Calliandra calothyrsus and *Arachis pintoi* supplementation effects on animal health and gastrointestinal nematodes infestation and condensed tannin extract effects on (*Haemonchus contortus*) larval motility

By
Karla A. Hernández Valentín

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
ANIMAL SCIENCE

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2007

Approved by:

Paul F. Randel Følling, Ph.D. Member, Graduate Committee	Date
Abner A. Rodríguez Carías, Ph.D. Member, Graduate Committee	Date
Rafael Ramos Santana, M.S. Member, Graduate Committee	Date
Elide Valencia Chin, Ph.D. President, Graduate Committee	Date
Lucy Bunkley Williams, Ph.D. Representative of Graduate Studies	Date
José R. Latorre Acevedo, Ph.D Chairperson of Animal Industry Department	Date

ABSTRACT

Experimentation, *in vivo* and *in vitro*, was conducted to determine the effects of condensed tannins (CT) in various tropical legumes and to evaluate their use as a means to reduce internal parasite infestation in goats. In Exp. 1, fresh leaves of Calliandra (*Calliandra calothyrsus)* and Arachis (*Arachis pintoi)* forages were used for dietary supplementation during 4 months and the results analyzed using repeated measures. Eight naturally parasite-infested, growing Boer goats, grazing *Panicum maximum* were randomly allocated, four to each of two supplemental forage treatments. Animals of one treatment with initial body weight (BW) = 18.8±0.5 kg received supplementation of freshly cut Arachis from September to December 2006; those of a second treatment 14.4±0.5 kg received Calliandra for 84 d. On day zero all animals were orally dewormed using the commercial anthelmintic *Ivomec*® (2mg/kg of BW). Fecal samples for determining egg counts and blood samples to determine packed cell volume (PCV) were collected every 21 days. Feces were obtained directly from the rectum and blood was collected in EDTA vaccutainer tubes via jugular venipuncture. Scoring for anemia by the FAMACHA method and BW measurements were also taken. Data were analyzed using the MIXED procedure of SAS and Tukey t-test for mean comparisons. The fecal egg counts (FEC) were log transformed prior to statistical analysis. As a part of this experiment *in vitro* dry matter digestibility (IVDMD) of the two supplemental forages was determined. Significant differences were detected between Calliandra and Arachis in content of dry matter (DM; 969.7 vs. 955.9 g/kg) and crude protein (CP; 18.4 vs. 14.9 g/kg), respectively. Calliandra showed lower IVDMD ($P < 0.001$) but higher CP and condensed tannins (CT; $P < 0.01$) than Arachis. Neutral detergent fiber (NDF)

percentages of 61.1 and 58.8 for Calliandra and Arachis, respectively, did not differ at $P = 0.05$. In Exp. 1, FEC (5262 vs. 7644 eggs/g; $P < 0.001$) and FAMACHA scores (2.5 vs. 2.9; $P < 0.02$) were lower, while average daily gain (ADG; 11.1 vs. -34.7 g; $P < 0.02$) and PCV (22.3 vs. 20.5; $P = 0.13$) were greater for Calliandra than for Arachis.

In Exp. 2, three purified tannins from Calliandra, Lespedeza (*Sericea lespedeza*), Prairie acacia (*Acacia angustissima* var. *hirta*), and three commercially available tannin monomers (ellagitannins, gallotannins and catechins) were used *in vitro* to determine their effect on larval migration inhibition rates (% LMI) of infective third-stage larvae of *Haemonchus contortus*, using a Sephadex LH-20 column. Calliandra was chosen to include in the *in vitro* study because of its anthelmintic activity (12.04% CT) measured in Exp.1, while Lespedeza and Acacia tannins were included because of their known anthelmintic activity.

In the larval migration assay (Exp. 2), LMI rates of *H. contortus* increased in the presence of 2 and 4 mg of purified tannin/ml as Lespedeza and Calliandra extracts. Acacia extract had no dose-related effect on LMI rates. *H. contortus* LMI exhibited a dose dependent response ($P < 0.01$) to 1, 2 and 4 mg/ml of ellagitannins and gallotannins. Ranking of monomers according to their inhibitory activity was: ellagitannins > gallotannins > catechins.

