole HCL,2.56% m/v*** | **Rafoxinide 5% m/v*** | **Trichlorfon 20% m/v*** |
|---|---|---|---|---|
| **Anthelmintic group** | 1 | 3 | 4 | 7 |
| **Trade name** | Ivermectin Injection | Prodose red oral solution | Nasalcur | Unidose |
| **Effectiveness of drug 2014 (%)** | 88 a Moderately effective | 50 b Insufficient effective | 85 a Moderately effective | 92 a Effective control |

*Mass volume$^{-1}$

*Values with different superscripts differ significantly* (P<0.05).

When comparing these results with the results shown in Table 4.1 (FECRT done in 2003 at Dundee Research Station), Levamisole was still moderately effective in 2003. Ivermectin gave insufficient control in the Dundee Research Station sheep flock in 2003 and was seldom used since then. With the recent test, Ivermectin showed moderate control. Levamisole was used often in the last few seasons (Dundee Research Station stock records). This comparison is only indicative, since management changed over time and new sheep were transferred to Dundee Research Station.

- ***Post treatment FEC trend: Day 0 to Day 63***

To investigate GIN infection following treatment, the same sheep were monitored till Day 63 (8 April), which is the start of autumn. These results will give a clear picture of what typically happens on a farm following drug administration. The results are displayed in the Figure 4.6.

Following treatment, EPG counts decreased as a reaction to the treatments and then slowly increased over time (Figure 4.6). According to animal ethics regulations selective dosing was done in sheep once in the Levamisole, Ivermectin and Rafoxinide treatments, when individual sheep showed an EPG of more than 6000 in two consecutive weeks. Those sheep were treated with their specific anthelmintic and left in the trial to graze with the rest of the experimental animals (Figure 4.6).

Due to the pattern of variance in the data (variance increase as means increased) $Log_e$ transformations were used stabilise variance (Figure 4.6). Over time the differences between treatments were non-significant, except in the case of treatments on Day 21 (18 February), following initial treatment, as described earlier.

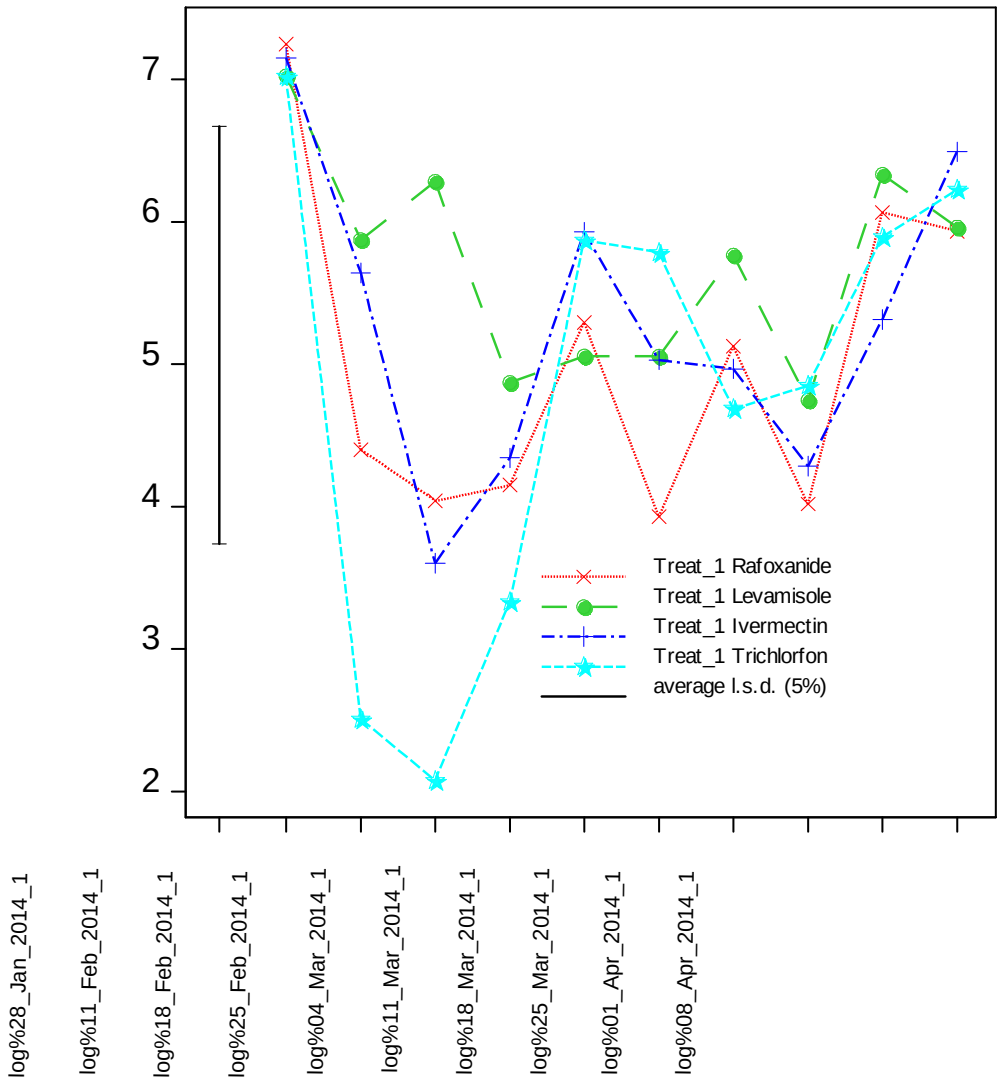


**Figure 4.6: Log$_e$ transformation of the EPG counts as effect of different anthelmintic treatments over time after treatment: Day 0 (11 February) to Day 63 (8 April).**

- ***Post treatment FEC trend:Day 0 to Day 63 including not treated group***

The FEC counts for the seven sheep that were not treated and excluded from the FECRT, was monitored with the rest of the trial groups. The sheep that were allocated to the NT group continuously showed a low FEC of 200 EPG and below during the initial three weeks before the FECRT. It was an indication of possible superior GIN resistance, compared to the rest of the trial group. These sheep were monitored over 65 days to test this observation. They grazed in one flock with the rest of the sheep described above and were exposed to the same conditions.

The FEC in each of the NT sheep stayed below 300 EPG for the duration of the experiment and their FEC did not follow the same trend as was measured in the treated groups. On Day 63 the FEC for all groups showed an increase, most probably as a result of the high rainfall in March. The trial was terminated after the dung sampling on Day 63. The $Log_e$ transformed data is displayed in Figure 4.7. Treatments over time differed significantly ($P<0.001$). The NT groups had significantly ($P<0.05$) lower FEC than other treatments on different days, e.g. Day 35 (11 March – lower than all other treatments except Rafoxinide) and Day 56 (1 April – lower than all other treatments).

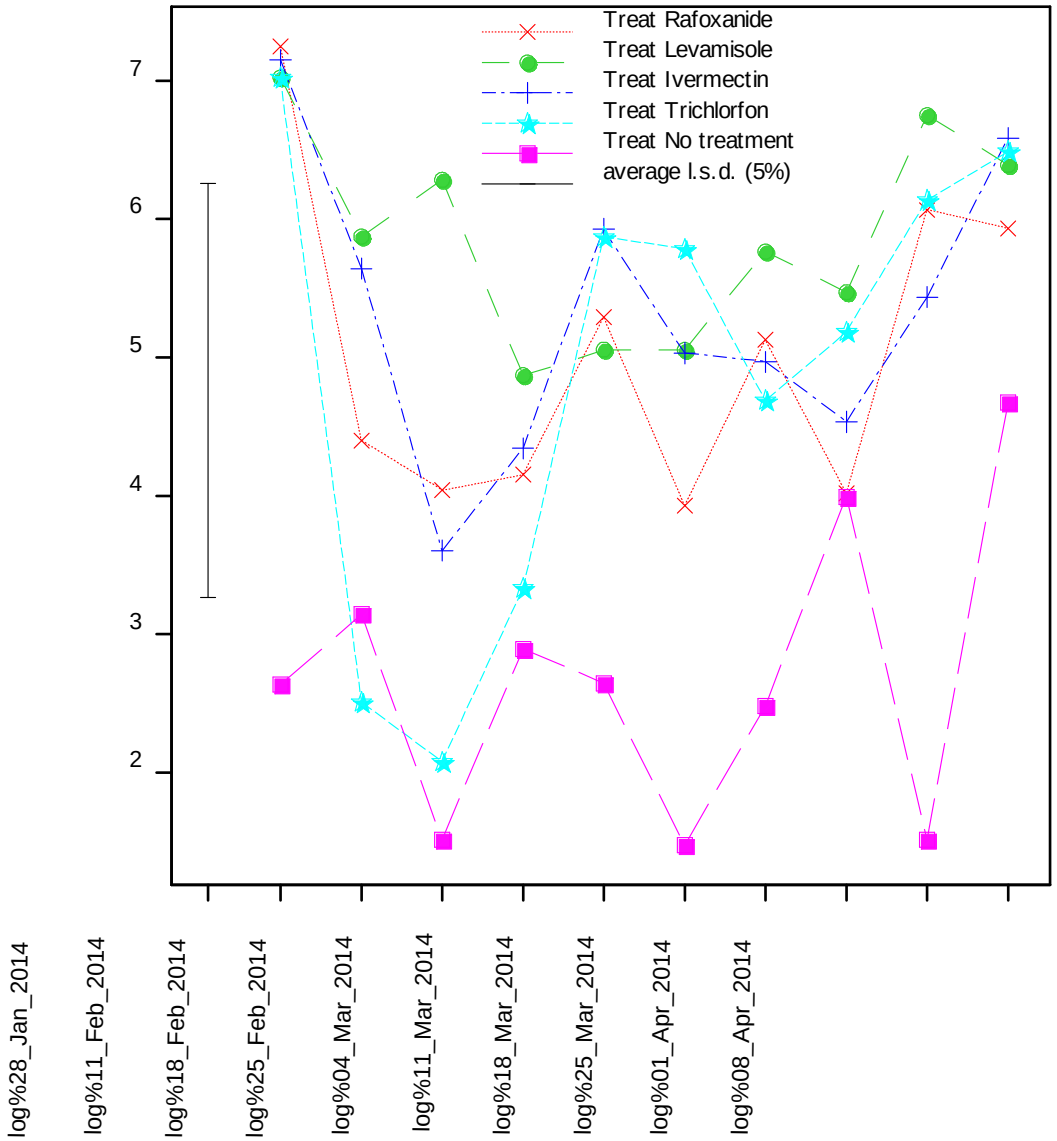


**Figure 4.7: $Log_e$ transformation of the EPG counts as effect of different anthelmintic treatments over time after treatment: Day 0 (28 January) to Day 63 (8 April) compared to the not treated group.**

GIN infestation compromises livestock production. One parameter of production is live weight changes. The weight (kg) changes between Day 0 to Day 63 for the different treatment groups, including the not-treated groups, are as follows: Not treated - 4.2 kg ± 2.3, n=7; Rafoxinide - 3.3 ± 2.7, n=8; Levamisole - 1.8 ± 5.0, n=8; Ivermectin - 2.9 ± 1.8, n=8 and Trichlorfon - 2.9 ± 1.8, n=8. The differences in weight changes, however, were not statistically significant. Due to the fact that the animals were mature ewes, grazing on an unfertilized pasture with only summer lick, major weight changes were not expected. Weight increase due to the growing foetus may also be expected.

Production was not compromised in the non-treated group and they had the best weight gain. In contrast with this result, the Levamisole treatment had the lowest gain (1.8 kg ± 5.0 kg), where inefficient control (Table 4.3) was measured during the FECRT, followed by a continued high GIN infestation (Figure 4.4), despite repeated treatment of highly infected animals.

## 4.6. Conclusion

The climatic conditions can give an indication as to when GIN infection can be expected, as shown in Experiment 1. In spring, naïve, post-weaned lambs on marginal feeding conditions, are at a high risk of becoming infected with GIN when climatic conditions become favourable for parasite development. Infections can increase rapidly over a short period, as was shown in this experiment. These circumstances provided the lambs with their first GIN challenge. To be able to cope as best as possible with the parasitism and develop their immunity, lambs will need supportive feeding, as was highlighted in the above literature review. Typical conditions like these are experienced annually at farm level and may lead to a severe malnutrition-infection condition which can result in the breakdown or incomplete development of immunity (Hoste *et al.,* 2006). Therefore, farmers should be alert to weather conditions in order to support naïve lambs timely through such a risk period and reduce losses. The lower FECs of ewes, exposed to more parasite-favourable conditions, support the phenomenon of better GIN resistance levels in older sheep. The evidence collected in Experiment 2 of this trial demonstrated the

value of a FECRT to identify effective chemotherapeutic products for GIN treatment. By using effective drugs, the frequency of dosing can be decreased and the speed of onset of anthelmintic resistance can be reduced (Bath and Van Wyk, 2009). Hansen and Perry (1994) advised to keep using the same class anthelmintic for at least a season or longer, provided it is effective.

Complete resistance to GIN can be the ultimate solution, but is generally ignored as a commercial reality (Saddiq *et al*., 2011). Out of the initial thirty–nine sheep, seven (18%) showed continued light infestation, indicating resistance to GIN infection, when compared to other animals with high infections, and even the need for re-treatment after eight weeks. These results supported the suggestion by Bath and Van Wyk (2009) that blanket treatment in a flock should be excluded. Hoste et al. (2006) stated that across a flock, parasites aggregate within a few individuals while some other acquired a degree of GIN resistance. This can form a basis for culling the susceptible animals. With more resistant animals the frequency of dosing can be effectively decreased (Bath and Van Wyk, 2009; Morgan *et al*., 2013) and future pasture contamination and re-infestation can most likely be reduced as well (Bisset and Morris, 1996; Bishop, 2012). This strategy is achievable. Recent research confirmed that regarding immune response, the heritability of nematode-specific IgA and IgE activity are moderate to high (Stear *et al.,* 2011), and regarding FEC, a heritable trait of typical range of 0.25 to 0.30 is established (Greef *et al*., 2014). Hansen and Perry (1994) already promoted the idea of selecting resistant individuals within a breed decades ago.

Research in breeding for resilience, rather than for resistance, is a controversial subject. Bisset and Morris (1996) indicated that the heritability of various measures examined, proved to be relatively low (ranging between 0.1 ± 0.03), but variation occurred between flocks as a result of the severity of nematode challenge. They found no positive association between resilience to nematode challenge and resistance to infection, but an implicated outcome showing better growth rates and lower dag scores in animals.

The adoption of methods to effectively monitor and timely detecting anthelmintic resistance is crucial for sustainable and effective ruminant production. At farm level wide scope exists to implement these strategies to identify susceptible and resistant individuals and manage effective treatment on a long-term basis. However, time, cost and labour restrictions are possible compromising factors at farm level.

# CHAPTER 5

# Yield, nutritional value and tannin level changes in *Lespedeza cuneata* under different defoliation frequencies and intensities.

## 5.1 Aim

To investigate *L. cuneata* in terms of:

- Dry Matter yield
- Forage quality, tannin levels and changes in tannin levels over the growing season.

## 5.2 Introduction

*Lespedeza cuneata* is a widely adapted, perennial, warm season, leguminous, fodder crop. The plant was previously known as *Sericea lespedeza* and later *L. sericea*. Both these names are still in use in the farming community, as well as the common name, poorman's lucerne (OTIS, 2013).

## 5.3 Literature review

### *5.3.1 Taxonomy, morphological description and diagnostic characteristics*

The taxonomy of L. cuneata is as follows (OTIS 2013):

Kingdom: *Plantae* – Plants
Subkingdom: *Tracheobionta* – Vascular plants
Superdivision: *Spermatophyta* – Seed plants
Division: *Magnoliophyta* – Flowering plants
Class: *Magnoliopsida* – Dicotyledons
Subclass: *Rosidae*
Order: *Fabales* (Leguminosae)
Family: *Fabaceae* – Pea family
Genus: *Lespedeza Michx.* – lespedeza
Species: *Lespedeza cuneata* (Dum. Cours.) G. Don – Sericea lespedeza

*Lespedeza cuneata* is an upright growing semi-woody herb with one to many slender stems. The plant can reach a height of up to 1.8 meters, but in South Africa, a maximum height of up to 1 m is more common. Stems are greyish green and can be hairy. Leaves compose three small (<2.5 cm), alternating leaflets that have wedge-shaped bases. *L. cuneata* is the only species in the genus *Lespedeza* with this type of leaf base (Dannhauser, 2002; Stevens, 2002).

Small, creamy white flowers with purple throats occur in summer. The flowers are scattered along the stems in clusters of two to four. The plant reproduces both vegetatively and sexually, and the seed produced by the outcrossed flowers do not germinate well, unless scarified (Stevens, 2002).

Food reserves are stored in the taproot for winter, when the stems die back completely. New stems develop from the root crown buds in spring and the number of stems increase each year. A single plant can therefore spread vegetatively; forming large stands and can live for over 20 years (Stevens, 2002).

The plant develops an extensive taproot that makes it drought tolerant. Dannhauser (2002) indicated a 600 mm rainfall $a^{-1}$ as the lower cut-off point for successful cultivation.

### *5.3.2 Yield, grazing norms and quality*

Very few local, scientifically verified grazing data are available for this plant in South Africa. A cutting trial, followed by a grazing trial, repeated for two years, and only published as poster abstracts, were carried out at the Cedarville Flats, southern KZN, during the 2000 to 2002 seasons (Cunningham and Van Niekerk, 2002). Yield, quality and grazing data, resulting from this trial, are shown in Table 5.1a and Table 5.1b. It is unclear in what year herbage quality was tested.

**Table 5.1a: Yield (t ha$^{-1}$) for the 2000/2001 and 2001/2002 growing seasons and herbage quality, expressed as ADF (%), NDF (%) and Crude Protein (%) under different cutting regimes of *L. cuneata* at Cedarville Flats.**

| Frequency of cutting | Yield | | Herbage Quality | | |
|---|---|---|---|---|---|
| | 2000/2001 | 2001/2002 | ADF (%) | NDF (%) | Crude Protein (%) |
| 8 weeks | 6.01 | 5.33 | 41.43 | 53.34 | 20.24 |
| 12 weeks | 8.04 | 6.13 | 44.77 | 61.86 | 17.45 |
| End of season | 7.22 | 6.01 | 48.07 | 69.98 | 12.23 |

**Source**: Cunningham and Van Niekerk, 2002.

**Table 5.1b: Grazing data: *L. cuneata* compared to burnt veld at Cedarville Flats during 2000/2001 and 2001/2002 growing seasons.**

| Years | 2000/2001 | | 2001/2002 | |
|---|---|---|---|---|
| Type | Sericea | Veld | Sericea | Veld |
| Grazing period: Start to end date | 13 Oct. 2000 to 22 Feb. 2001 | | 29 Oct. 2001 to 22 April 2002 | |
| Total grazing days | 132 | | 179 | |
| SR (SSU ha$^{-1}$) | 16.9 | 2.5 | 12 | 2.5 |
| ADG (g day$^{-1}$ SSU$^{-1}$) | 96 | 41 | 89 | 62 |

SR (SSU ha$^{-1}$) = Stocking rate per small stock unit per ha
ADG (g day$^{-1}$ SSU$^{-1}$) = Average daily gain (g) per day per small stock unit
**Source:** Cunningham and Van Niekerk., 2002.

This trial showed that the pasture was able to carry four to six times more sheep for the summer, compared to the veld. Sheep performance (live weight gain per day) on the pasture was in the first trial year twice as good as on veld, but the second year not as high, but still better compared to growth rates on veld.