RESUMEN

Se realizó experimentación, *in vivo* e *in vitro*, para determinar los efectos de los taninos condensados (TC) en varias leguminosas y evaluar su uso como un método para reducir la infestación con parásitos internos en los cabros. En Exp. 1, se usaron hojas frescas de Calliandra (*Calliandra calothyrsus)* y Arachis (*Arachis pintoi)* como suplementos dietéticos durante 4 meses y se analizaron los resultados usando un análisis de medidas repetidas. Ocho cabros de la raza Boer en crecimiento, infectados naturalmente con parásitos y que pastaron *Panicum maximum*, fueron asignados aleatoriamente, cuatro a cada uno de dos tratamientos basados en sendas forrajeras suplementarias. Los animales del primer tratamiento, de peso vivo inicial (PV) = 18.8±0.5 kg, recibieron suplementación con forraje fresco de corte de *Arachis pintoi*; los del segundo tratamiento (PV) = 14.4±0.5 kg recibieron Calliandra. Se aplicaron ambos tratamientos durante 84 días desde Septiembre a Diciembre de 2006. El día cero, se desparasitaron todos los animales oralmente utilizando el desparasitante comercial *Ivomec*® (2mg/kg de peso vivo). Se recogieron muestras de heces fecales para el conteo de huevos, y muestras de sangre para determinar el volumen de células compactada (PCV) cada 21 días. Se obtuvieron las heces directamente del recto, mientras la sangre fue recogida utilizando tubos con solución EDTA, vía la vena yugular. También se efectuó la evaluación FAMACHA para el grado de anemia y el pesaje de los animales. Los datos fueron analizados utilizando el procedimiento MIXED del paquete estadístico SAS y el ensayo "t" de Tukey para la comparación de medias. Para el conteo de huevos en las heces (CHH), se transformaron los datos a forma logarítmica, previo a los análisis estadísticos.

Como parte de este experimento se determinó la digestibilidad *in vitro* en base seca (DIVBS) de los dos forrajes tropicales suplementarios. Se detectaron diferencias significativas entre Calliandra y Arachis en contenido de materia seca (MS; 969.7 vs. 955.9 g/kg) y proteína bruta (PB; 18.4 vs. 14.9 g/kg), respectivamente. La Calliandra mostró una DIVBS menor ($P < 0.001$) pero contenidos mayores de PB y TC ($P < 0.01$) que Arachis. En el porcentaje de fibra detergente neutro (FDN), de 61.1 y 58.8 para Calliandra y Arachis, respectivamente, no hubo diferencia a $P = 0.05$.

En Exp. 1, el CHH (5262 vs. 7644 huevos/g; $P < 0.001$), y los valores para FAMACHA (2.5 vs. 2.9; $P < 0.02$) resultaron ser menores, mientras la ganancia diaria media de peso 11.1 vs. -34.7 g; $P < 0.02$ y el PCV (22.3 vs. 20.5; $P = 0.13$) fueron mayores para Calliandra que para Arachis.

En el Exp. 2, se ensayaron *in vitro* tres taninos purificados provenientes de Calliandra, Lespedeza (*Sericea lespedeza*) y Prairie acacia (*Acacia angustissima* var. *hirta*) y tres monómeros de taninos disponibles comercialmente (ellagitannins, gallotannins y catechins), para determinar su efecto sobre la tasa de inhibición de la migración de la larva (% IML), en la tercera etapa infectiva de *Haemonchus contortus,* mediante uso de una columna Sephadex LH-20. Se escogió a Calliandra para incluir en el estudio *in vitro*, por su actividad antihelmíntica (12.04% TC), medido en el Exp.1, mientras, taninos de Lespedeza y Acacia fueron incluidos por su también conocida actividad antihelmíntica.

En relación al ensayo de la migración de la larva (Exp. 2), la tasa de LMI de *H. contortus* aumentó en presencia de 2 y 4 mg de tanino purificado/ml, procedente de extractos de Lespedeza y Calliandra. El extracto de Acacia, no tuvo efecto dependiente de la dosis en

la tasa de IML de *H. contortus*. La IML de *H. contortus* exhibió una respuesta dependiente de la dosis ($P < 0.01$) para 1, 2 y 4 mg/ml de los monómeros de taninos ellagitannins y gallotannins. El orden de los monómeros según su actividad inhibitoria de mayor a menor fue: ellagitannins > gallotannins > catechins.

DEDICATION

God, who gives me the life, knowledge and patience to continue and never gave up.

Cecilia Valentín Ruíz, Carlos A. Hernández Martínez and Carlos A. Hernández Valentín for supporting me in this project and in my entire life.

Luis Fernando Hernández Martínez, Fidela Martínez, Fernando Hernández Laguna and María Aponte.

Thanks for being part of my life and for giving me all the tools to make it possible.

ACKNOWLEDGEMENTS

During these two and a half years as a graduate student of the University of Puerto Rico, Mayagüez Campus, many persons collaborated with the development of this research work. Their support was essential and without them it would have been hard to finish. I want to recognized their help, time and support. They all know that they have an important place in my heart and in my successful career as a professional.

Dr. Elide Valencia Chin, for the challenge and the opportunity to work in this project.

Dr. Abner Rodríguez Carías for his help in this project.

Dr. Paul F. Randel Følling for being part of this project.

Dr. Byeng Ryel Min at Texas A &M experiment station in Vernon, TX, for being a real guide during this year 2007. Thanks for all your help, support during this time. You are such a special person in my life.

Dr. William E. Pinchak who gave me the opportunity to work for him during the months May - August 2007.

Dr. Lucy Bunkley Williams for all her collaboration on this project.

Mr. Rafael Ramos Santana for all his collaboration on this project.

Margarita Olivencia for being my support in hard moments and giving me the most beautiful smile to survive when nobody believed in my potential as a graduate student.

Jacqueline Rivera for all her support and collaboration.

Ruth Amanda Acero Camelo, who helped me in the experimental phase of my thesis and who was a real friend.

To my friends, Suzika Pagán Riestra, Jonael H. Bosques, Verónica Díaz Carcache, Bayrex M. Rosa, Orlisa Crespo Rodríguez, Ricardo Prieto, Bismarck Sandoval and Jason Rivera Cruz.

Small Ruminant Project personnel at the Agricultural Experiment Station at Mayagüez Campus. Especially Don "Cheo" and José Ariel Muñoz Rivera.

Daliannisse (“Dalia”) González and Germán Valentín for their weekly help in the experimental phase of this project. Thanks for being my students in the Veterinary Physiology Laboratory (2006).

My Texas mom: Sherry Lawrence for being the most important person during all those eight months in Texas. Thanks for all your support.

Diane Conover… simply thanks… I will never forget all your help.

Finally, but not less important, I want to give thanks to a special person; my lovely buddy; Ed Rhodes. Thanks for being there, for all your support and your coolest personality that made me happy during all this time. I do not have words to describe you, because you are amazing. I am really glad to met you.

To all of you, who made this project possible.
¡THANKS!
God bless you all…

TABLE OF CONTENTS

Pages

List of Tables

List of Figures

List of Abbreviations

ADG	Average daily gain
BW	Body weight
CT	Condensed tannins
Cu	Copper
COWP	Copper oxide wire particles
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EAA	Essential amino acids
ECT	Extractable condensed tannins
epg	Eggs per gram feces
FCT	Fiber-bound condensed tannins
FEC	Fecal egg counts
GIN	Gastrointestinal nematodes
HT	Hydrolysable tannins
IVDMD	*In vitro* dry matter digestibility
L_3	Third stage infective larvae
LMI	Larval migration inhibition
Mo	Molybdenum
NDF	Neutral detergent fiber
NPAA	Non-protein amino acids
OM	Organic matter
P	Phosphorous
PCV	Packed cell volume
PCT	Protein-bound condensed tannins
SL	*Sericea lespedeza*
TCT	Total condensed tannins
v/v	volume/volume

List of Appendices

CHAPTER ONE

1.1. INTRODUCTION

Agriculture in Puerto Rico is undergoing great change. Of the traditional plantation crops from Spanish colonial times - sugarcane, coffee and tobacco, only coffee remains as a significant agricultural commodity. Today, sugarcane and tobacco have been replaced by the livestock industries, exotic fruit crops, and ornamental plants.