*L. cuneata* is known as a high tannin containing forage. The positive and negative effects of tannins have been discussed in Chapter 3, paragraph 3.4.6. The condensed tannin (CT) content of *L. cuneata* in comparison with other forage species is shown in Table 5.2.

**Table 5.2: The Condenced tannin content of *L. cuneata* in comparison with other forage species.**

| Forage | CT (g/kg of DM) | % DM |
|---|---|---|
| *L. cuneata* | 46-152 | 4.6-15.2 |
| Birdsfoot trefoil | 48 | 4.8 |
| Big trefoil | 77 | 7.7 |
| Lucerne (alfalfa) | 0.5 | 0.05 |
| Sainfoin | 29 | 2.9 |
| Perennial ryegrass | 1.8 | 0.18 |
| Chicory | 3.1 | 0.31 |

**Source**: Coffey *et al*., 2007.

### *5.3.3 Cultivars*

There are several cultivars on the market internationally. Besides tannin content differences between cultivars, individual plants within cultivars, can differ also in tannin content. The so-called lower tannin containing cultivars are only varieties with average lower tannin compared to the other varieties that have plants with a more uniform high tannin level. The low-tannin cultivars are very sensitive to overgrazing, which will eventually kill the sward (Mosjidis *et al.,* 2014). The cultivar, AU Lotan, known as one of the lower tannin cultivars, is commercially available in South Africa, in the de-hulled form.

### *5.3.4 Anthelmintic properties*

Coffey *et al.,* (2007) summarized several research trials proving the positive anthelmintic properties of *L. cuneata* (Table 5.3).

**Table 5.3: Summarized trials on the anthelmintic properties of *L. cuneata* (LC).**

| Animals used | Treatments | Results | Notes |
|---|---|---|---|
| Goats, confined and fed hay (75% of diet) and grain (25%) | Ground LC (0, 25, 50, 75%) and/or Bermuda grass (75, 50, 25, 0%) in combinations equaling 75% hay; levels testing dose of LC needed, 6 weeks | FEC reduced for those fed LC at all levels, greater reduction as % LC increases and with time; at 6 weeks, 75% LC hay, 91.9% reduction | Optimum level of LC hay appeared to be 50-75% of total diet (Dykes *et al.,* 2006), but 25% was also beneficial, reducing number of adult barber pole worms in the stomach by 58% (unpublished data) |
| Lambs, fed hay; natural and experimental *Haemonchus contortus* infections | LC hay Bermuda grass hay, 7 weeks, Bermuda grass an additional 2 weeks | FEC reduced for those receiving LC (67-98%); FEC increased after LC feeding stopped. LC also reduced worm numbers. | LC fed as hay reduced naturally infected worm burdens 67%; reduced establishment of incoming larvae 26%. (Lange *et al.,*2006) |
| Goats, confined and fed hay and grain | *L. cuneata* hay or Bermuda grass hay, 7 weeks | FEC reduced, number of adult worms reduced, hatchability of eggs into L-3 larvae reduced in goats fed LC hay | Egg counts dropped by about 80% one week after LC feeding started; reduction increased to almost 90% by end of trial. Both abomasal and small intestinal worms reduced and female worms reduced more than male worms. Male and female *H. contortus* were reduced by 61 and 76%, respectively (Shaik *et al*., 2006) |
| Angora does, grazing | LC or crabgrass/tall fescue grazing, 81 days | Goats on LC had reduced FEC and fewer adult worms. Inhibited larval activity. Improved weight gain and immune responses. No adverse effect on does and kids (3.6 kg/kid). | Goats grazing LC reduced both *H. contortus* (89%) and *Trichostrongylus* Parasites (50%). (Min *et al.,*2005) |

**Source**: (Coffey *et al*., 2007).

## 5.4 Materials and methods

### 5.4.1 Study area

#### 5.4.1.1 Site

The field trials were conducted at the Dundee Research Station, Department Agriculture and Environmental Affairs, KZN. The Research Station is situated about 10 km east of Dundee (latitude 28° 10'S and longitude 30°31'E) at an altitude of 1219 m (2830AB). The long-term mean annual rainfall is 782.8 mm $a^{-1}$ and the distribution is a typical summer rainfall pattern with convectional rainfall in the months from October to March.

#### 5.4.1.2 Soil

The trial was conducted on a Hutton soil form with effective depth of more than 900 mm, which is categorized as a marginal or high risk soil for annual cash crop cultivation in the Dundee area. The local summer rainfall is highly variable and erratic with regular drought spells, due to the convectional summer rainfall pattern. Under irrigation, this soil is highly rated for cash crop production, but under dry land conditions, exposed to the unreliable and sometimes, erratic rainfall, highly risky due to good internal drainage and thus poor soil water retention. Due to these soil characteristics and prevailing environmental conditions, these soils are normally allocated to planted pastures under dry land conditions, (Soil Classification Working Group, 1991).

#### 5.4.1.3 Climate

The long-term mean minimum summer temperature is 12.4°C and the maximum 26.1°C. Mean long-term relative humidity is above 90% maximum and below 36 % minimum for summer months. Mean long-term minimum winter temperature is 3.3°C with a maximum of 21.1°C. Relative maximum humidity for winter is above 85% and below a minimum of 25%. The long-term mean rainfall is 782.8 mm $a^{-1}$, distributed in a

typical summer rainfall pattern with convectional rainfall in the months from October to March. Climatic conditions of the study area are given in Table 5.4.

**Table 5.4: The long-term climatic data for Dundee Research Station.**

| **Key** | **Evapo-transpi-ration daily** | **Frost (days)** | **Rain (mm)** | **Rel. Hum. Min. (%)** | **Rel. Hum. Max. (%)** | **Sun Shine hours daily** | **Min. air temp. (°C)** | **Max. Air temp (°C)** |
|---|---|---|---|---|---|---|---|---|
| Years | *32* | *32* | *13* | *23* | *23* | *27* | *32* | *32* |
| Jan | 198.0 | - | 120.82 | 36.0 | 92.5 | 6.7 | 15.2 | 27.3 |
| Feb | 172.1 | - | 91.61 | 34.7 | 92.9 | 7.0 | 14.9 | 27.0 |
| Mar | 159.5 | - | 84.71 | 34.1 | 93.4 | 8.9 | 13.5 | 28.2 |
| Apr | 128.3 | 1.1 | 42.5 | 28.2 | 92.8 | 7.1 | 9.8 | 24.1 |
| May | 118.2 | 11.8 | 9.00 | 25.9 | 88.3 | 7.5 | 5.3 | 22.1 |
| Jun | 104.8 | 9.6 | 11.16 | 20.4 | 82.7 | 7.7 | 1.4 | 20.0 |
| Jul | 115.4 | 2.8 | 12.37 | 20.3 | 81.7 | 7.7 | 1.9 | 20.1 |
| Aug | 156.9 | 0.4 | 20.47 | 21.1 | 81.6 | 7.6 | 4.5 | 22.1 |
| Sep | 184.6 | - | 34.52 | 24.8 | 82.5 | 7.1 | 8.5 | 24.7 |
| Oct | 191.0 | - | 82.71 | 32.8 | 88.5 | 6.8 | 10.8 | 24.9 |
| Nov | 198.4 | - | 105.81 | 33.0 | 88.4 | 6.7 | 12.8 | 25.0 |
| Dec | 211.4 | - | 165.61 | 34.4 | 90.9 | 6.8 | 14.3 | 27.1 |
| Total annual | 1938.6 | - | 781.29 | - | - | - | - | - |
| **Mean** | - | - | - | 28.7 | 88.1 | 7.1 | 9.6 | 24.3 |

*Average first frost: 28 May* *Average last frost: 23 August*
*Average frost season: 89 days* *Average frost days day year$^{-1}$: 26*
*Percentage years with frost: 100*
**Source**: Agrometeorology, 2014.

### *5.4.2 Pasture establishment and management*

Six hectare of *L. cuneata,* cultivar AU Lotan, was established during late summer 2011 at a seeding rate of 15 kg seed ha$^{-1}$ under dry land conditions. The pasture was not grazed for the first growing season, but only used for haymaking. Cutting trials was conducted in the 2012/2013 and the 2013/2014 growing seasons as explained below (Paragraph 5.4.3)

### 5.4.3 Treatments applied

A defoliation trial with a two-way design in randomized blocks was applied in an existing sward of *L. cuneata*. The nett plot size was 9.16 $m^2$ for the first season and 16 $m^2$ for the second season. The difference was due to the replacement of the motorized mower with one with a different cutting width.

Cutting treatments were:
Frequency - 6 week cutting frequency,
8 week cutting frequency and
12 week cutting frequency.

Height above ground - 5 cm
15 cm

### 5.4.4 Data collection

#### 5.4.4.1 Dry Matter (DM) yield

To determine dry matter (DM) yield per plot, the material from the nett plot was weighed with an electronic hanging scale directly post cutting on the field. The wet weight of a subsample of each plot was taken, oven dried at 60°C to a constant mass and again weighed for dry matter yield calculation and expressed as kg DM $ha^{-1}$.

#### 5.4.4.2 Feed Quality and Tannins

The samples collected for the DM yield determination, as explained above, were milled through a 2 mm sieve and sent for full feed chemical analysis according to methods described by De Figueirero and Thurtell (1998), based on the Van Soest (1965) methods. The analyses were done at the Cedara Feed laboratory of the Department KwaZulu-Natal Department of Agriculture, Environmental Affairs and Rural Development, which is a fully accredited laboratory.

A portion of the samples was divided, by hand, into leaf and stem fractions to determine the leaf/stem ratio. This extra information might be worthwhile in the calculation of grazable material, since sheep tend to selectively browse the leaves and avoid the stems. These separated samples were also chemically analysed for nutritional value, using the same procedure as described above.

After cutting of the six and 12 week treatments (only at the 5 cm cutting height treatments) were cut, a random sample was collected from the harvested material and air dried in the shade. This was done to avoid any effects of heat treatment (oven drying, as mentioned above) on the tannin content of the samples. Samples were once again divided by hand into stem, leaf and whole plant samples. These samples were analysed for tannin content by the Cedara Feed laboratory, according to a method described by Reed *et al.* (1982) and Waterman and Mole (1994), whereby the condensed tannins were extracted from the samples with aqueous acetone. Afterwards butanol-HCl and ferric reagent were added, followed by the measuring of absorbance at 550 nm with a spectrophotometer. Tannin levels are expressed as g CT $kg^{-1}$ DM.

### *5.4.5 Statistical analysis*

The data on DM yield were analysed by using standard Analysis of Variance. Fisher's test of least significant differences (LSD) was conducted at a 5% significance level. Linear regressions were used to analyse the effect of rainfall on the CT levels in plants, whole plant and separated in leaves and stems.

## *5.5 Results and discussion*

### *5.5.1 Climatic conditions*

#### *5.5.1.1 Rainfall*

Climatic data were sourced from the Agricultural Research Council (ARC) (Agro meteorology, 2014*)* from the weather station (Comp. 30109) based at the Dundee Research Station. The rainfall data in both trial seasons, compared to the long-term average per month over 68.5 years, are displayed in Figure 5.1.

The 2012/13 season was characterized by above normal rainfall for the first part of the growing season. The December 2012 rainfall was exceptionally high. January received slightly above normal, with February normal and March below average, but April above average rainfall again.

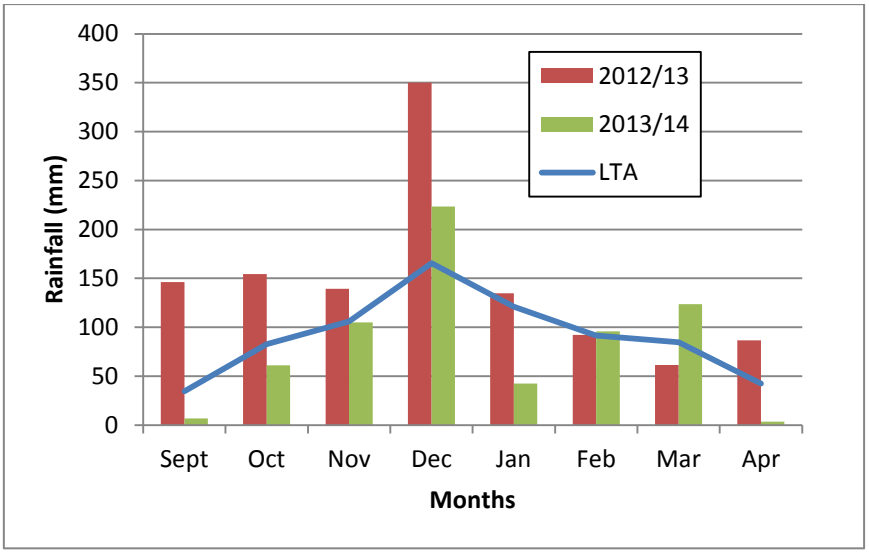


**Figure 5.1: Monthly rainfall (mm month$^{-1}$) for Dundee Research Station, compared to the mean long-term rainfall for growing seasons 2012/13 and 2013/2014.**

The 2013/14 growing season was characterized by drought, with erratic showers. Again, December was abnormally wet and January was dry again. During March above normal rainfall was measured. The long-term total rainfall for the growing season (September to April) is 728.29 mm. In the 2012/13 season a total of 1164.6 mm (or 160% of long-term rainfall) was measured, compared to the 661.41 mm (or 90.8% of long-term rainfall) of the 2013/14 season, however with poor distribution.

### *5.5.1.2 Temperatures*

Except for September 2012, which was cooler than normal, the maximum temperatures for the first season did not show serious deviation from the long-term maximum temperatures. In the second growing season, September and November 2013 and January and February 2014 were warmer than normal but all other months again showed normal temperatures (Figure 5.2).

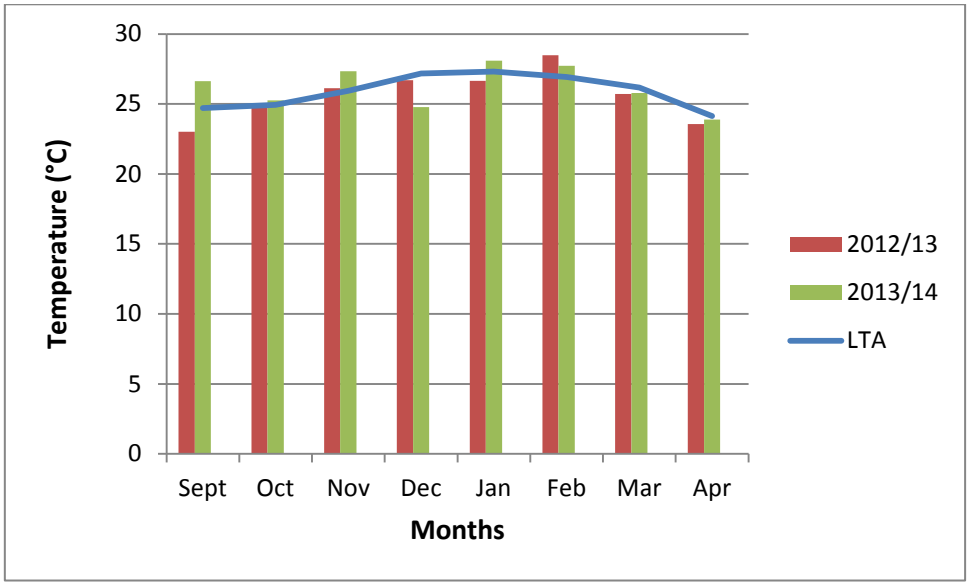


**Figure 5.2: Long-term monthly mean daily maximum temperature (°C) for Dundee Research Station compared to mean daily maximum temperatures for the 2012/13 and 2013/14 growing seasons.**

Above normal minimum temperatures were recorded for the first part of the growing season in 2012/13, but these drop to the mean or below mean for the second part (autumn) of the first growing season (Figure 5.3).

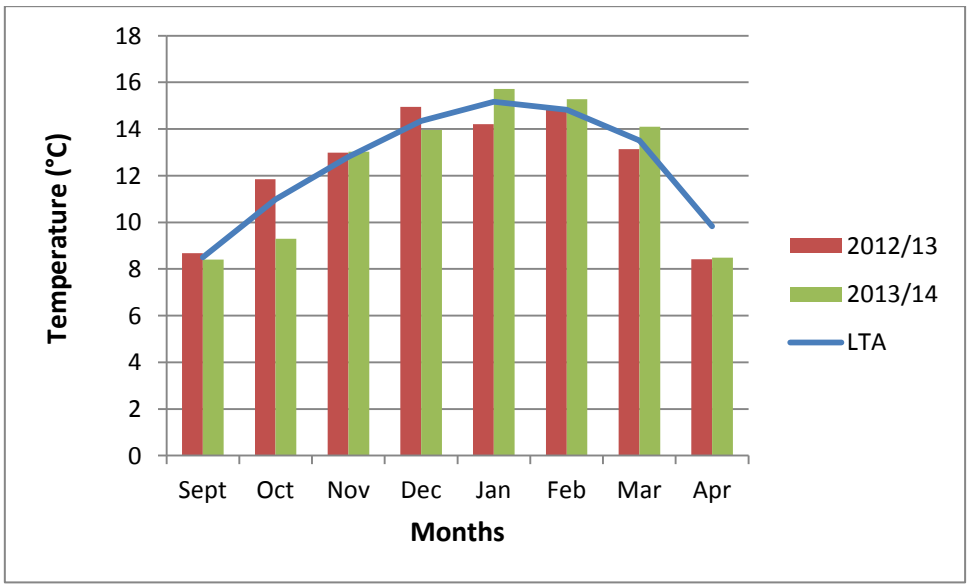


**Figure 5.3: Long-term monthly mean daily minimum temperature (°C) for Dundee Research Station, compared to daily minimum temperature for the 2012/13 and 2013/14 growing seasons.**

During the second season the opposite trend was recorded. Below normal minimum temperatures were recorded for the first part of the growing season, where after above normal minimum temperatures were recorded for the last part of the growing season.

### *5.5.2 Plant production*

#### *5.5.2.1 Dry Matter (DM) yield*

To determine DM yield, the different treatments were cut on dates given in Table 5.5.

**Table 5.5: Dates for the 6, 8 and 12 week cutting frequencies for the 2012/13 and 2013/14 growing seasons.**

| Cutting frequencies | Season 1 | | | | Season 2 | | | |
|---|---|---|---|---|---|---|---|---|
| 6 week | 22-Nov-2012 | 07-Jan-2013 | 18-Feb-2013 | **01-Apr-2013** | 03-Dec-2013 | 16-Jan-2014 | 25-Feb-2014* | **08-Apr-2014** |
| 8 week | 10-Dec-2012 | 08-Feb-2013 | **05-Apr-2013** | | 03-Dec-2013 | 28-Jan-2014 | 25-Mar-2014 | |
| 12 week | 07-Jan-2013 | 02-Apr-2013 | | | 03-Dec-2013 | 25-Feb-2014** | | |

*Dates in bold indicate that no cut was done, due to lack of regrowth.*
**This cutting date was delayed to 4 March 2014 due to bad weather*
*** This cutting date was delayed to 12 March 2014 due labour restrictions*

The grand mean yield for the 2012/13 growing season was 8.3 t DM $ha^{-1}$. Highly significant differences ($P<0.001$) were found between the different cutting frequencies and cutting heights. The total DM yield recorded in the 6 week cut frequency was 5.66 t $ha^{-1}$, and for the 8 and 12 week frequency, respectively 8.99 and 10.26 t $ha^{-1}$. Mean yields of 9.62 and 6.98 t $ha^{-1}$ were respectively measured in the 5 and 15 cm cutting height treatment. In terms of interaction between cutting heights and cutting frequencies, the DM yield did not differ significantly ($P>0.05$) (Table 5.6).