For livestock industries, the most common problem in small ruminant production pertains to the presence of intestinal parasites (e.g. *Haemonchus contortus* and *Trichostrongylus colubriformis*). Gastrointestinal parasite infections are characteristic of pastoral grazing systems and many internal parasite species have developed resistance to anthelmintic drugs (Kaplan, 2004). Infestation with internal parasites causes significant production losses, ranging from 13 to 33% in grazing ruminants, with the greatest reduction occurring in young, non-suckling ruminants, which typically have limited immunity to nematode parasites (Craig, 1988; Kaplan, 2004; Stuedemann et al., 2005). Sub-clinical intestinal parasite infections occasionally depress feed intake and animal production (death and weight loss), and can impair tissue deposition and skeletal growth (Parkins and Holmes, 1989). Alternatives to chemical anthelmintics are necessary in conventional livestock systems because of increasing parasite resistance to contemporary synthetic anthelmintics and, of course, are required in organic systems (Prichard, 1994; Kaplan, 2004). Therefore, research to develop alternative strategies for internal parasite control should be a high priority.

New methods to control internal parasites in small ruminants such as sheep and goats have been developed. The effects of condensed tannins (CT) contained in temperate forage species on internal parasite control in ruminants have been well documented (Min et al., 2003; Hoste et al., 2005; Githori et al., 2006). Feeding tannin-containing forages reduces parasite infestation directly, and by increasing the amount of by-pass protein, improves the nutritional status of the host animal, indirectly improving immune responses (Min et al., 2003, 2005).

New investigations are focused on establishing a direct effect of CT on helminth control. Some tropical legumes, such as *Leucaena leucocephala, Cratylia argentea* and *Calliandra calothyrsus*, have been used for their chemical composition and elevated nutritional value to improve animal feed intake and performance. However, the consumption of these forages can cause toxicity problems and reduction of palatability and digestibility with negative impacts on animal health and production (D'Mello and Devendra, 1995; Walton et al., 2001).

The working hypothesis of this research was that natural plant tannin extracts or tannin-containing tropical legumes can effectively control internal parasites and improve animal performance; and that exposure to purified CT and tannin monomers can have inhibitory effects on the larval migration activities of infective third-stage larvae of *Haemonchus contortus.*

1.2. OBJECTIVES

1. Determine effects on the growth and health of goats receiving condensed tannins (CT) in the tropical legumes *Calliandra calothyrsus* and *Arachis pintoi* and evaluate this alternative method to reduce internal parasitism.

2. Determine the effects of purified tannins extracted from three high tannin-containing forages and of three commercially available tannin monomers on the larval migration inhibition rates (% LMI) of infective third-stage larvae of *Haemonchus contortus*.

CHAPTER TWO

LITERATURE REVIEW

This review, of published literature summarizes data on alternative internal parasite control strategies in ruminant animals fed forage diets. Secondly, structure and reactivity with various other substances of forage CT are presented. Finally, detailed consideration is given to the relationship of CT and forage nutritive value, including effects on voluntary feed intake, nutrient digestion, rumen microbial activity, and animal production.

Ruminants grazing forages are subject to a number of disease and environmental impacts, some of which have nutritional and metabolic causes. Two major considerations are ruminal gas formation (bloat and combustible gas i.e. methane production) and parasite infection in grazing ruminants. Bloat is caused by high solubility of certain forage proteins leading to the development of stable foam in the rumen (Min et al., 2005, 2006). Parasitism of the abomasum and small intestine causes extensive protein losses in ruminants and is a significant economic burden to the animal industries in many countries. However, of greater importance is the development of parasite resistance to anthelmintic drenches (Prichard, 1994; Kaplan, 2004), which has been reported in sheep, goats and cattle; and the increasing concern about anthelmintic residues in animal products. Alternative non-chemical parasite control strategies have been developed recently based on CT-containing forages, in addition to grazing management, and use of nematophagous fungi, and copper oxide particles. These strategies may reduce the dependence on anthelmintic drugs as the sole method of controlling internal parasites.

Anthelmintic resistance has been described as the situation in which normal dosage of a drug does not promote a consistent reduction in number of parasitic worms and their excreted eggs. Anthelmintic resistance can be spread from one farm to another through purchase of animals and by grazing in pastures shared by flocks from several farms. An overuse or misuse of anthelmintics increases anthelmintic resistance of the nematodes in the gastrointestinal tract (GIN) of goats, sheep and cattle (Prichard, 1994).

Nematode resistance has been reported in different parts of the world. Frequent use of the same drug, under-dosing, lack of consideration of the size of the nematode population in refugia at the time of treatment, and poor pasture management are the principal causes of anthelmintic resistance.