The high yields that were recorded for the 2012/13 season were influenced by the above normal rainfall (Figure 5.1) received up to midsummer. Above normal

minimum temperatures (Figure 5.3) were recorded for the first part of the growing season in 2012/13. The first six week cut could already be done on the 22nd November 2012. In this regard, Gucker (2010) stated that seasonal variations in temperature and genotype play an important role in early season's regrowth in *L. cuneata,* but its effect on late growth and regrowth needed to be ascertained. Munger (2004) also categorized *L. cuneata* as relatively slow regarding to regrowth in spring compared to competing plants.

**Table 5.6: Yield of *L. cuneata* (t ha$^{-1}$) under different defloliation frequencies, namely 6, 8 and 12 weeks, and different cutting heights, 5 and 15 cm for the 2012/13 growing season.**

| **Cutting height (H)(cm)** | **Yield (t ha$^{-1}$) with different defoliation frequency (F)** | | | **Mean of cutting heights (H)** |
|---|---|---|---|---|
| | 6 weeks | 8 weeks | 12 weeks | |
| 5 cm | 6.8 | 10.30 | 11.77 | 9.62$^{a}$ |
| 15 cm | 4.52 | 7.67 | 8.75 | 6.98$^{b}$ |
| Means of defoliation frequency | 5.66 $^{a}$ | 8.99 $^{b}$ | 10.26 $^{c}$ | |
| CV % | 13.0 % | | | |
| LSD (5%) | Frequency (F): 1.146 (SIGNIFICANT)<br>Height (H): 0.936 (SIGNIFICANT)<br>H X F interaction: 1.621 (NS) | | | |

*CV% = Coefficient of variation; LSD=Least significant difference;*
*Values with different superscripts within rows or columns differ significantly.*

Two more cuts could be harvested in the 6 week treatment before regrowth ceased in the late summer. For the last part of the season, rainfall (Figure 5.1) dropped below normal and minimum temperatures (Figure 5.3) dropped in March below 13 °C and regrowth ceased. The last cut was planned for 8th April 2013, but there was insufficient regrowth to be cut, despite the good rains received in April.

The yield for the 6 week cut frequency at 5 cm height was, as expected, higher than harvested in the 15 cm cut treatment, namely 6.8 t DM ha$^{-1}$ compared to 4.5 t DM ha$^{-1}$ respectively. The DM yield trend from the 6 week cut from cutting height treatments was repeated in the 8 and 12 week frequency cuts, respectively, with

10.30 and 11.77 t DM ha$^{-1}$ in the 5 cm cutting height and the lower yields of 7.67 and 8.75 t DM ha$^{-1}$ in the 15 cm cutting height treatment. Only two cuts could be done in the 8 and 12 week treatments during this season. Insufficient regrowth occurred after the second cutting. These differences in yield for cutting heights and cutting frequency were highly significant (P<0.001), but not for the interactions between cutting height and cutting frequency.

The longer the cutting frequencies, the better the yield recorded in the first season. The mean yield of the 8 week cut frequency produced 158.8 % of the 6 week cut and the 12 week cut 181.3 % of the 6 week cut. These yields were better than the yields reported by Cunningham and Van Niekerk (2002), which were in the order of 5.3 to 8.04 t ha$^{-1}$.

The grand mean yield for the 2013/14 season was 2.56 t DM ha$^{-1}$. The early season rainfall, apart from being below normal (Figure 5.1), was erratic and characterized by showers from less than one mm to more than 30 mm with dry spells in between. The maximum temperatures tended to be slightly above normal (Figure 5.2), with minimum temperature below normal (Figure 5.3). The combination of these climatic conditions resulted in a slow early summer regrowth. It was difficult determining the first cutting dates in the treatments due to the influence of the erratic rainfall and therefore all treatments were cut on 3 December 2013. Highly significant differences (P<0.001) were measured between the total DM yield of the 12 week cut frequency (3.52 t DM ha $^{-1}$), compared to the 8 (2.09t DM ha$^{-1}$) and 6 week (2.07 t DM ha$^{-1}$) frequencies. The differences between total yields in cutting height treatments were non-significant (P>0.05), as well as in the interaction between cutting height and cutting frequency (Table 5.7).

The trend regarding cutting heights, recorded in the first season, continued in the second season, except for the 6 week cut where a higher yield was measured in the 15 cm cut than in the 5 cm cutting height treatment. The reason for this is unkown. However, the mean yields in cutting height treatments followed the expected trend, with non-significant (P>0.05) differences of 2.73 t DM ha$^{-1}$ for the 5 cm cutting height treatment and 2.39 t DM ha$^{-1}$ for the 15 cm cutting height treatment, respectively.

The grand mean yield for the 2013/14 season of 2.56 t DM ha$^{-1}$ was only 30.8 % of the grand mean yield for the 2012/13 season. The poor rainfall in the second season (69.3 % of the long-term average for the growing season) played a major role in the low yields recorded for this season. These yields were lower that the yields reported by Cunningham and Van Niekerk (2002), which were in the order of 5.3 to 8.04 t ha$^{-1}$.

**Table 5.7: Yield of *L. cuneata* (t ha$^{-1}$) under different defoliation frequencies, namely 6, 8 and 12 weeks, and different cutting heights, namely 5 and 15 cm, for the 2013/14 growing season.**

| Cutting height (H)(cm) | Yield (t ha$^{-1}$) with different defoliation frequencies (F) | | | Mean of cutting heights(H) |
|---|---|---|---|---|
| | 6 weeks | 8 weeks | 12 weeks | |
| 5 cm | 1.88 | 2.67 | 3.65 | 2.73 |
| 15 cm | 2.27 | 1.51 | 3.4 | 2.39 |
| Means of defoliation frequency | 2.07$^{a}$ | 2.09 $^{a}$ | 3.52 $^{b}$ | |
| CV % | 22.2 % | | | |
| LSD (5%) | Frequency (F): 0.610 SIGNIFICANT<br>Height (H): 0.498 NS<br>H X F interaction: 0.863 NS | | | |

*CV% = Coefficient of variation; LSD=Least significant difference;*
*Values with different superscripts differ significantly.*

On the other hand, the good yields in the first season were stimulated by the abnormally high rainfall (162.3% of long-term mean). The soil type, namely a Hutton, with specific characteristics of being a good potential irrigation soil, but with marginal potential under dry land conditions, nevertheless enhanced the yield differences between years of abnormally high and below average low rainfall.

### *5.5.2.2 Growth distribution over the season*

The growth distribution of *L. cuneata* is displayed in Figures 4a to 4f. The growing season was divided into the following categories to simplify the graphs:

- Begin: October,
- Early: November and December,
- Mid: January and February,

- Late: March and
- End: April.

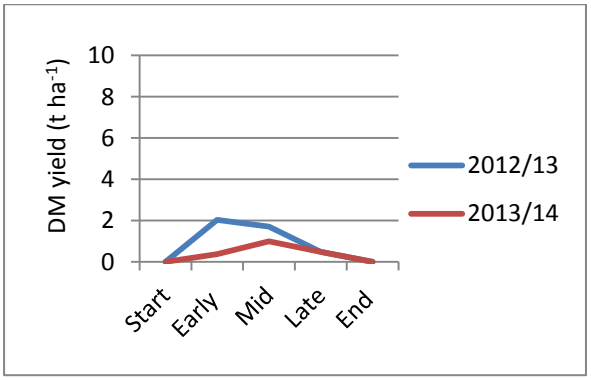


**Figure 5.4a: Yield (t DM ha$^{-1}$): 6 week cut at 5 cm height**

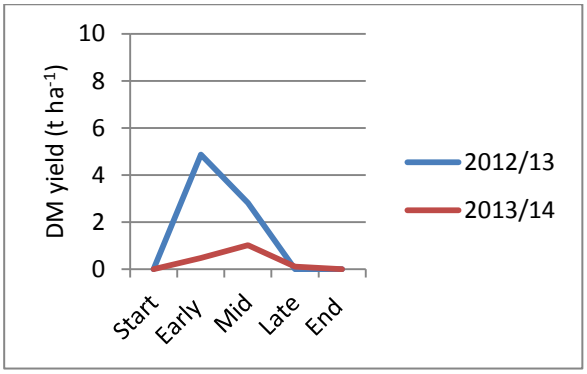


**Figure 5.4d: Yield (t DM ha$^{-1}$): 8 week cut at 15 cm height**

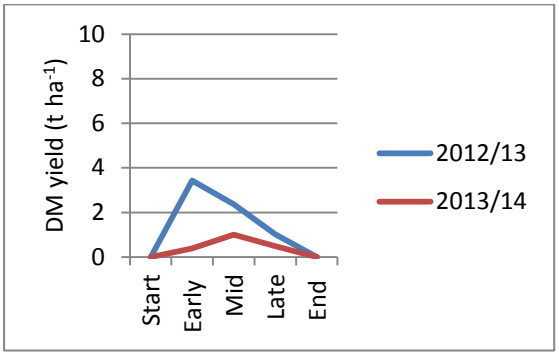


**Figure 5.4b: Yield (t DM ha$^{-1}$): 6 week cut at 15 cm height**

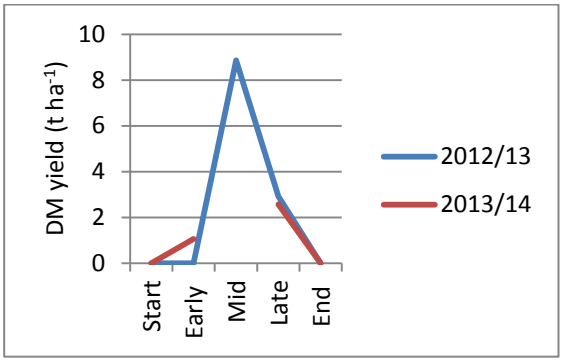


**Figure 5.4e: Yield (t DM ha$^{-1}$): 12 week cut at 5 cm height**

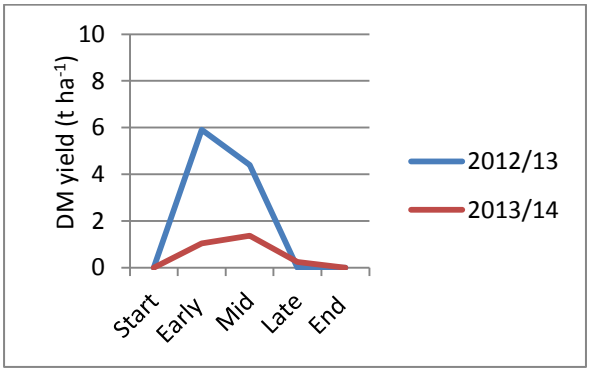


**Figure 5.4c: Yield (t DM ha$^{-1}$): 8 week cut at 5 cm height**

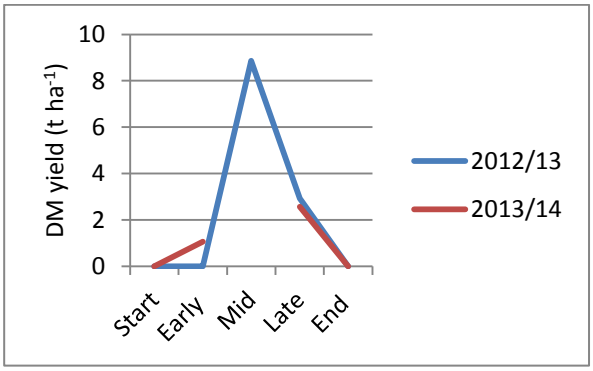


**Figure 5.4f: Yield (t DM ha$^{-1}$): 12 week cut at 15 cm height**

The yield distribution for all treatments showed a typical summer growth distribution, normally seen in subtropical pastures. Initial regrowth after spring is influenced by climatic conditions, but is especially moisture dependant.

#### *5.5.2.3 Leaf: Stem ratio*

The dried samples were divided into stem and leaf portions by hand selection. The results are displayed in Table 5.8 as leaf: stem ratio.

**Table 5.8: Mean leaf: stem ratio for *L. cuneata*, at different cutting frequencies.**

| Cutting frequency | Leaf: Stem ration |
|---|---|
| 6 weeks | 65:35 |
| 8 weeks | 58:42 |
| 12 weeks | 39:61 |

The results in Table 5.8 showed that leaf: stem ratio's changed as the plant matured from 65% leaves and 35% stems at the 6 week cut frequency to a 39% leaf to 61% stem ratio in the 12 week frequency. The 12 week cut is a typical haymaking stage. These results indicated a large percentage of woody stems in hay, which are not utilized by animals, and can be regarded as wastage, when fed as hay (Figure 5.6).


**Figure 5.6: The amount of woody stems left after sheep have been given free access to utilize a bale of *L. cuneata***

### 5.5.3 Chemical analysis

#### 5.5.3.1 Chemical feed analysis

The crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) are good parameters for evaluating forage quality (Table 5.9). In general, if CP levels are 13%, animals maintain their weight, above 13% they gain weight and below 6-8% appetite is depressed and pasture intake will drop. ADF represents the lower digestible cell wall components namely hemicelluloses, cellulose, lignin, cutin, silica and tannins. Forages lower in ADF are usually higher in energy. As ADF increases, digestibility normally decreases. An ADF value of lower than 31% is considered as prime quality and above 45% is consider as low quality. NDF is the portion of the plant sample that contains the soluble cell content namely sugars, starch, lipids and real protein. Forages, low in NDF, are usually high in quality and have high levels of intake. A NDF value of lower than 40%, is considered prime quality and above 54% of low quality (Shaker, 2009).

**Table 5.9: Typical changes in forage composition showing the CP, ADF and NDF ranges from prime quality to low quality.**

| Quality Standard | CP (%) | ADF (%) | NDF (%) |
|---|---|---|---|
| Prime | >19 | <31 | <40 |
| Good | 17-19 | 31-35 | 40-46 |
| Moderate | 14-16 | 36-40 | 47-53 |
| Low | 11-13 | 41-42 | 54-60 |
| Lower to poor | 8-10 | 43-45 | 61-65 |
| Poor quality | <8 | >45 | >65 |

**Source:** Van der Merwe and Smith (1991); Shaker (2009).

The CP content of herbage samples in the trial showed that in each treatment, an acceptable CP standard was obtained with values above 10% in all 6 week frequency treatments (Figure 5.7.1). The CP values in the 12 week cutting frequency treatments for the 2012/13 season were 10% or below, but still above the lower limit (Figure 5.7.3). The CP level measured in the 8 week cutting frequency in the 2012/13 growing season was below 10% in the early cut, but just below 16% in the mid season's cut. However, all treatments showed a CP level of more than 9%.

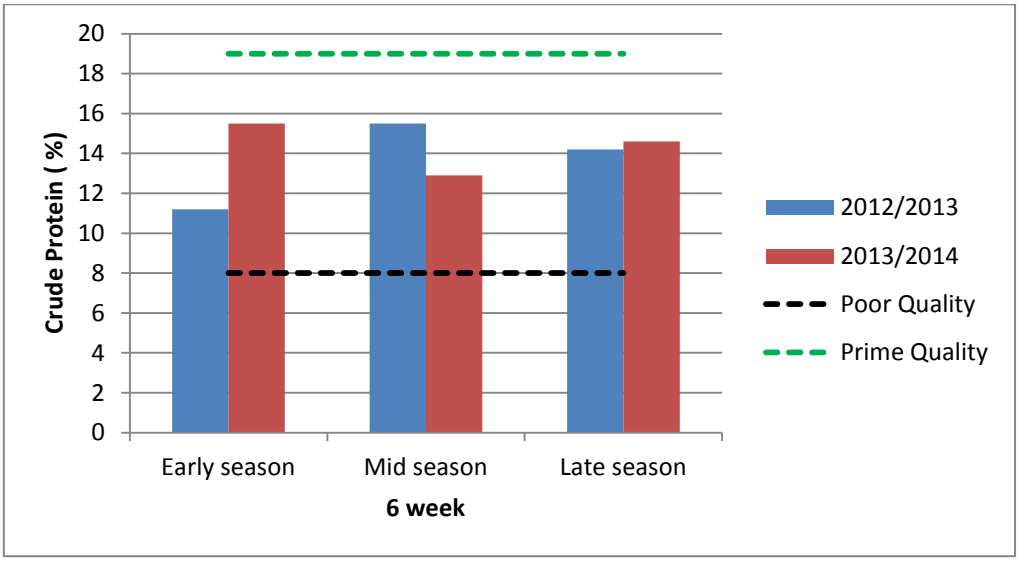


**Figure 5.7.1: The CP (%) values for *L. cuneata* as an effect of 6 week cutting frequency with indication of standard prime quality 19 % and above and poor quality 8 % and below for the 2012/13 and 2013/14 growing seasons.**

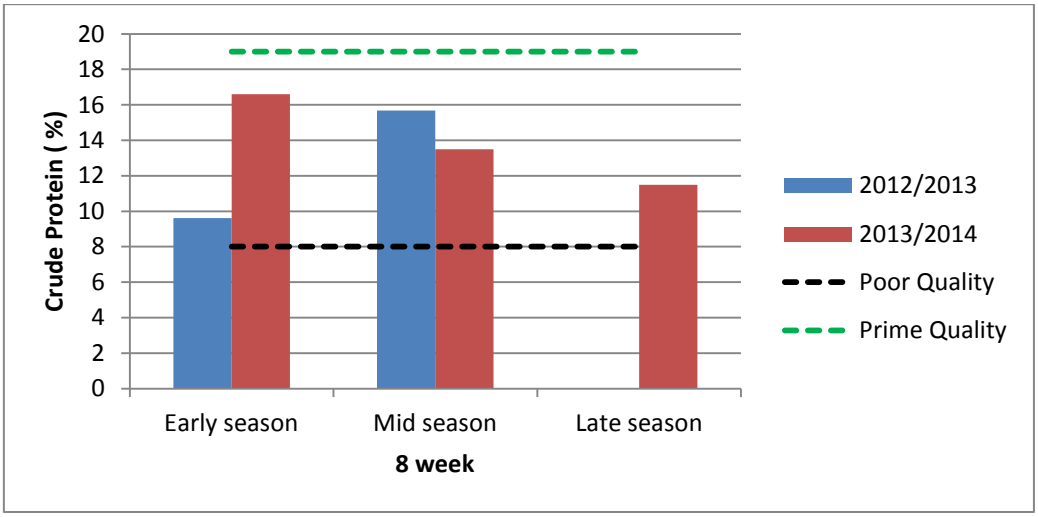


**Figure 5.7.2: The CP (%) values for *L. cuneata* as an effect of 8 week cutting frequency with indication of standard prime quality 19 % and above and poor quality 8 % and below for the 2012/13 and 2013/14 growing seasons.**

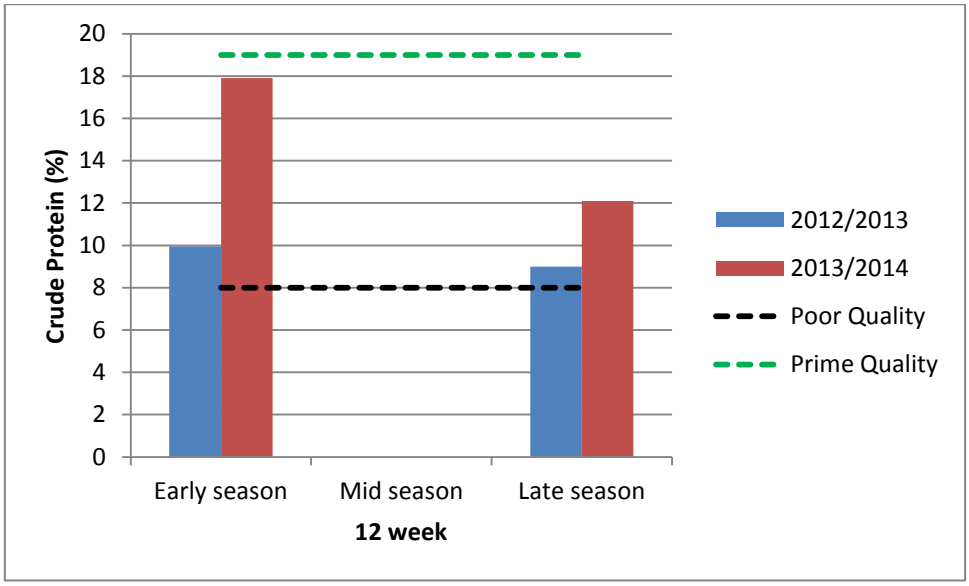


**Figure 5.7.3: The CP (%) values for *L. cuneata* as an effect of 12 week cutting frequency with indication of standard prime quality 19 % and above and poor quality 8 % and below for the 2012/13 and 2013/14 growing seasons.**

Most of the ADF values were in the lower quality range, namely 40% and above, but lower than 50% (Figures 5.8.1-5.8.3). The ADF values in the mid season's cut in both the 6 week and 8 week cuts were above 36%, but lower that 40% during the 2012/13 growing season and can be classified as moderate quality. The late season cut in 2013/14 showed a poor forage quality of 50% ADF (no late season cut took place in the 2012/13 season). The ADF values in the 12 week cut were better than expected for mature herbage and compared well with the values in the 6 and 8 week cuts in both growing seasons.

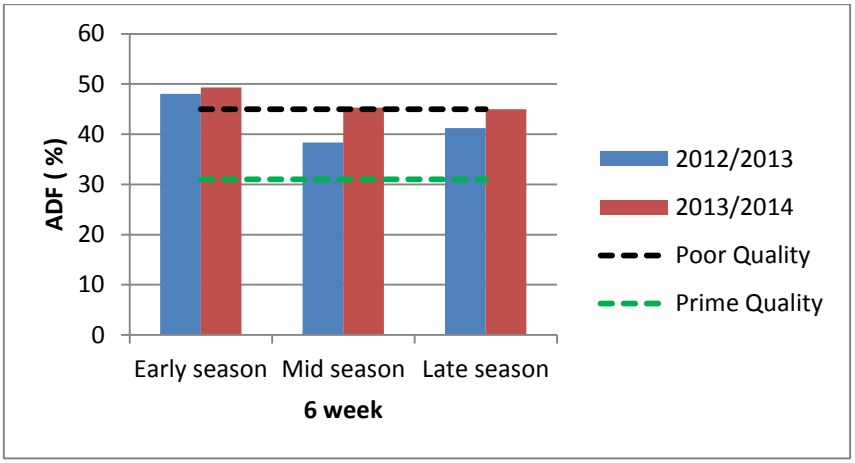


**Figure 5.8:1 The ADF values for *L. cuneata* as an effect of 6 week cutting frequency with indication of standard prime quality 31% and below and poor quality above 45% for the 2012/13 and 2013/14 growing seasons.**

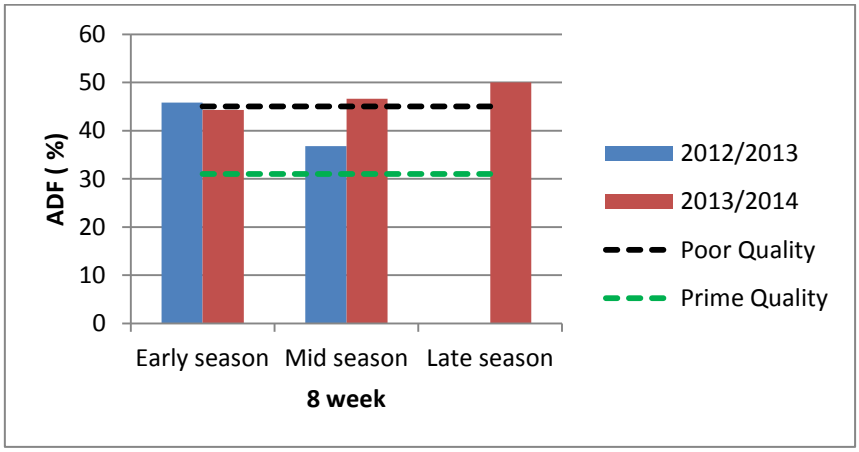


**Figure 5.8:2 The ADF values for *L. cuneata* as an effect of 8 week cutting frequency with indication of standard prime quality 31% and below and poor quality above 45% for the 2012/13 and 2013/14 growing seasons.**

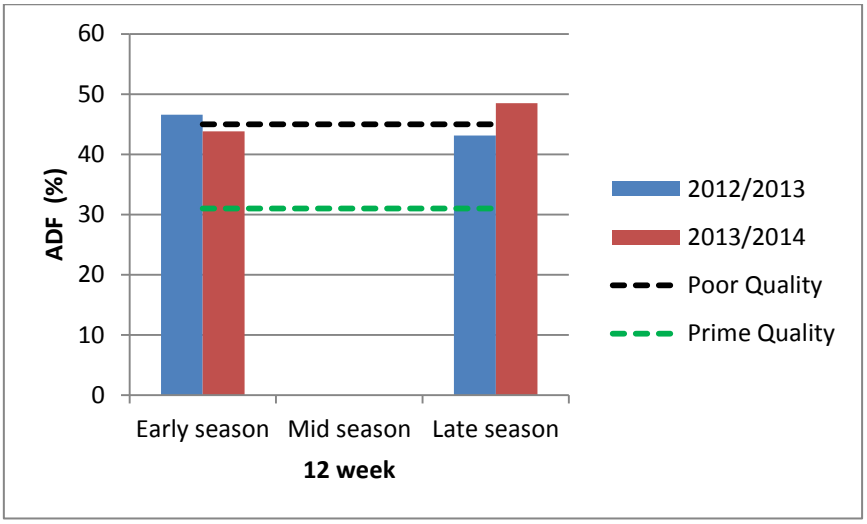


**Figure 5.8:3 The ADF values for *L. cuneata* as an effect of 12 week cutting frequency with indication of standard prime quality 31% and below and poor quality above 45% for the 2012/13 and 2013/14 growing seasons.**

The NDF values (Figures 5.9.1-5.9.3) in all cutting frequencies in the 2012/13 season, (the season with higher rainfall) for the first cuts exceeded the maximum preferred level and were regarded as low NDF quality. In 2013/14, the drier year, the values for the first cuts were below 60%. The later cuts never exceeded the upper limit, but all samples showed low, but not poor, forage quality.

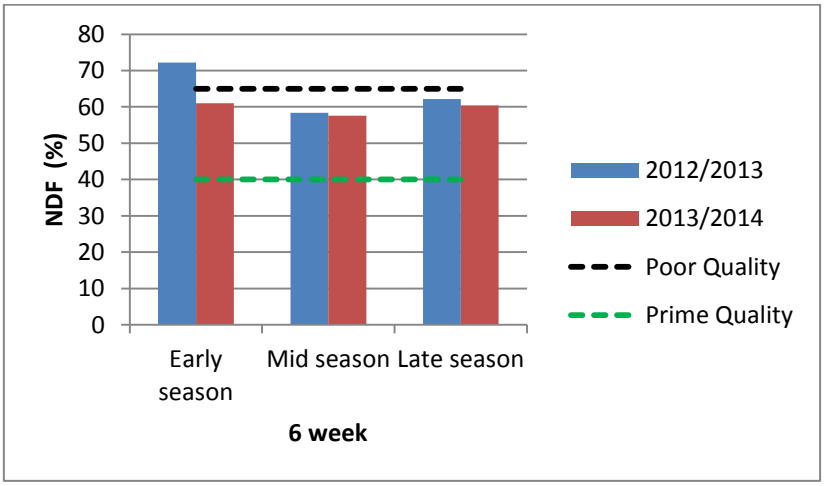


**Figure 5.9:1 The NDF values for *L. cuneata* as an effect of 6 week cutting frequency with indication of standard prime quality 40 % and below and poor quality above 65 % for the 2012/13 and 2013/14 growing seasons.**

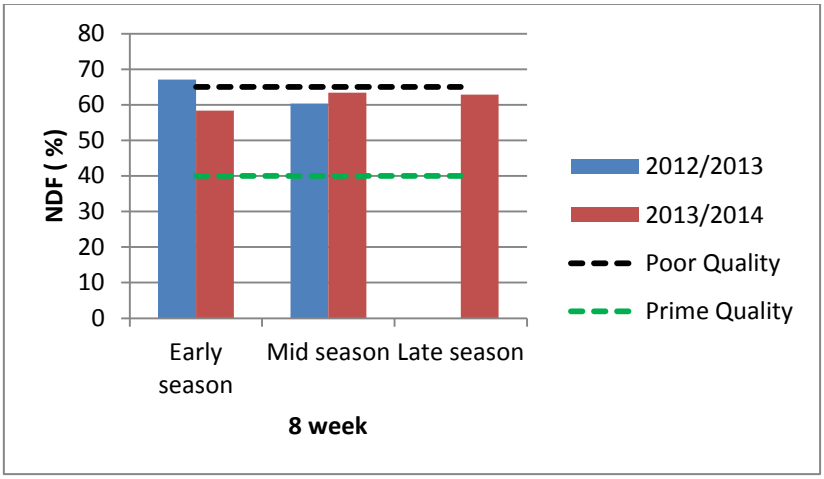


**Figure 5.9:2 The NDF values for *L. cuneata* as an effect of 8 week cutting frequency with indication of standard prime quality 40 % and below and poor quality above 65 % for the 2012/13 and 2013/14 growing seasons.**

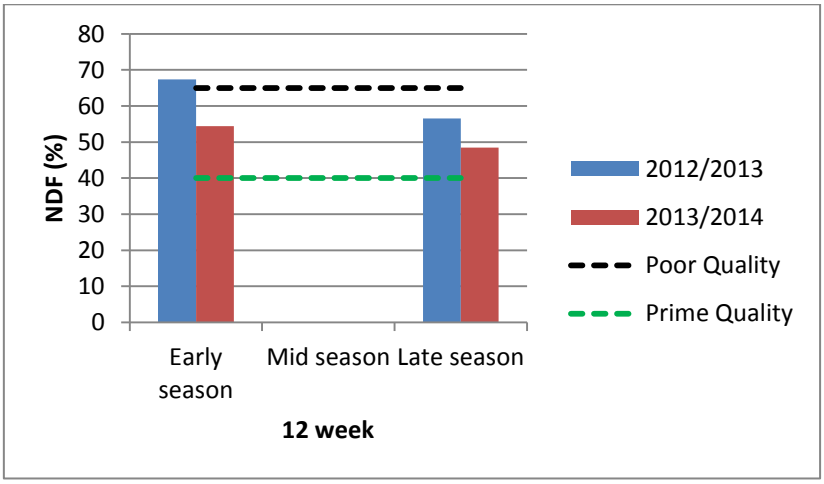


**Figure 5.9:3 The NDF values for *L. cuneata* as an effect of 12 week cutting frequency with indication of standard prime quality 40 % and below and poor quality above 65 % for the 2012/13 and 2013/14 growing seasons.**

Average international standards on the chemical composition of fresh aerial parts of *L. cuneata* refer to CP levels of 12.9 to 17% and NDF levels of 46.0 - 54.2%, and for hay a CP of 11.35% (INRA CIRAD AFZ and FAO, 2012, 2013). These are in a better range than the values obtained in the Dundee trial, however, these are general norms given by the authors and no indication of growing conditions (rainfall, plant maturity or climatic conditions) are given. The ADF and NDF levels reported by

Cunningham and Van Niekerk (2002) showed increasingly poorer quality with older material; ADF value increased from 41.43 to 48.07% and NDF levels of 53.34 to 68.98%, which corresponds to a certain extent, especially the CP values, with results from this study.

Hand-cut samples failed to be a true representation of herbage selected by grazing herbivores. It is well documented that grazing animals can select a higher quality diet compare to hand-cut samples (De Waal, 1990; Shaker, 2009). Sheep are selective grazers and selectively graze or "browse" *L. cuneata* (Figure 5.10) and concentrate on the leaf parts and as a result, select a diet that would be better represented by the leaf analyses. Therefore, a few samples were separated by hand into leaves and stems fractions, but with the primary aim of determining leaf: stem ratio. These were also chemically analysed for quality. The results of these separated samples are shown in Figure 5.11a to Figure 5.11e. The results show clearly the higher nutritional values of the leaves, compared to the stems and the whole plant.

The chemical feed analysis of the leaf material separated from the rest of the sample, for comparison (Figure 5.11a-e) show that in 6 week old material, CP content was above 17%, between 13.7 and 19.3% in the 8 week and 11.8% the 12 week old material. The ADF levels in all these leaf samples were lower than 38% and NDF values were lower than 60%. These levels were much more favourable, when compared to the analysis of the whole plant.

**Figure 5.10: Sheep selectively "browse" *L. cuneata*.**

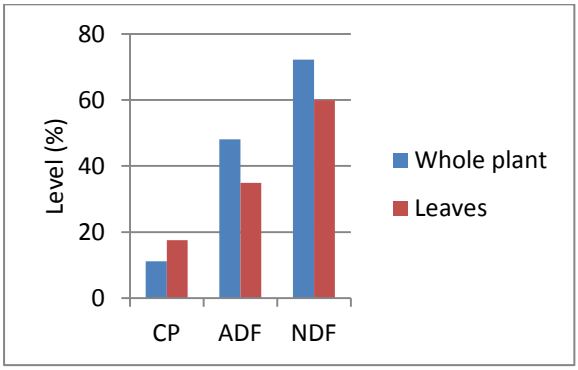


**Figure 5.11a: The CP, ADF and NDF values for *L. cuneata* as an effect of 6 week cutting frequency – Early 2012/13 season.**

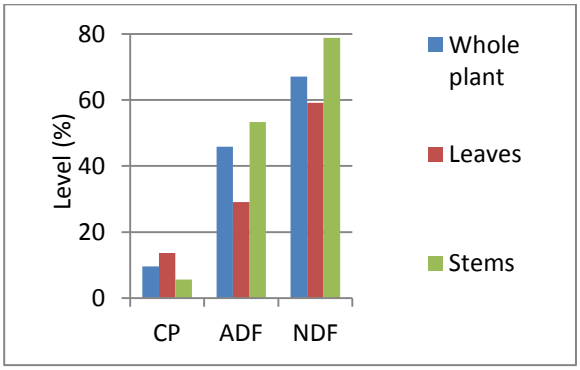


**Figure 5.11c: The CP, ADF and NDF values for *L. cuneata* as an effect of the 8 week cutting frequency – Early 2012/13 season.**

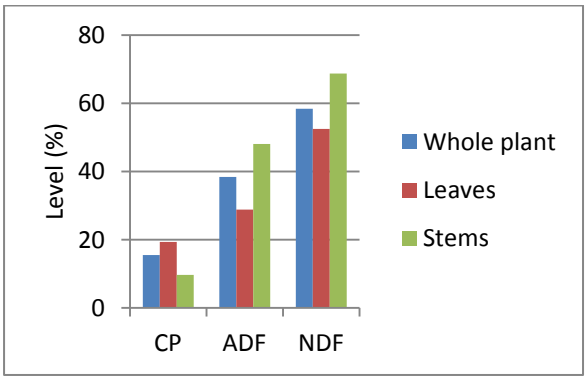


**Figure 5.11b: The CP, ADF and NDF values for *L. cuneata* as an effect of 6 week cutting frequency – Mid 2012/13 season.**

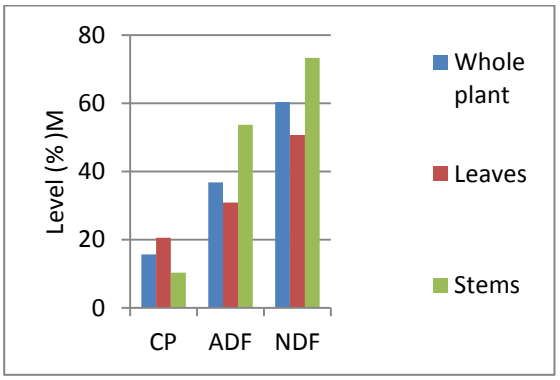


**Figure 5.11d: The CP, ADF and NDF values for *L. cuneata* as an effect of 8 cutting frequency – Mid 2012/13 season.**

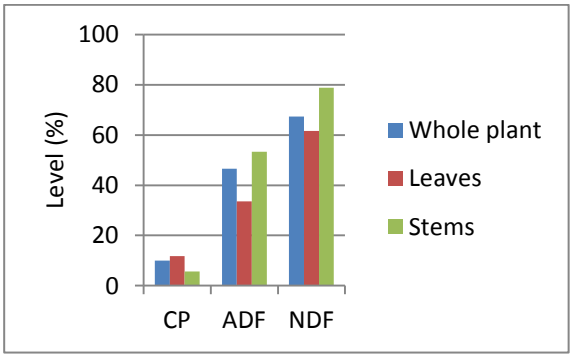


**Figure 5.11e: The CP, ADF and NDF values for *L. cuneata* as an effect of 12 week cutting frequency – Early season 2012/13.**

Very often, *L. cuneata* is referred as a pasture with low palatability and quality, frequently leading to poor animal performance. To counteract this, it is advisable to graze plants at a young stage. This may however compromise the longevity of the sward, in effect of over grazing. Cultivars, like AU Grazer™, released in 1997 in the United Stated of America, were developed under grazing conditions and it is classified to be tolerant to grazing (Mosjidis *et al.,* 2013).

Live mass gain in weaned lambs was simulated in a feed ration model to predict live mass gain. When using the average six week chemical feed analysis of the leaves, an ADG of 52.3 g day$^{-1}$ lamb$^{-1}$ was predicted (Jacobs, 2000). Cunningham and Van Niekerk (2002) reported an ADG of 96 and 89 g day$^{-1}$ SSU in different years. The results of the feed analysis confirmed that *L. cuneata* does not provide a high quality pasture. A general, but not clear trend in the results showed that the six week cutting treatment's material is of better quality and therefore represents the best grazing option. These proposals have to be verified by taking environmental conditions, such as soil type and moisture conditions into consideration.

#### *5.5.3.2 Condensed tannin analysis*

Samples were collected for condensed tannin analysis in the 6 and 12 week cutting treatments in the different treatments mentioned above (Table 5.10). The eight week treatments were excluded in the first season, but not in the second season due to the availability of the analyses service. Samples were dried in the shade and hand divided into stems and leaves to imitate the selective grazing habits of sheep. CT analyses were done on whole plant-, leaves- and stems samples.