Tables 1 and 2 present information on the taxonomic classification of helminth parasites and the major pathogenic gastrointestinal helminth parasites in domestic ruminants. Table 3 gives some detail on the main internal parasite genera affecting cattle, sheep and goats. Table 4 summarizes the number of references in the literature pertinent to this topic. The abomasum is the organ most commonly affected by parasites that decreased the feed efficiency and animal productivity.

Table 1. Taxonomic classification of helminth parasites.

Taxonomic classification	General characteristics	Reference
Kingdom: Animalia Phylum: Platyhelminthes Class: Trematoda, Cestoda	Flatworms, tapeworms, flukes; many free-living predatory forms, many parasites. Causes great economic losses in sheep and cattle.	Overend and Bowen, 1995.
Kingdom: Animalia Phylum: Nematoda Class: Adenophorea, Secernentea	Roundworms, common free-living, parasitic (e.g. *Haemonchus contortus*)	Lichtenfels et al. 1997.
Kingdom: Animalia Phylum: Protozoa Class: Cnidaria, Molusca and Arthropoda	The most abundant animals in the world in terms of numbers and biomass.	IVRI Annual reports (1986-1998).

Table 2. Major pathogenic gastrointestinal helminth parasites of domestic ruminants.

Location	Genera
Abomasum	*Haemonchus, Ostertagia, Trichostrongylus*
Small intestine	*Nematodirus, Trichostrongylus, Cooperia, Bunostonum*
Large intestine	*Oesophagostomum, Chabertia*
Liver	*Fasciola*

Source: James et al. (1989)

Table 3. Characteristics of the principal internal parasite genera in cattle, sheep and goats

Parasite	Description	Organ Infected	Life Cycle	Signs
Haemonchus	M: 10-20 mm red F:18-30 mm red and white	Abomasum	IS: 4-6 days PP: 3 weeks	Anemia, soft swelling under jaw and abdomen, weakness, no weight gain.
Ostertagia	M: 6-9 mm, brown F: 8-12 mm	Abomasum	IS: 4-6 days PP: 3 weeks	Same as *Haemonchus* and also lack of appetite and weight loss.
Trichostrongylus	M: 4-5.5 mm F: 5-7 mm, light brown	Abomasum, Duodenum	IS: 3-4 days PP: 2-3 weeks	Same as *Haemonchus* and also diarrhea and weight loss.
Cooperia	Red M: 5-7 mm F: 6-9 mm	Duodenum	IS: 5-6 days PP: 15-20 days	Same as *Haemonchus*.
Bunostomum	M: 10-30 mm	Duodenum	PP: 30-56 days	Edema, anemia, weight loss, diarrhea.
Strongyloides (young animals)	M: 4-6 mm	Small intestine	IS: 1-2 days PP: 8-14 days	Anorexia, enteritis and diarrhea.
Chabertia	M: 13-14 mm F: 17-20 mm	Large intestine	IS: 5-6 days PP: 42 days	Anemia, diarrhea with blood.
Oesophagostomum	M: 12-17 mm F: 15-22 mm	Large intestine	IS: 6-7 days PP: 41-45 days	Dark green diarrhea, edema.
Protostrongylus	M: 16-28 mm F: 25-35 mm	Lungs	IS: 12-14 days PP: 30-37 days	Pneumonia
Dictyocaulus	M: 30-80 mm F: 50-100 mm	Lungs	IS: 6-7 days PP: 3-4 weeks	Sticky nasal discharge, difficulty breathing and cough.

Source: Duval, 1997.

Legend: M = Male; F = Female; IS = Infectious stage: minimum number of days for parasite to reach infectious larvae stage (L3) after hatching of eggs; PP = Prepatent stage: period up to appearance of first eggs in dung after host is infected.

Table 4. Number of reported investigations on ruminants, their helminthes, use of anthelmintics, and resistance to these treatments.

Host species	Total of number of references	References on helminthes (%)	References on anthelmintics (%)	References on resistance to anthelmintics (%)
Goats	32555	2276 (7%)	601 (2%)	184 (0.06%)
Sheep	109759	11570 (11%)	3398 (3%)	745 (0.07%)
Cattle	230005	10778 (5%)	3370 (2%)	202 (0.01%)

Source: Cabaret, 2000.

Sheep and goats are more susceptible to internal parasites than other livestock, due to their grazing behavior and poor natural immunity (Cabaret, 2000). The anthelmintic resistance in goats is especially high. This might be due to introductions of goats from resistant to non-resistant areas. Of the studies published on different classes of caprines, 54% concerned dairy goats, 29% meat goats and 17% fiber goats. Resistance was reported mostly against benzimidazoles (Elard et al., 1999) and, secondarily, against levamisole (Robertson et al., 1999).

2.1. Biological cycle of nematodes

The stages of the direct life-cycle of many important gastrointestinal helminth parasites in small ruminants are summarized in Figure 1. Knowledge of the life cycle and characteristics of parasitic worms is essential for anyone wishing to reduce the use of dewormers. Although, Figure 1 shows only the direct life cycle common to most nematode parasites, some parasites have an indirect cycle (Digenea), which involves an

alternate host animal. For example, the liver fluke (*Fasciola* sp.) spends part of its life in certain snail species before infecting ruminants.

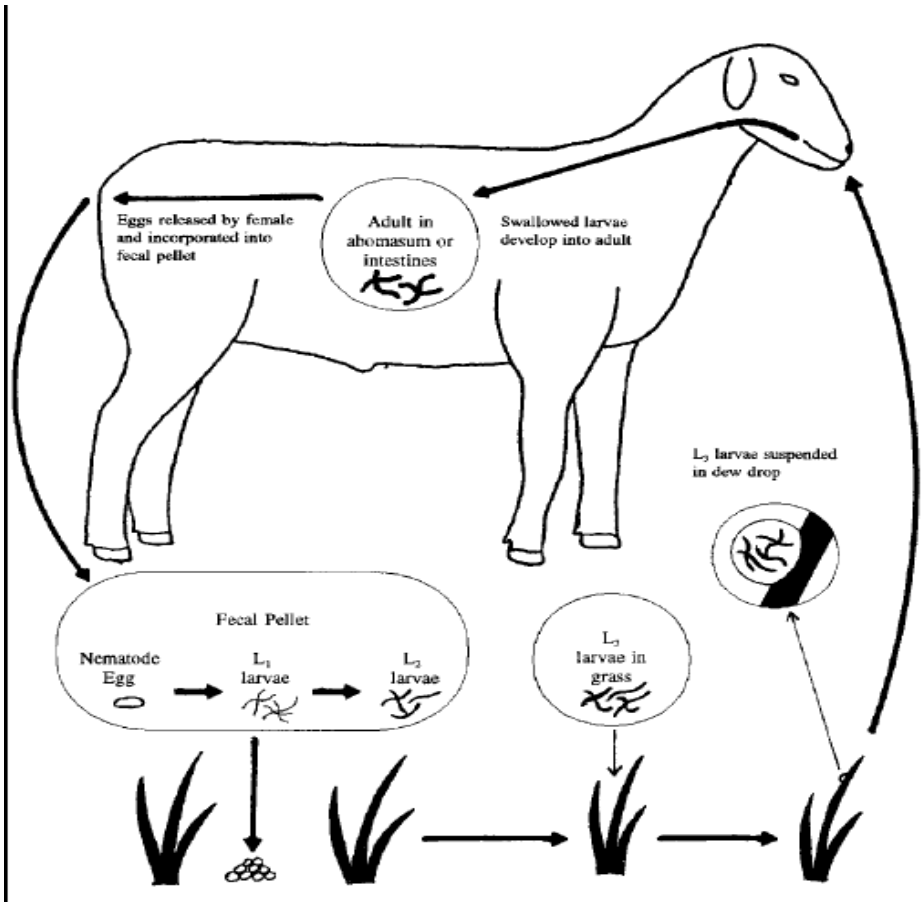


Figure 1. Direct life-cycle of important gastrointestinal nematode parasites of sheep**.** Source: Machen, 1993.

The prevalence of different types of nematode infections has been studied for many years and the clinical signs and parasitological and pathological aspects of the major infections have been reviewed (Sykes, 1983; Holmes, 1985). *Haemonchus contortus* is broadly similar to other abomasal Trichostrongyle infections, but it is the third larval stage (L_3) that possesses the hematophagic habit and causes the resultant blood loss.

Trichostrongyle infections of the small intestine associated with *H. contortus* cause severe mucosa damage. Most of the damage occurs in the proximal third of the small intestine and the worst infections are associated with severe enteritis.