**Table 5.10: Sampling dates of *L. cuneata* material, used for Condensed tannin analyses.**

| Cutting frequencies | Season 1 | | | | Season 2 | | | |
|---|---|---|---|---|---|---|---|---|
| 6 week | 22-Nov-2012 | 07-Jan-2013 | 18-Feb-2013 | 16-Apr-2013 | 03-Dec-2013 | 16-Jan-2014 | 4- March 2014 | |
| 8 week | | | | | | 28-Jan-2014 | | |
| 12 week | 07-Jan-2013 | | | | | | 12-March 2014 | |

The CT content of *L. cuneata* can vary between 46 and 152 g $kg^{-1}$ of DM (Table 5.2). The results obtained for the tannins in this study, varied between 24.5 and 122.5 g $kg^{-1}$ DM, obtained from the 6 week cut on 3 Dec 2013 in stems and 6 weeks cut in leaves on 16 January 2014. The lowest CT level in leaves, (32.5 g CT $kg^{-1}$ of DM) was obtained from the 6 week cut, on 7 January 2013 (Table 5.11).

**Table 5.11: Condensed tannin content (g $kg^{-1}$ DM) of *L. cuneata* for different sampling dates and rainfall (mm) for four pentadals prior to sampling.**

| Plant part | Year | Cutting frequency | Date | CT content (g $kg^{-1}$ DM) | Pentade -1 (mm) | Pentade -2 (mm) | Pentade -3 (mm) | Pentade -4 (mm) |
|---|---|---|---|---|---|---|---|---|
| **Leaves** | 2012/13 | 6 weeks | 22 Nov | 80.2 | 5.85 | 26.92 | 24.4 | 17.52 |
| | | | 7 Jan | 32.5 | 0.76 | 13.12 | 66.29 | 86.1 |
| | | | 18 Feb | 64.4 | 86.1 | 38.35 | 19.18 | 7.11 |
| | | | 16 April | 90.9 | 1.02 | 2.79 | 31.5 | 14.73 |
| | | 12 weeks | **7 Jan | 32.8 | 0.76 | 13.12 | 66.29 | 86.1 |
| | 2013/14 | 6 weeks | 3 Dec | 102 | 57.4 | 13.47 | 26.67 | 7.36 |
| | | | *16 Jan | 122.5 | 2.79 | 2.79 | 0 | 24.64 |
| | | | 4 Mar | 88 | 1.02 | 42.92 | 14.22 | 0 |
| | | 8 weeks | 28 Jan | 101.9 | 0.25 | 28.19 | 2.79 | 2.79 |
| | | 12 weeks | 12 Mar | 73.5 | 28.45 | 74.68 | 1.02 | 42.92 |
| **Stems** | 2012/13 | 6 weeks | 22 Nov | **65.2** | 5.85 | 26.92 | 25.4 | 17.52 |
| | | | 7 Jan | 35.8 | 0.76 | 13.12 | 66.29 | 86.1 |
| | | | 18 Feb | 35.9 | 86.1 | 38.35 | 19.81 | 7.11 |
| | | | 16 April | 83.9 | 1.02 | 2.79 | 31.5 | 14.73 |
| | | 12 weeks | 7 Jan | 77.1 | 0.76 | 13.12 | 66.29 | 86.1 |
| | 2013/14 | 6 weeks | *3 Dec | 24.5 | 57.4 | 13.47 | 26.67 | 7.36 |
| | | | 16 Jan | 42.0 | 2.79 | 2.79 | 0 | 24.64 |
| | | | 4 Mar | 63.8 | 1.02 | 42.92 | 14.22 | 0 |
| | | 8 weeks | 28 Jan | 44.4 | 0.25 | 28.19 | 2.79 | 2.79 |
| | | 12 weeks | 12 Mar | 64.0 | 28.95 | 74.68 | 1.02 | 42.92 |
| **Stems** | 2012/13 | 6 weeks | 22 Nov | 35.9 | 5.85 | 26.92 | 25.4 | 17.52 |
| | | | 7 Jan | 29.0 | 0.76 | 13.12 | 66.29 | 86.1 |
| | | | 18 Feb | 35.9 | 86.1 | 38.35 | 19.81 | 7.11 |
| | | | 16 April | 52.7 | 1.02 | 2.79 | 31.5 | 14.73 |
| | | 12 weeks | 7 Jan | 83.5 | 0.76 | 13.12 | 66.29 | 86.1 |
| | 2013/14 | 6 weeks | 3 Dec | 102.6 | 57.4 | 13.47 | 26.67 | 7.36 |
| | | | 16 Jan | 92.1 | 2.79 | 2.79 | 0 | 24.64 |
| | | | 4 Mar | 80.0 | 1.02 | 42.92 | 14.22 | 0 |
| | | 8 weeks | 28 Jan | 76.8 | 0.25 | 28.19 | 2.79 | 2.79 |
| | | 12 weeks | 12 Mar | 98.0 | 28.95 | 74.68 | 1.02 | 42.92 |

*Highest and lowest CT content are highlighted in bold red. **Lowest leaf CT content is highlighted in red

To investigate the effect of rainfall on the CT levels, the recorded rainfall prior to sampling was analysed per rainfall pentadal (five day period) for four pentadals prior to the sampling date, using linear regressions. Regression analyses of the CT levels over plant parts (leaves, stems, whole plant) showed that the effect of Pentadal 1 rainfall on CT levels were not significant (P>0.05) (Fig 5.12). The cumulative precipitation of pentadals 1 and 2 over all data was also not significant (P>0.05). The cumulative precipitation of pentadals 1, 2 and 3 was also not significant (P>0.05), but showed a clear trend that CT levels decreased with increasing rainfall. The cumulative effect of precipitation of pentadals 1, 2, 3 and 4 on CT levels was significantly (P<0.05) different. The influence of rainfall on CT level was inversely related with a "more rain, lower CT level" trend. The influence of rainfall on CT content was most pronouncing on the leaves (Figure 5.12)

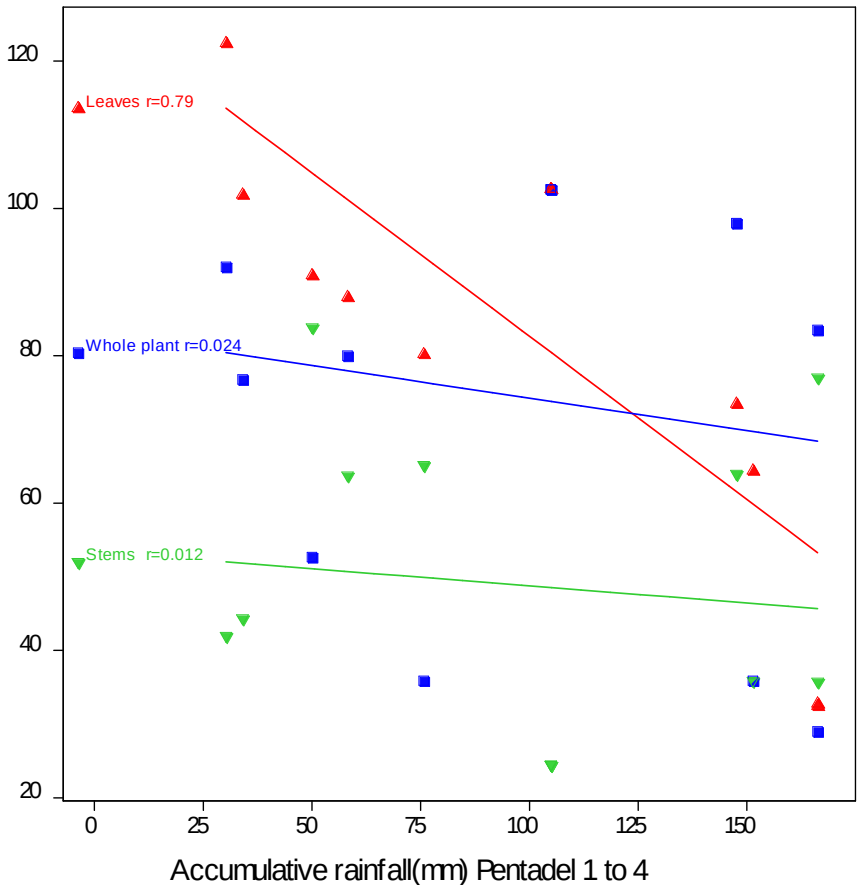


**Figure 5.12: The effect of cumulative rainfall, Pentadals 1 to 4 prior to sampling, on the Condenced tannin content (g kg$^{-1}$DM) in leaves, stem and whole plant of *L. cuneata*.**

The regression analyses of the data established that the effect of rainfall (accumulative pentadals 1 to 4) on CT levels was not significant (P>0.05) for stems and whole plants, but highly significant (P< 0.001) for leaves (Figure 5.13), with an inverse relationship.

The relationship between tannins and rainfall, for leaves, can be described as:

CT level = 127 – 0.443 (Accumulative rain for pentadals 1 to 4)

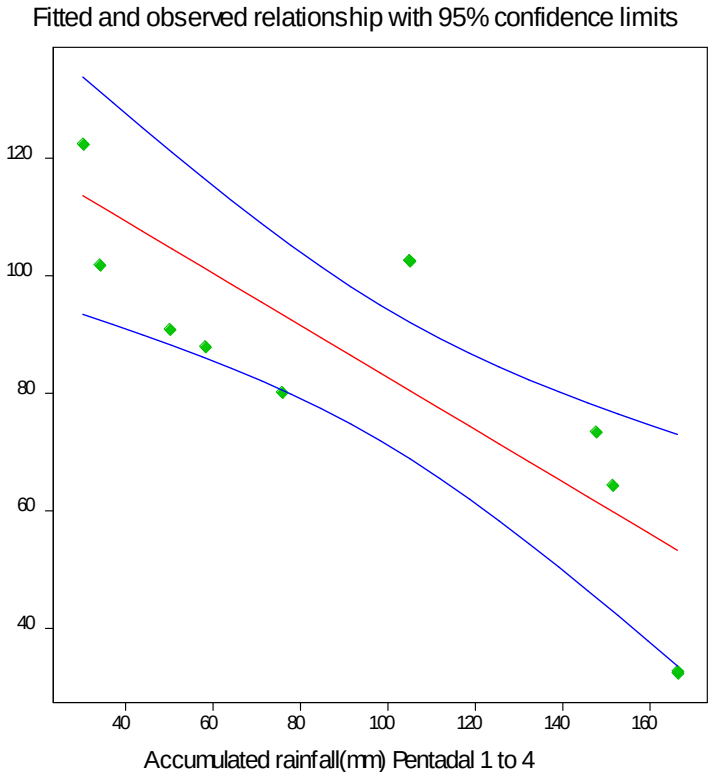


**Figure 5.13: The cumulative effect of rainfall (mm) in pentadals on the Condensed tannin content of leaves of *L. cuneata*.**

The effects of different CT levels on ruminants were described in detail in Chapter 3, Paragraph 3.2.6. According to Min and Hart (2003) and Hoste *et al.* (2006), moderate concentrations of CT (20-40 g CT $kg^{-1}$ DM) have positive effects in animal nutrition, while high forage CT concentrations (>55 g CT $kg^{-1}$ DM) may have negative effects, such as decreased voluntary feed intake. High CT levels in *L. cuneata* (plus high NDF and ADF levels) explained why the pasture is sometimes branded as "unpalatable". From the data collected in this study, it is clear that rainfall conditions

influenced the CT levels in *L. cuneata* within a season. With a decrease in precipitation, the CT levels increased, with leaves most affected.

## 5.6 Conclusion

During the two trial years, high variability in rainfall was experienced. During the first year, above expected DM yields of *L. cuneata* were recorded, whereas in the second year, below average yields were recorded. For fodder flow planning, early summer grazing is weather dependent and late season grazing will not be available later than the end of March, since regrowth then ceased. To obtain reliable yield norms, yields over more than two years need to be quantified. Soil fertility was not taken into account in this study and needs investigation in the future.

Due to rainfall variation, over the trial's growing seasons, it was not possible to make definite conclusions on the effects of the 5 and 15 cm cutting height on plant survival, as measured by yield. It is suspected that the 5 cm cutting height would compromise plant survival, but that could not be demonstrated in this trial, since the total mean yields for cutting heights did not differ significantly in the second year. More research is necessary to clarify this.

Nutritional value and other chemical characteristics of the whole plant were low. When analyses of the separated plant material are considered, the quality of the leaf material was within an acceptable range and therefore theoretically should not compromise animal performance, due to the selective grazing habits of sheep. Since the stems are of low quality and sheep tend to graze the higher quality leaves, the total DM yield needed to be adjusted downwards by at least a third for sustainable fodder flow planning purposes. The changes in leaf:stem ratios as the plant matures, are important to take into account when calculating grazing capacity or utilizable hay available. To overcome the texture problem of the hay, milling might be an option, but by mixing the lower quality stem material with the leaf material, lower the quality of the hay.

Tannins protect the plant against pathogens and defoliation, lowering the palatability and interfering with digestion of the browser. The data collected in this

study showed clearly how CT levels in the plants increased under moisture stress conditions. The CT levels in leaves, which seemed to be most affected by moisture changes, are targeted during defoliation and will therefore have a major influence on animal production off this pasture. Therefore, although *L. cuneata* appears to have a good potential based on high yields and adaptability to low fertility soils, its actual feeding value may be substantially lower, during certain times, such as drought, than expected due to the resultant high levels of CT.

To mitigate the effect of drought, the choice of soil type is important. Investigations into the cultivation of this pasture on other soil types are needed.

To conclude, the results from this study indicate that:

- The amount and distribution of rainfall in spring and early summer will determine the early season's regrowth.
- The forage must be utilized when young to have the best quality available to livestock.
- Moisture stress will increase CT level and is advisable to withdraw livestock from grazing during times of moisture stress, to curb poor animal production.
- The growing season of this fodder crop is short, especially towards late summer.
- *L. cuneata* have huge potential as dryland pasture, being a legume, which implies lower fertilizer costs.
- It requires a high level of management skills to be successfully incorporated in a fodder flow program.

# CHAPTER 6

# Direct anthelmintic effects of feeding *Lespedeza cuneata* hay (leaf material) on gastrointestinal parasites in sheep: *in vivo* studies

## 6.1 Aim

To determine the effect of *Lespedeza cuneata*-leaf hay on an established gastro-intestinal parasite infection in Merino sheep.

## 6.2 Introduction

Increased drug resistance to modern anthelmintics and the increased public awareness of drug residues in animal products, are two major driving forces in the initiative to find alternative endoparasite-control methods. Many natural homemade plant remedies are used in third world countries (Hutchings *et al*., 1996; Githiori, 2004) to address helminth infections, but not all of them are effective (Githiori, 2004). Rahmann and Seip *(*2007) declared a lack of scientific, verified information in this regard. He suggested that effective plant remedies needed to be distinguished from ineffective ones by means of *in vitro* testing, which will save time and costs. Once done, plants with proven potential, can be tested *in vivo* to confirm results obtained, to evaluate risks and side effects.

## 6.3 Literature review

Shaik *et al.* (2004) did a study on the anthelmintic properties of *L. cuneata* hay with 6-8 month-old Boer goats, which were trickle infected with 500 *Haemonchus contortus* larvae per animal three times per week during the trial to simulate natural infection. These authors compared the faecal (FEC) and blood Packed cell volume (PCV) of the goats, fed for five weeks in confinement on *long* stem Bermuda grass (*Cynodon dactylon L*.) plus a concentrate, with half of the trial animals switched to long-stem *L. cuneata* hay for an additional seven weeks. The outcomes were a significantly ($P < 0.01$) reduced FEC and increased PCV in the *L. cuneata* hay group and a lower percentage of ova in faeces from *Lespedeza* fed goats that

developed into infective (L3) larvae. Coffey *et al.,* (2007) summarised several research trials which focus on the investigation of the anthelmintic properties of L. *cuneata* (Table 6.1).

The literature also mentions that, when field dried, the tannins in tannin-rich forages become more polarised, resulting in a lower number of free hydroxyls available for binding protein. In practice, this implies that, in hay form, intake could he higher, opposed to green grazing (Hovarth, 1981; Reed *et al*., 1982; Barry and McNabb, 1999)

A pilot trial to investigate the effect of grazing *L. cuneata* on the GIN burden in Merino sheep was conducted at Dundee Research Station (Van Zyl and Oosthuizen, 2010). After good rains, pregnant Merino ewes, grazing on Nile grass (*Acroceras macrum*) pasture, naturally became heavily infected with GIN. Sheep (n=9) were moved to a *Lespedeza/Eragrostis* pasture (*Lespedeza* 50 % / *Eragrostis curvula* 50%). The rest (n=9) stayed on the Nile grass pasture and were treated with an injectable Ivermectin according to prescription. Sheep were weighed every two weeks and EPG from individual sheep were determined from their faeces via the Mc Master technique.

**Table 6.1: Summarized trials on the anthelmintic properties of *L. cuneata (SL)**.**

| Animals used | Treatments | Results | Notes |
|---|---|---|---|
| Goats, confined and fed hay (75% of diet) and grain (25%) | Ground SL (0, 25, 50, 75%) and/or Bermuda grass (75, 50, 25, 0%)in combi-nations equaling 75% hay; levels testing dose of SL needed, 6 weeks | FEC reduced for those fed SL at all levels, greater reduction as % SL increases and with time; at 6 weeks, 75% SL hay, 91.9% reduction | Optimum level of SL hay appeared to be 50-75% of total diet (Dykes et al., 2006), but 25% was also beneficial, reducing number of adult barber pole worms in the stomach by 58% (unpublished data) |
| Goats, confined and fed hay and grain | SL hay or Bermuda grass hay, 7 weeks | FEC reduced, number of adult worms reduced. Hatchability of eggs into L-3larvae reduced in goats fed SL hay | Egg counts dropped by about 80% one week after SL feeding started; reduction increased to almost 90% by end of trial. Both abomasal and small intestinal worms reduced and female worms reduced more than male worms. Male and female *H. contortus* were reduced by 61 and 76%, respectively (Shaik *et al.,* 2006) |
| Angora does, grazing | SL or crabgrass/tall fescue grazing, 81 days | Goats on SL had reduced FEC and fewer adult worms. Inhibited larval activity. Improved weight gain and immune responses. No adverse effect on does and kids. | Goats grazing SL reduced both *H. contortus* (89%) and *Trichostrongylus* parasites (50%). (Min *et al.,*2005) |
| Kiko-Spanish kids fed ground hay and pellets; natural infection | SL hay in ground and pelleted forms, ground hay | Pelleted SL reduced FEC 78%; increased PCV 32% compared with Bermuda grass | Pelleting increased effectiveness of SL hay against parasitic worms; reduced adult *H. contortus* 75% (Terrill *et al.,* 2007) |
| Lambs, fed hay; natural and experi-mental *H. contortus* infections | SL hay bermudagrass hay, 7 weeks, Bermuda grass an additional 2 weeks | FEC reduced for those receiving SL (67-98%); FEC increased after SL feeding stopped. SL also reduced worm numbers | SL fed as hay reduced naturally infected worm burdens 67%; reduced establishment of incoming larvae 26% (Lange *et al*.,2006) |

- These researchers referred to *L. cuneata* as *Sericea lespedeza*

**Source**: Coffey *et al.,* (2007)

After the chemical anthelmintic administration, a decrease in EPG was measured; however, the degree of control was insufficient (60.7%) and EPG increased again after treatment. Parasite resistance against the anthelmintic was most possibly the reason for this result. The EPG decrease on the *Lespedeza* pasture was more effective and continued to be low for the duration of the experiment; however the differences between treatments were not significant (P>0.05). The effect of the treatments is displayed in Figure 6.1. The weight increase on the *Lespedeza* group was significantly better (P<0.001) than the weight changes in sheep on the Nile grass (Figure 6.2).

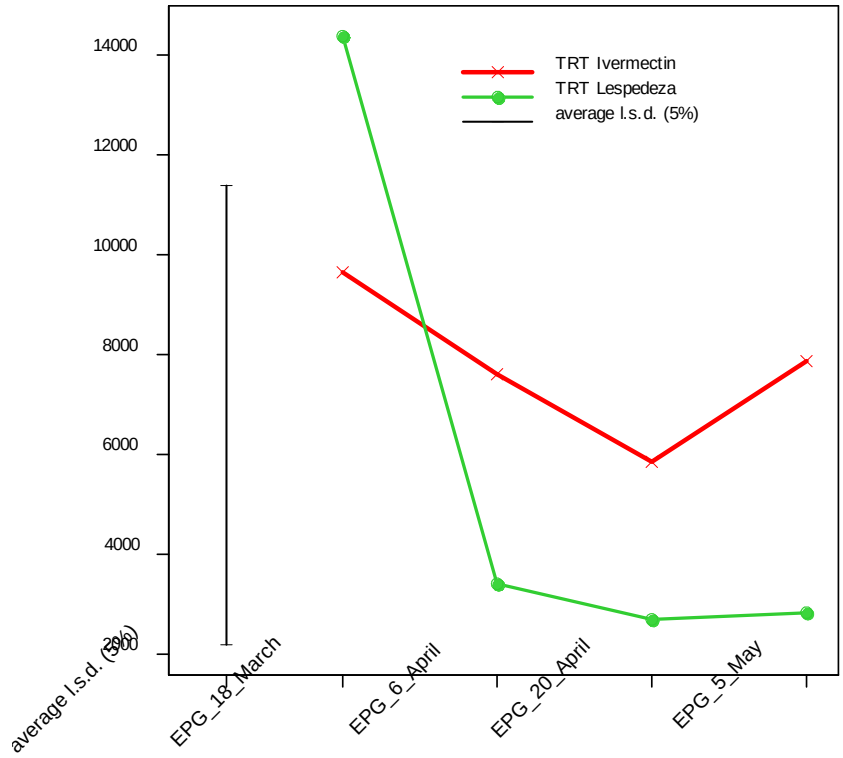


**Figure 6.1: Differences in EPG counts between sheep grazing *Lespedeza cuneata* pasture and sheep treated with Ivermectin and grazed on *Acroceras macrum* (TRT=treatment) (Van Zyl and Oosthuizen, 2010)**

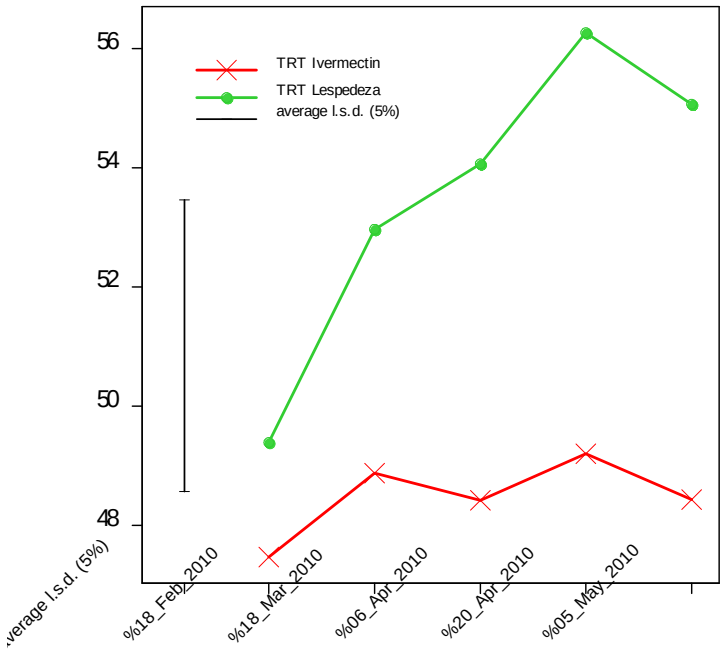


**Figure 6.2: Differences in weight (kg) between sheep grazing *Lespedeza cuneata* and sheep treated with Ivermectin and grazed on *Acroceras macrum* (TRT=treatment) (Van Zyl and Oosthuizen, 2010)**

Min and Hart (2003) compared the results of several corresponding studies of the effect of CT levels on FEC reduction (Figure. 6.3). They concluded that FEC's were reduced by 50% with CT containing forages (45 to 55 g of CT $kg^{-1}$ DM) relative to non-CT containing forages. When CT levels increase above 55 g $kg^{-1}$ DM and decreased below 45 g $kg^{-1}$ DM the FEC responses become variable. *L. cuneata*, referred to as *Sericea* by the authors, Figure 6.3, with CT content of 50 g $kg^{-1}$ DM, showed a FEC reduction of 60%. Compared to other forage types, *Medicago sativa* (commonly named Lucerne or alfalfa) with a zero CT content and no FEC reduction properties is popular for inclusion as control forage in parasite-forage studies.

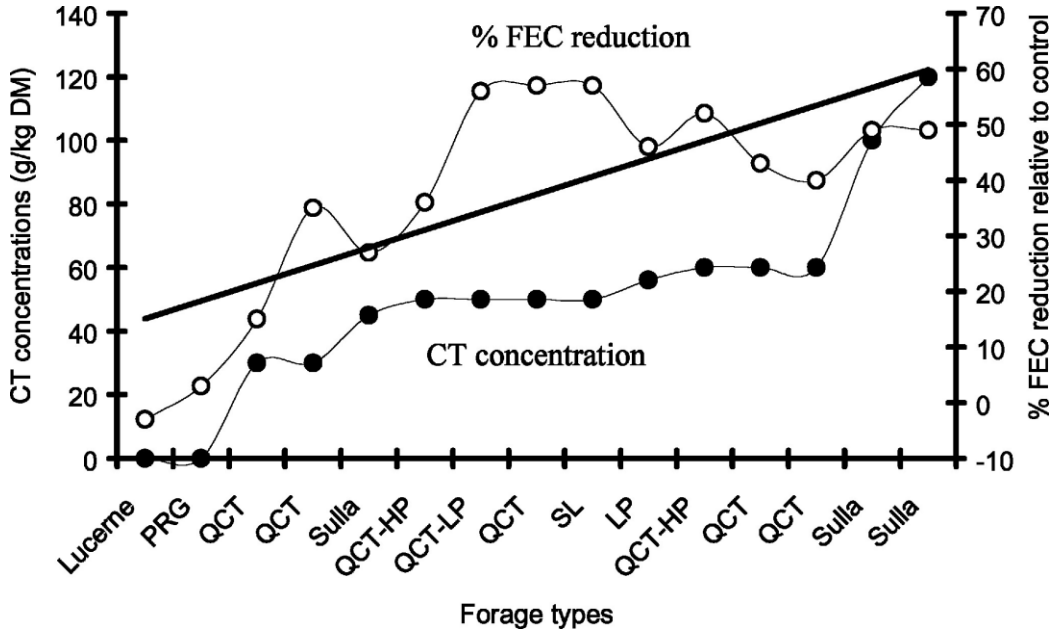


**Figure 6.3: The effect of forage condensed tannins (CT) concentration on percentage feacal egg count (FEC) reduction relative to control.**

PRG =perennial ryegrass/white clover, QCT = Quebracho CT, QCT-HP = QCT-high protein, QCT-LP = QCT-low protein, SL = *Sericea lespedeza*; LP = *Lotus pedunculatus*.

**Source**: Min and Hart (2003)

## 6.4 Materials and methods

### *6.4.1 Animals and feed*

A confinement-feeding experiment was completed with fourteen Merino ewes at the Dundee Research Station. All husbandry practices and experimental procedures were approved by various applicable authorities. The *L. cuneata* leaves were obtained from *L. cuneata* hay, cut the previous late summer with stems removed semi-mechanical for reasons explained in Chapter 5: Paragraph *5.5.3.* Commercially available *M. sativa* hay was bought in from a local producer.

The ewes became naturally infected with GIN by grazing contaminated rain fed Nile grass (*Acroceras macrum)* pastures. The animals were on a salvaged deworming protocol, which was deworming with Unidose, (Active ingredient Trichlorfon 20% m/v, Merial). This anthelmintic group was identified in Chapter 4 (Paragraph 4.5.2) as being effective against GIN on Dundee Research Station. Treatment would take

place if FEC in individual sheep would exceed 6000 EPG in two consecutive weeks. No animals required salvage deworming throughout the study.

Weekly FEC were done to verify the parasite infection status prior to the commencement of the trial. Animals were ranked according to the FEC counts in their dung samples and assigned to one of two treatment groups to provide a similar mean EPG in each group (*n*= 7). Animals in each treatment were then assigned to two pens with either three or four, sheep in each pen. The treatment groups were fed *ad libitum* as follows:

- *L. cuneata* hay, leaf fraction only (L group) and
- *Medicago sativa* hay (M group).

Animals were housed in pens of 4m x 6m, with similar temperature and sunlight conditions in a covered barn with open sides and concrete flooring. The pens were carefully cleaned each day to prevent GIN re-infection.

The sheep were fed the rations *ad libitum* on a pen basis once per day to allow 10% remaining feed and were given access to fresh water *ad libitum* daily. The rations were fed for a total of 35 days.

### *6.4.2 Sample collection and analysis*

Fresh dung samples were taken directly from the rectum of individual sheep to determine their FEC levels. These collections were made prior to the start of the experiment to aid in the blocking of the animals and were thereafter made weekly until Day 35 when the experiment was terminated.

Faecal samples (10-15 g) were analysed for FEC, using the modified McMaster and Visser slide techniques (Reinecke, 1983). Three grams of the collected faeces were diluted into 30 ml of a saturated sugar solution. After mixing the solution a sample was taken with a pipette and dropped into the McMaster slide chambers. Using a microscope, eggs were counted on both sides of the chamber and multiplied by 50 to estimate the total number of eggs in a sample, as eggs per gram (EPG) of faeces.

Live animal weights and Famacha© scorings were collected weekly, when dung samples were collected. Rectal body temperature was also taken with a handheld thermometer (Digiflash, Phizer).

### 6.4.3 *Statistical analysis*

The data on FEC, live mass and rectal temperatures were analysed by using a Repeated Measures Analysis of Variance. Fisher's test of least significant differences (LSD) was conducted at a 5% significance level.

## 6.5 Results and discussion

### 6.5.1 *Chemical composition of feed and intake*

To nutritionally balance the two rations, the ration of the L group had to be supplemented and consisted of 80% *L. cuneata leaves,* 12% HPC (High protein concentrate) and 8% molasses meal [Molasses meal from Molotek: Protein=40g $kg^{-1}$; ME (MJ $Kg^{-1}$=105)]. The ration of the M group consisted out of only *Medicago sativa* hay. Both the rations were offered at 2 kg DM sheep $^{-1}$ day $^{-1}$. The chemical analyses of the rations are displayed in Table 6.2**.** Voluntary intake was similar for both L and M groups.

**Table 6.2: Composition of the rations fed to Merino sheep.**

| % | L group ration | M Group ration |
|---|---|---|
| CP | 17.17 | 16.55 |
| NDF | 41.91 | 44.52 |
| ADF | 30.45 | 36.12 |
| Ca | 0.96 | 0.84 |

By offering completely whole *L. cuneata* bales, sheep tend to select the leaves (See Chapter 5, Figure 5.6). Therefore, only the leaf fraction of the hay was used in this experiment. The grinding and pelleting of dried *Lespedeza cuneata* hay (leaves and

stems) has been investigated with positive results as dewormer (Terrill *et al.,* 2007), but nutritional qualities were not considered.

The CT level of the hay used in this experiment was not analysed. The small plot trial, which was reported on in Chapter 5 (Paragraph 5.3.4) is situated adjacent to the area where the hay was cut. The haymaking took place in the same week of the cutting trial. The CT level of the leaves of the 12 week cut, taken on 12 March 2014 was 80 g $kg^{-1}$ DM and is a good indication of the CT level of *L. cuneata* material used in this experiment. *M. sativa* has a known low CT and is often used as contrasting forage to high-tannin forages (Valderrábano *et al.,* 2010).

### *6.5.2 Data collected from animals*

#### *6.5.2.1 FEC*

The FEC counts decreased rapidly in both groups, following commencement of feeding up to Day 14. A gradual increase in FEC in the L group was observed from Day 14 to Day 21, followed again by a slight decrease and increase, respectively on Days 28 and 35. FEC levels in the M group followed the same trend, but on higher levels of FEC. The final FEC levels at termination of the trial were 53% and 105.9%, respectively in L group and in the M group, compared to the levels at commencement of the trial (Figure 6.4).

Shaik *et al,* (2006) did a study to compare the influence of feeding *L. cuneata hay* compared to *Bermudagrass* hay on GIN infection levels in goats. The results showed also how FEC in- or decreased to a certain level over the trial period, as was observed in the data discussed above.

The FEC levels, compared in the two treatments, were not significantly ($P>0.05$) different up to Day 28, but become significantly different on Day 35 ($P<0.05$), but with high variability in the data. $Log_e$ transformations were then used to stabilise variance in FEC. After transformation, differences between treatment groups on Day 35, became not significantly ($P>0.05$), with P value of 0.054. FEC change over time, in

both treatment groups was highly significant (P<0.01). The analyses of the significance of data are shown in Table 6.3.

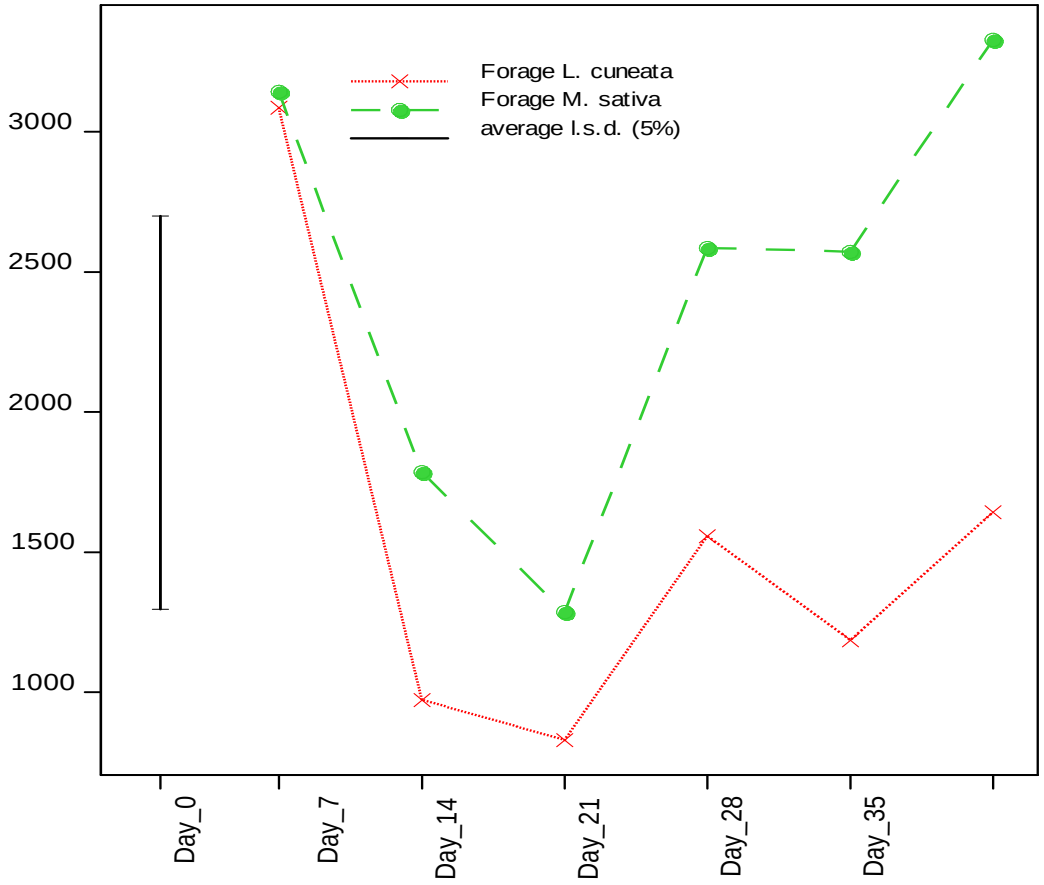


**Figure 6.4: Mean feacal egg counts (FEC) of merino sheep fed a *L. cuneata* hay ration (leaves only) or *M. sativa* hay.**

As discussed above, the data showed that FEC levels were consistently lower in the L group, compared to the M group, although not always significantly. However, these differences are of agricultural importance. Colditz (2008) expected blood loss of 10 ml $day^{-1}$ in a parasitized host with EPG count of 1600 and stated that blood loss in excess of this norm, will result in anaemia in mature sheep. Animals with FEC above 1600 therefore will be negatively affected by the infection. The FEC results presented in Figure 6.4 show that from Day 7, the EPG in the L group were consistently below this level, which was not the case in the M group. The agricultural value of these

results is that the sheep in the M group was in line for anthelmintic treatment, compared to sheep in the L group where treatment is not required.

**Table 6.3: The FEC results of sheep(Log$_e$ transformationed) on a *Lespedeza* (L group) or *Medicago (*M group) ration.**

| *Different days-Time (T)* | FEC (Natural log) for sheep fed different rations (R) | | Means FEC on different days |
|---|---|---|---|
| | Lespedeza | Medicago | |
| Day 0 | 7.628 | 7.672 | 7.650 |
| Day 7 | 6.596 | 7.130 | 6.863 |
| Day 14 | 6.316 | 7.064 | 6.690 |
| Day 21 | 6.647 | 7.664 | 7.155 |
| Day 28 | 6.744 | 7.608 | 7.176 |
| Day 35 | 7.139 | 7.981 | 7.560 |
| Means of T | 6.688 | 7.428 | |

Least significant differences of means: (5% level)

| | | |
|---|---|---|
| T | 0.4678 | Highly significant |
| R | 0.9374 | NS* |
| T x R interaction | 1.1271 | NS*, |
| T x R interaction | 0.6616 | when compared in the same ration |

**NS = not significant.*

### *6.5.2.2 Live weight*

Sheep were not blocked for initial live mass. Ewes in the L group had an average initial live body mass of 53.5 ± 5.9 kg sheep$^{-1}$ and showed an average daily gain (ADG) of 0.2 g sheep$^{-1}$ day$^{-1}$ over the trial period. The M group had an initial live body mass of 50.4.5 ± 6.5 kg sheep$^{-1}$ and showed an average daily gain (ADG) of 0.1 g sheep$^{-1}$ day$^{-1}$ over the trial period. Live body mass of sheep in the two treatments did not differ significantly ($P>0.05$), but the interaction between treatments and time became significant ($P<0.05$) at termination.

### 6.5.2.3 *Rectal temperature*

The normal rectal temperature of sheep differs between 38.3 and 39.9 °C (Merck, 2014). Gastrointestinal parasitism results in a loss of haemoglobin, causing anaemia (Bath and De Wet, 2000; Taylor *et al*., 2007) and anaemia is associated with below normal body temperature. Anaemia results in a restricted oxygen and iron supply to cells in the body, which in turn interferes with the ability of the body to regulate its temperature (Anon, 2014).

The rectal temperature of the sheep in this trial was monitored to investigate whether differences between the two treatment groups, with different parasite loads, could be detected (Figure 6.5). For the duration of the experiment, the rectal temperature measured was within the normal range. Due to apparatus failure, the temperatures were only measured from Day 7 till Day 28.

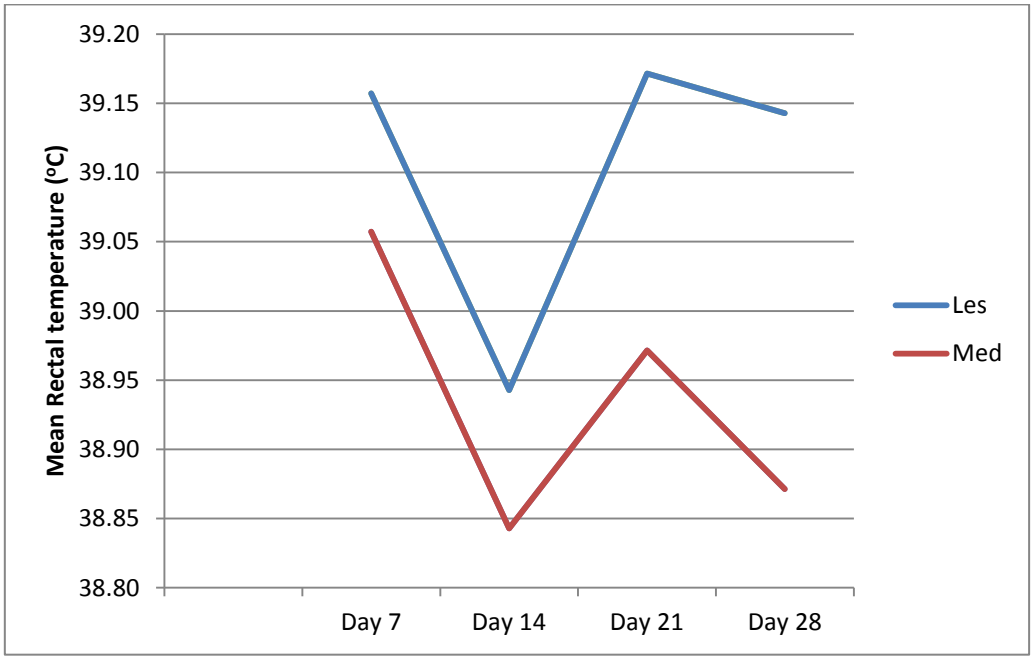


**Figure 6.5: Mean rectal temperature (°C) of merino sheep fed a *L. cuneata* hay ration (leaves only) or *M. sativa* hay.**

The rectal temperature in the M group was consistently lower for the duration of the experiment, compared to the L group, although the difference was not significant (P>0.05) with repeated measures. A noticeable decrease in rectal temperature is

observed on Day 14 of the experiment, which co-incided with a low level in FEC. The reason for this observation is unclear.

The differences in mean rectal temperature between the treatments almost became significant on Day 21 (P<0.007) and on Day 28 (P<0.008). When the relationship between rectal temperature and FEC were analysed, significant (P<0.05) differences were found between FEC on Day 14 and rectal temperature on Day 28, but other than that, no significant (P> 0.05) relationship existed. The trend of higher rectal temperature in the L group, compared to the M group, however non-significant (P>0.05), suggested a higher level of anaemia in the M group, which could relate to the higher parasite-worm burden. Specht (1982) investigated the effect of GIN infections on host-body temperature, but concluded that the body temperature results did not show as close a relationship to the infection as expected.

#### *6.5.2.4 Famacha©*

Famacha© scoring showed no significant (P>0.05) differences in the data. The scoring also did not pick up the changes in infection as shown in the FEC results. This can be due to the relatively low infection rate (and therefore anaemia) in these sheep.

The results in this study corresponds well with a similar experiment that was conducted with *Hedysarum coronarium* (sulla), comparing the FEC of lambs fed *H. cornarium*, a high CT-containing forage (CT of 110 g $kg^{-1}$ DM) and *M. sativa*, with low CT content (CT of 1.5 g $kg^{-1}$ DM). The results did not differ significantly (P> 0.05) for weight changes, but significantly (P< 0.001) for FEC differences of respectively 1320 EPG for H. *coronarium,* compared to 2200 EPG for *M. sativa,* as well as a total worm burden *of* 10 553 for *H. coronarium,* compared to 18 676 for *M. sativa* (Niezen *et al*., 1995; Niezen *et al*., 1998).

The reduction in FEC observed in this study, confirms results from previous studies where *L. cuneata,* fed as fresh material or grazed, or fed in dried form (hay, leaf

meal, pellets) (Table 6.1) resulted in reduced EPG. Kommuru *et al.*, (2014) reported a decreased EPG within one week of feeding a 90% *L. cuneata* containing ration to GIN infected kids, but referred to a delayed reduction in EPG reported on in several other similar studies referenced. Burke *et al.* (2011) did a *L. cuneata* dose titration study on lambs. *L. cuneata* meal was fed to *H. contortus* infected lambs in diets containing respectively 0, 25, 50 and 75% *Lespedeza* and concluded that FEC and PCV were not influenced by the *L. cuneata* fraction in the diet.

The lasting anthelmintic effects on GIN, after cessation of the CT-containing feed, are unclear. Heckeldoorn (2007) reported a sustainability reduction in FEC. Other studies showed that reduction in EPG disappeared when CT-containing feeding was stopped (Min *et al.,* 2004; Lange *et al.,* 2006). Athanasiadou *et al.* (2000) suggested that CT only temporarily reduced female worm fecundity.

CT content, as discussed earlier, plays a cardinal role in the anthelmintic properties shown by tanniferous forages. In many studies, the CT content is not referred to. In a study where sheep were dosed with wattle tannins at the rate of 0.08 and 1.6 g tannins $kg^{-1}$ bodyweight of the sheep with varying frequencies, the differences between tannin levels and frequencies of dosing varied ($P<0.05$) over time, but EPG consistently decreased with increased tannin level and frequency of dosing (Ahmed, 2010).

The effects of CT-containing forages on GIN are not fully understood and can be either through direct effects on the parasites themselves, or mediated through improved nutrition, as has been discussed in Chapter 3, Paragraph 3.2.6. Min and Hart (2003) showed that with higher CT levels, results in FEC reduction became variable (Table 6.4). Also, anthelmintic results reported on goats, cannot be directly applied to sheep, since sheep and goats differed regarding the reaction to GIN infections (Hoste *et al.,* 2008).

## 6.6 Conclusion

The results from this study, although some values did not differ significantly, indicated that dried *L. cuneata* leaves can reduce the GIN infestation in sheep. This finding is supported by several scientific studies. *L. cuneata*, therefore, can play a major role in reducing the contamination of pastures with ineffective larvae, and result in a reduced need for anthelmintics. The possibility of using the hay as dewormer offers exciting possibilities, for example:

- Post-weaned lambs, which are highly susceptible to GIN infection, can be supplemented on veld with *Lespedeza* hay in early summer, when helminth activity commenced to mitigate the summer's first parasite challenges (See Chapter 4 (Paragraph 4.5.2).
- *L. cuneata* hay can be offered to livestock, grazing pastures, to reduce the FEC infection in sheep and as secondary effect, the contamination of pastures with GIN.

# CHAPTER 7

# The efficacy of acetone leaf extracts of *Lespedeza cuneata* on egg hatching of *Haemonchus contortus: in vitro* studies

## 7.1 Aim

To determine the efficacy of leaf extracts of *Lespedeza cuneata*, containing different tannin levels, on the egg hatching of *Haemonchus contortus*.

## 7.2 Introduction

Due to successive reports of resistance covering all generic classes of anthelmintics that have been done worldwide, including South Africa (Van Wyk and Gerber, 1980; Van Wyk and Malan, 1988), a need exists to find effective alternatives for the control of GIN. Plants containing anthelmintic properties, including tanniferous plants, such as *L. cuneata,* are some of the most promising alternatives for GIN control (Waller *et al*., 2001; Gillian *et al*., 2004).

## 7.3 Literature review

In order to enhance extraction of all biologically active substances in plant material, different solvents with different polarities can be used, e.g. water used in the preparation of traditional medicine (Sparg *et al.*, 2002). Many other studies used acetone (70%) and ethanol, as well as other solvents (Kandu-Lelo, 2009; Ademola and Eloff, 2011b). Acetone has been shown to be a good extractant of metabolites in plants, due to its miscibility with polar and non-polar solvents (Eloff, 1998).

Investigation into the extracts of *L. cuneata* has shown that ethanol extracts had good larvical inhibition, even at a 10% concentration (Ahmed, 2012). However, no mentioning was made of the tannin levels of plant materials used in the experiments, nor the effect on the viability of eggs and free-living larvae stages.

Differences in the tannin levels of leaves and stems exist and are also reported on in Chapter 5. The anthelmintic properties of tanniferous plants have been discussed earlier in the dissertation (Chapter 3, Paragraph 3.2.6. and Chapter 5, Paragraph 5.3.5). Sheep selected the leaves and conceivably soft tips of stems when grazing, ignoring the fibrous stems [Personal observation (E van Zyl, 2012-2014); Figure 5.11]. Therefore, it was decided that the properties of leaves are more important than those of the stems.

## 7.4 Materials and methods

### *7.4.1 Experimental site*

The *in vitro* work was done at the Faculty of Veterinary Sciences University of Pretoria, Onderstepoort (Phytomedicine Programme, Department of Paraclinical Sciences).

### *7.4.2 Plant collection and processing*

In 2014 plant material from *L. cuneata* was collected by hand during an agronomic evaluation; reported on in Chapter 5 of this dissertation. The plants were dried in-house under dry conditions at room temperature. After drying, leaves were stripped from their stems by hand and were then sorted into stems and leaf portions. The material was stored in brown paper bags, away from direct sunlight until analysis. Prior to analysis, leaves were ground to a fine powder in a Macsalab Mill (Model 200 LAB Eriez®) and then stored in dark bottles until used.

### *7.4.3 Plant extraction*

A standard procedure was followed for leaf extractions (Eloff, 1998; Bizimenyera *et al*., 2006; Kandu-Lelo, 2009; Ademola *et al*., 2011a, b; Van Wyk C (2012). Leaf materials with predetermined CT levels were used in the assays, namely samples with condensed tannin (CT) levels of 75.3, 88, 102 and 122 g $kg^{-1}$ DM.

Acetone (70%) was used as extractant. Three grams of powdered plant material of each sample was extracted with 30 ml of the acetone (10:1ml $g^{-1}$ solvent to dry weight ratio), vigorously shaken for 30 minutes (Labotec Model 20.2 shaker),

sonicated for 30 minutes and shaken again for 30 minutes. The extracts were then centrifuged at 4000 rpm for 15 minutes. The supernatant was retained and the sediment was suspended in 10 ml of acetone, shaken for 30 minutes, centrifuged and was then mixed with the first batch of supernatant. Honey jars were weighed and the extract was poured into the honey jars and was then dried in a flow of cold air in a fume cupboard. After drying, the bottles were weighed again to determine the yield.

### *7.4.4 Nematode egg recovery*

According to a method originally described by Hubert and Kerboeuf (1992), *H. contortus* eggs were recovered from faeces. A sample of faeces (10 g to 15 g) was collected from donor sheep that was previously infected with larvae of *H. contortus*. The faecal pellets were mashed and suspended in water and the slurry was then washed through a series of sieves with meshes of decreasing aperture sizes. The filtration through the 150 µm sieve cleared the slurry from much of the plant residues. Filtration through both 63 µm and 20 µm sieves followed.

The eggs collected on the 20 µm sieve and smaller were cleared of organic debris by backwashing the material with 40% sucrose off of the sieve, for transferring into centrifuge tubes. The tubes were centrifuged for 5 minutes at 1000 rpm to separate the floating eggs from debris that precipitated. The supernatant was decanted again onto a series of sieves, with meshes of decreasing aperture sizes up to the 20 µm sieve, to collect the eggs and then transfer them to a 50 ml tube.

The concentration of eggs in the suspension was estimated by counting the number of eggs in a 200 µl sample and then the concentration was adjusted to give a final concentration of 100 eggs per 200 µl. To avoid proliferation of fungi, 5 µg amphotericin B solution (Sigma, Germany) was added per millilitre of suspension.

### *7.4.5 Egg hatch assay*

A standard procedure used by Ademola and Eloff (2011a, b), originally described by Coles *et al.* (1992), was used for this assay. An egg suspension (0.2 ml), containing

approximately 100 fresh eggs, was allocated in each well of a 24-flat-bottomed microtitre plate. The same volume of plant extracts, dissolved in dimethyl sulfoxide (5% DMSO), at concentrations of 0.63 to 20 mg $ml^{-1}$ in six serial dilutions, were added to the egg suspension. In all cases, eggs were subjected to a final 2.5% DMSO, due to the mixing with the same volume of aqueous suspension. Albendazole (Valbazen Ultra Albendasole 7.6% m $v^{-1}$, Phizer) was used as a positive control. Albendazole was dissolved in dimethyl sulfoxide (5% DMSO) and evaluated at concentrations between 0.78 and 25 µg $ml^{-1}$. Phosphate buffered saline (PBS) and 5% DMSO were used as negative control. All results were compared with PBS (Phosphate buffered saline, 10% solution) as the negative control.

Eggs were incubated in this mixture for 48 h at 27°C and 70% relative humidity. After incubation, a drop of Lugol's iodine solution (Reidel de Hae, Germany) was added to stop the unhatched eggs from hatching. All the eggs and first-stage larvae ($L_1$) in each plate were counted under an inverted microscope (see Addendum A for images of eggs and larvae). The test was replicated three times for each concentration.

### *7.4.6 Statistical analysis and calculations*

The data on the egg hatch assay of different CT containing extracts of *L. cuneata* were analysed by using Repeated Measures Analysis of Variance. Fisher's test of least significant differences (LSD) was conducted at a 5% significance level.

To calculate the percentage of egg hatch inhibition, the following formula was used (Bizimenyera *et al.,* 2006):

$$\text{Egg hatch inhibition \%} = 100\ (1 - P_{test}/P_{control}) \quad \text{where}$$

$P_{test}$ is the number of eggs hatched (or larval forms in the EH assay) in the test extracts and $P_{control}$ is the respective numbers in the negative control.

## 7.5 Results and discussion

### *7.5.1 Yields of extracts*

The yields and Condensed Tannin (CT) levels of the extracts are displayed in Table 7.1.

**Table 7.1: The Condensed tannin levels and yields of different acetone extracts of *L. cuneata* used in the study.**

| Extracts | CT content (g $kg^{-1}$ DM) | Extractant Yield (g) |
|---|---|---|
| UT (Ultra highest CT) | 122 | 6.33 |
| HT (High CT) | 102 | 7.88 |
| MT (Medium CT) | 88 | 5.66 |
| LT (Lowest CT) | 73.5 | 5.89 |

### *7.5.2 Egg hatch assay*

The hatched larvae and unhatched eggs in the microtitire plates were counted to calculate the egg hatch inhibition (EHI) %. An adaptation to the method described was made as follows: A grid pattern was drawn on the bottom of the microtitire plates with a dissection needle, which simplified and increased the accuracy of counting of eggs and larvae in the wells. Adding the Lugol's iodine, besides from stopping egg hatching, inactivate larvae. To have the opportunity to distinguish between live and moribund larvae, the Lugol's iodine was not added. This again assisted in the accuracy of counting, since debris in the extracts complicated counting. Plates were all counted within 3 h following incubation. The results are displayed in Figure 7.1 and Table 7.2.

The percentage inhibition of eggs hatched in the negative control was 5% ± 2.21 and in DMSO 5.7% ± 2.97. The positive control showed complete inhibition of egg hatching.

EHI increased significantly ($P< 0.001$) with an increase in concentration of all four different extracts of *L. cuneata*. At concentrations 20 to 5 mg $ml^{-1}$ all extracts

displayed dose-response profiles. For lower concentrations, except for the lowest concentration, the UT still followed the dose-response profile, but the other extracts varied.

At the highest concentration, all four extracts were completely ovicidal and larvicidal. No $L_1$ live larvae or eggs were observed in the wells incubated at 20 mg $ml^{-1}$; the extracts caused complete lyses of the eggs and larval ($L_1$) forms. At the second highest concentration, 10 mg $ml^{-1}$, an EHI of 97% was measured in all four extracts; in all the extracts a few live larvae with low activity were observed per well.

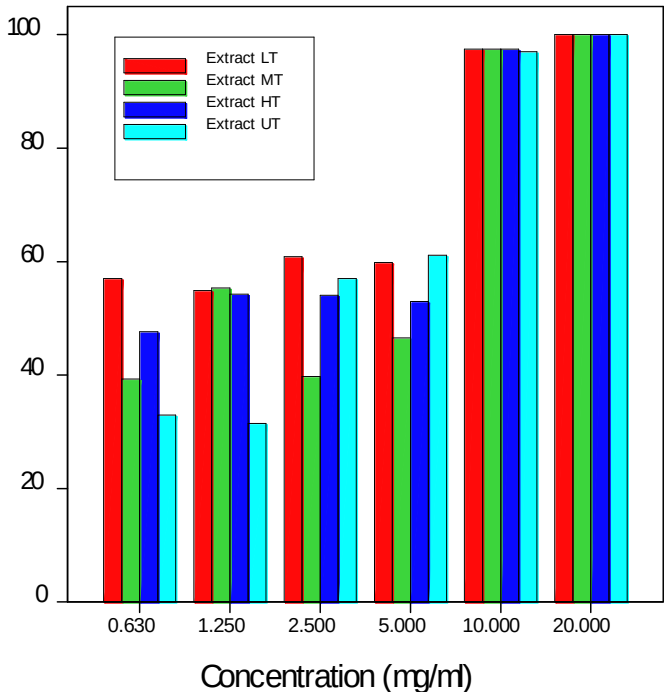


**Figure 7.1: Mean inhibition of *H. contortus* eggs hatching (%) by four different Condensed tannin containing leaf extracts in different concentrations (mg $ml^{-1}$)of *L. cuneata***
(UT: Ultra high CT; HT: High CT; MT: Medium CT and LT: Low CT)

A sharp decrease in EHI% was measured between the 10 mg $ml^{-1}$ and lower concentrations. At the 5 $mg^{-1}$ ml concentration, EHI% decreased to 61% and lower. An EHI of 61% was observed in the UH extract, compared to 59.83%, 52.99% and 46.48%, in the LT, HT and MT extracts, respectively. No significant differences ($P > 0.05$) existed between the EHI% of UT, HT and LT extracts, but these extracts all gave significantly ($P<0.05$) higher EHI% than the MT extract. Larvae that showed no activity

were classified as moribund larvae and the MT concentration was characterized by the presence of moribund larvae. The number of these moribund larvae in the UT and HT exceeded the number of live larvae. The lowest presence of moribund larvae was found in the MT concentration.

At the 2.5 mg ml$^{-1}$ concentration, all the extracts still gave an egg inhibition of over 50%, except the MT extract. At lower concentrations, results were more varied. The UT extract, other than expected, gave the lowest EHI% in the 1.25 and 0.63 mg ml $^{-1}$ concentrations, whereas the LT gave the best EHI%.

**Table 7.2: Egg hatch assay of different Condensed tannin level containing extracts of *L. cuneata* against *H. contortus*.**

| Concentration (C) (mg ml$^{-1}$) | EHI% of extracts of *L. cuneata (E)* | | | | Means of C |
|---|---|---|---|---|---|
| | UT | HT | MT | LT | |
| 0.63 | 33.0 | 47.7 | 39.3 | 57.1 | **44.3$^{a}$** |
| 1.25 | 31.5 | 54.3 | 55.3 | 54.9 | **49.0$^{ab}$** |
| 2.5 | 57.0 | 54.1 | 39.7 | 60.9 | **52.9$^{b}$** |
| 5.0 | 61.1 | 53.0 | 46.6 | 59.8 | **55.1$^{b}$** |
| 10 | 97.0 | 97.4 | 97.4 | 97.4 | **97.3$^{c}$** |
| 20 | 100.0 | 100.0 | 100.0 | 100.0 | **100.0$^{c}$** |
| **Means of E** | **63.3$^{a}$** | **67.7$^{a}$** | **63.1$^{ab}$** | **71.7$^{b}$** | |
| CV % | | 11.8 % | | | |
| LSD: Concentration (C): 6.453 (P< 0.001); Extracts (E): 5.269 (P< 0.05); C x E interaction: 12.906 (P< 0.05) | | | | | |

*CV% = Coefficient of variation; LSD=Least significant difference;*
*Values with different superscripts within rows or columns differ significantly*

It was expected that the highest CT containing extract would show the highest EHI. There were however no differences (P>0.05) between 73.5 and 122 g kg$^{-1}$DM CT content. Min and Hart (2003) compared the results of several corresponding studies of the effect of CT levels on FEC reduction (Chapter 6, Figure 6.3). When CT levels

increase above 55 g $kg^{-1}$ DM and decrease below 45 g $kg^{-1}$ DM the FEC responses become variable. The variability in EHI% in lower concentrations was also observed in studies on other plants (Hounzangbe-Adote *et al.,* 2005; Bizimenyera *et al*., 2006).

The anthelmintic properties of tanniferous plants are not restricted to EHI, but also affect the infective third stage $L_3$ larvae. Both *in vivo* and *in vitro* studies showed that the first stages of infection were negatively affected by tannins (Paolini *et al*., 2005 Bahaud *et al.,* 2006), which will further contribute to the control of GIN.

Because there were no differences (P> 0.05) between the 73.5 and 122 g $kg^{-1}$ DM CT content as far as EHI is concerned, it is possible that the control by tannin content is not related to egg hatching, but possibly to larval development of a direct toxic effect on other stages on the infection. Depending on the type of tannin, acetone may not readily extract tannins and this could explain the lack of differences. Further studies are needed for clarification of this point.

## 7.6 Conclusion

The aim of this study was to determine the efficacy of leaf extractions of *L. cuneata*, containing different tannin levels, on the egg hatching of *Haemonchus contortus*. It was found the extracts were all ovicidal and larvicidal at the highest concentration 20 mg/ml tested. No substantial correlation between CT level and EHI% was found.

The ovicidal and larvicidal action of *L. cuneata* extracts observed in this study is of particular relevance since the intention is to use this plant as forage for grazing livestock. In pasture cultivation, environmental conditions, such as drought, affects the CT content of the plant. However, no significant (P>0.05) differences in EHI% existed at the two highest concentrations, 20 and 10 mg $ml^{-1}$ and at the third highest concentration, 5 mg $ml^{-1}$, only the MT was significantly (P<0.05) lower. To clarify the result, a broader range of CT levels needed to be tested, starting at lower levels of CT.

The results, however, clearly show that extracts of *L. cuneata* had a substantial inhibitory effect on *H. contortus* egg hatching, at concentrations of 20 and 10 mg ml$^{-1}$. Even at the lowest concentration, EHI% from the LT extract still resulted in a 57.1% inhibition. Therefore, if the effects shown *in vitro* could apply *in vivo,* administration of the extracts to animals infected with the adult worms would be followed by a reduction in FEC and therefore lowered pasture contamination. This will result in a reduced need for anthelmintics. To a certain extent, this statement is supported by the findings in Chapter 6 of this dissertation.

# CHAPTER 8

## Thesis overview and discussion

The existence of livestock is closely bound to that of parasites (Villalba *et al.,* 2014). Increased concentration of livestock and grazing on more mono-culture forages had enhanced parasite populations to such a level that livestock production has, to a large extent, become dependent on anthelmintic chemotherapy. Increased public awareness of chemical drug residues in agricultural products, together with the increasing development of resistant strains of parasites, has enforced the search for sustainable alternative methods to complement or replace anthelmintics. More and more calls are made for a more holistic management solution. Recent studies on breeding of resistant livestock and the use of bio-active forages highlighted the potential of these to contribute towards holistic parasite control.

The first part of this study examined the parasite challenge in the sheep flock at Dundee Research Station in terms of gastrointestinal nematode (GIN) resistance to standard anthelmintics and resistance to GIN. In spring, naïve, weaned lambs on marginal feeding conditions, have a high risk to be subjected to an installation of parasite infection, which escalated sharply over a short period. To be able to cope as successfully as possible with the parasitism and develop their immunity, lambs need supportive feeding and timely treatment. Therefore, farmers should be alerted to the effect of weather conditions to support naïve lambs successfully through such a risk period and to cut losses.

The FECRT was used to identify the effective chemotherapeutic products for GIN treatment in the sheep flock at Dundee Research Station. Anthelmintic resistance in the flock was quantified and related to the resistance level some years prior to the experiment. Complete resistance to GIN can be the ultimate solution, but is generally ignored as a commercial reality (Saddiq *et al*., 2011). Out of the initial thirty–nine sheep, seven (18%) showed continued light infestation, indicating resistance to GIN infection. Hoste *et al*., (2006) stated that across a flock, parasites aggregate within a few individuals while some other acquired a degree of GIN resistance.

The second part of the study was dedicated to investigate, according to literature, a promising plant identified as a tanniferous bio-active forage, namely *Lespedeza cuneata*. The pasture is relative well-known, but not widely cultivated in South Africa due to a perception of poor animal production on *L. cuneata*. Small plot trials were conducted to established DM yield under local environmental conditions and to investigate the forage quality, tannin levels and changes in tannin levels during the growing season. DM yields were variable, but relatively promising, given the soil quality and dry land conditions. The first season was abnormally wet, and yielded 8.3 t DM $ha^{-1}$ (grand mean yield). The next season had a poor rainfall distribution and below normal rainfall and yielded 2.56 t DM $ha^{-1}$. To mitigate the effect of drought, the choice of soil type is important. Growing season is short and regrowth ceased late in March.

Mature herbage showed an unfavourable leaf: stem ratio, resulting in very coarse hay. Nutritional value and other chemical characteristics of the complete plant were low, except crude protein; the latter being between 10 and 18%. Acid detergent fibre (ADF) levels were above 45%, with few exceptions. Neutral detergent fibre (NDF) levels were in most cases above 55%, and even rose up to 60+%. In recognition of the selective grazing habits of sheep in *L. cuneata*, the leaves were analysed separately. The quality of the leaf material was within an acceptable range and therefore theoretically should not compromise animal performance.

Samples, separated into leaf and stem parts, were analysed for condensed tannins (CT). The results showed that CT levels increased under moisture-stress conditions, predominantly in the leaves. The CT content in leaves, targeted during defoliation, can have major influences on animal production.

Therefore, although *L. cuneata* appears to have a good potential based on high yields and adaptability to low potential soils, its actual feeding value may be substantially lower than expected during certain times, such as drought, due to the high levels of CT.

In the third part of the study, the effect of *L. cuneata* leaf hay on an established gastrointestinal parasite infection in sheep was examined. Merino ewes, in

confinement, were fed for 235 days, either a ration consisting of *L. cuneata* hay (leaf fraction) or a *Medicago sativa*-hay ration with known zero anthelmintic properties, as control. The level of faecal egg count (FEC) in the sheep showed a significantly FEC reduction on Day 35, ($P<0.05$), with FEC levels constantly lower in the Lespedeza group, compared to the control. Rectal temperatures tended to correlate with the FEC, but need further investigation.

The aim of the last part of this study was to determine the efficacy of leaf extractions of *L. cuneata*, containing different tannin levels (73.5, 88,102 and 122 g CT $kg^{-1}$ DM), on the egg-hatching efficacy of *Haemonchus contortus*. It was found that all the extracts were ovicidal and larvicidal at the highest concentration namely 20 mg $ml^{-1}$. No significant ($P>0.05$) differences existed between the extracts in concentrations of 20 and 10 mg $ml^{-1}$. In the concentration, 5 mg $ml^{-1}$, only the 88 g CT $kg^{-1}$ DM containing extract, showed a significantly ($P< 0.05$) lower EHI%. No substantial trend was found between the CT level and the EHI%.

## 8.1 Conclusions

- The FECRT is invaluable to identify effective chemotherapeutic products for GIN management at farm level. By excluding blanket treatment and identifying resistant individual animals (18% in the Dundee Research Station flock) in a flock, the use of anthelmintics can be decreased.

- *L. cuneata* appears to have a good forage potential based on DM yields and CP, but ADF and NDF are marginal. Sheep selectively grazed *L. cuneata*; leaf analyses, therefore, are a more appropriate norm. These resulted in better values in CP, ADF and NDF. CT levels increased during moisture stress, especially in leaves, and reached unfavourable levels of above 100 g $kg^{-1}$ DM.

- Feeding a ration containing *L. cuneata* hay (leaf parts only), resulted in FEC reduction in sheep, compared to the FEC of sheep on a *Medicago* ration. This difference was statistically significant ($P<0.05$) only after 35 Days. These results ere of practical importance, since it indicated that alternative GIN treatment

was unnecessary for the *Lespedeza* group at termination of the trial, but needed for the *Medicago* group.

- Acetone extracts of *L. cuneata*, containing respectively 73.5, 88,102 1nd 122 g CT $kg^{-1}$ DM, were all ovicidal and larvicidal at a concentration of 20 mg/ml. No significant (P> 0.05) differences existed between egg hatch inhibition (EHI)% in the 20 and 10mg/ml concentration. In the 5 mg/ml concentration, only the MT was significantly (P<0.05) lower, compared to other extracts. No substantial trend was found between the CT level and the EHI%.

## 8.2 Recommendation

*L. cuneata* has a tremendous advantage over many other plants with anthelmintic properties, because it can be directly used on farm to be consumed by animals as grazing (or in hay form), since it is already established as a planted pasture with commercially available seed. With its many other agronomic advantages, including that it is relatively drought resistant, the inclusion of *L. cuneata* as bio-active forage, can play an invaluable role in a holistic GIN control programme. Except for providing forage, strategically it can be offered to animals when a GIN challenge is expected, such as in young sheep. When weather turns favourable for GIN infection it can curb the installation of a GIN infection and reduce an infection or result in reduced FEC to minimise pasture contamination. Incorporation of *L. cuneata* in a fodder flow, supported with strategies such as selecting resistant animals in a flock for breeding material and culling susceptible animals, holds exciting possibilities to use it as an alternative strategy for chemotherapy.

Several new and exciting parasite-detecting methods, (*Haemonchus* dipstick test, Flotec apparatus and lectin staining for identification of *Haemonchus*) are in use, even at farm level, in countries such as New Zealand and Australia. South Africa lags behind on these and the implementation of these methods must be addressed in the battle against GIN.

## 8.3 Further research

- Breeding with identified GIN resistant animals, needs further investigation.

- The level of anthelmintic properties of *L. cuneata* needs further verification, either as dried product, or as green grazing.

- Management strategies to optimise quality challenges in *L. cuneata*, but still contain the CT levels for anthelmintic properties, need to be developed. In this regard a near infra-red (NIR) spectroscopy offers an opportunity to determine quality instantly and at affordable cost to the farmer.

- The use of pelleted *L. cuneata* as deworming agent is an exciting possibility and needs to be investigated further.

- Clarification of the effect of CT on *H. contortus* EHI and larval inhibition with extracts containing a broader range of CT levels need to be tested, starting at lower levels of CT, than the levels tested in this study. The effect on more parasite species also needs further investigation.

# CHAPTER 9

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# Addendum A

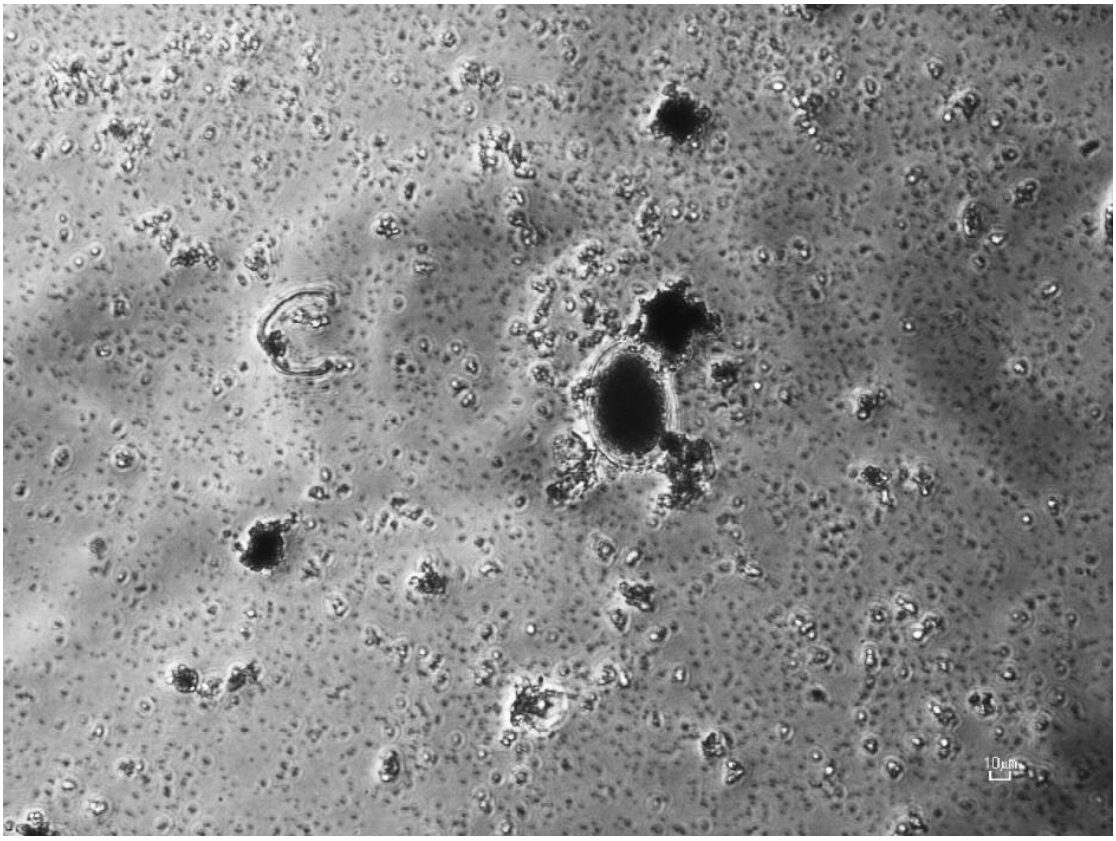
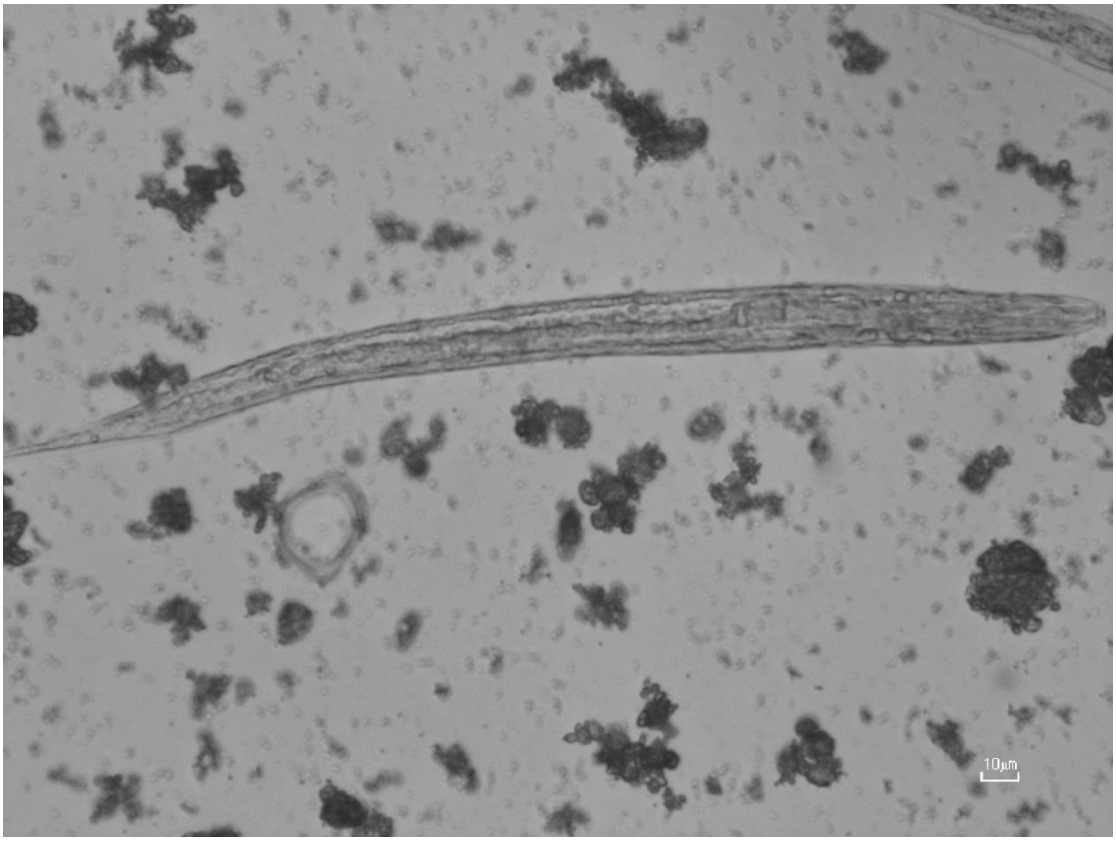
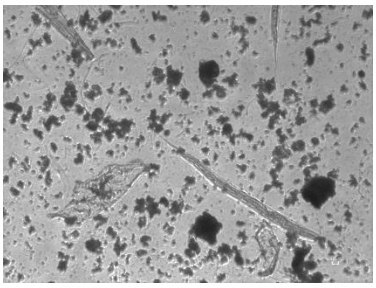
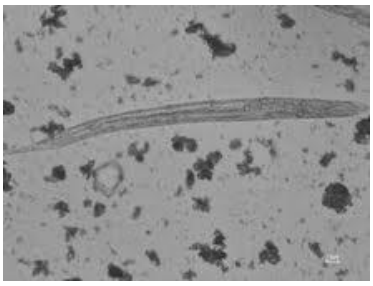
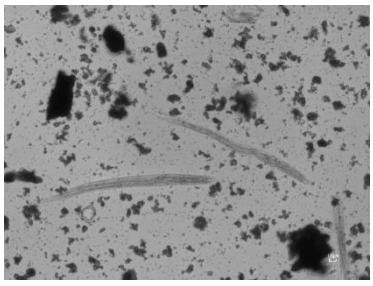



Figure A.1: Unhatched *Haemonchus contortus* egg fixated with Lugol's Iodine







Figures A.2: Hatched *Haemonchus contortus* stage $L_1$ larvae fixated with Lugol's Iodine