2.2. Factors affecting internal parasite control in ruminants

Anthelmintics are an indispensable component of gastrointestinal worm (roundworms, nematodes, stomach worms) management. In tropical and subtropical regions, anthelmintics are used intensively, causing parasite resistance, that has made most of them ineffective. This is the principal reason why research is aimed at finding other solutions to the parasite problem in small ruminants. The major alternative parasite control strategies in small ruminants are:

- Good grazing management
- Use of clean or safe pastures
- Pasture rest and rotation
- Multi-species grazing
- Alternative forages or dewormers
- Cooper oxide or nematophagous fungi
- Nutritional management
- Proper anthelmintic use
- Refugia populations and vaccines

2.2.1. Grazing management

One of the key factors for controlling internal parasites involves management of pastures.

- ***The height of the pasture sward*** can affect parasites. The majority of infective parasite larvae move slowly and are able to ascend only about 5 cm above the ground onto the pasture sward. Thus, not allowing animals to graze below that level will cut down infestation. However, Jones (1993) recommended that letting animals graze very close to the ground is a better method for use with newly established pasture because the sunlight can dry the ground and sward quickly and thus diminish the chances of survival of parasites brought in with the animals. A new pasture is considered a field where animals have not been grazing for a number of years. It may be a pasture seeded in the spring or a hay or silage field that is used as pasture after harvest.

- ***Rotational grazing*** generally does not help to control internal parasites unless pasture rest periods are long enough (> 90 days; Castells and Bonino, 2001). In tropical climates, a good control of digestive nematodes has been obtained with maximum grazing duration of four days and 30-day rest periods (Barger et al., 1994). The reason thereof is that larvae develop between 4 and 6 weeks after contamination (Barger et al., 1994; Sani et al., 1995). Also, rotational grazing alternating with hay production has been found effective for reducing the burden of parasites in ruminants. As soon as a grazing area is depleted all the animals are moved to new grazing paddocks. The first area is then left to produce harvestable

hay which, when baled and removed, eliminates most of the infective larvae (Sani et al., 1995).

- ***Controlled grazing methods*** allow pastures to rest and soil life to function well, thus contamination with parasites can be reduced. This reduction occurs because soil organisms, including earthworms, dung beetles, and nematophagous fungi will destroy parasite eggs and larvae.

- ***Multi-species grazing*** of two or more ruminant animals has been shown to be effective in controlling some parasite species. For example, cattle, sheep and goats seldom compete for the same type of grazing plants because these species prefer forages of different lengths and textures. Cattle and sheep or goat herds can be combined in three ways: 1) graze the cattle to "clean" the pasture after the lambs have grazed; 2) graze the cattle before the sheep to control pasture quality and; 3) graze the cattle and sheep or goats together where vegetation is abundant. Running cattle in pastures that have had sheep or goats grazing on them helps break up the life cycle of sheep parasites, since sheep and cattle are not infested by the same species of worms (Wells et al., 2000). This affects parasite loads of both grazing species as transmission is dependent on ingesting the parasite larvae on certain parts of the forage.

2.2.2. Nematophagous fungi

Nematophagous fungi have been of value for nematodes biocontrol. Due to frequent drug resistance and the problem of drug residues in animal tissues and in pastures which result from the intensive use of anthelmintics, the demand for nematode bioagents, such as nematophagous fungi, for application in management programs has increased in recent years. The fungus is administered via the feed and is ultimately passed in the feces to complete its effects in the pasture area. Nematophagous fungi have been classified into three major groups: nematode-trapping fungi, endoparasitic fungi and egg-parasitic fungi.

A nematode-trapping fungus is a facultative fungus that forms a structure designed to trap nematodes (Barron, 1977). The endoparasitic fungus is an obligate parasite that grows to a limited extent in soil outside the colonized nematode cadaver (Kerry and Abbey, 1997). This type of fungus infects nematodes by producing adhesive spores which attach to the cuticle of passing nematodes. An egg-parasitic fungus is a facultative parasite that attacks sedentary female and egg stages of nematodes (Kerry and Abbey, 1997). This type is an opportunistic fungus commonly found in the soil.

Experiments have shown *Duddingtonia flagrans* to be among the most successfully used nematophagous fungi. *D. flagrans* survives passage in the chlamydospore stage and can kill from 45.5 to 99.5% of the larvae in feces (Baudena et al., 1998). Good survival rates and larval control have been obtained in cattle, pigs, horses and sheep (Larsen et al., 1998). When *D. flagrans* fungi were fed to sheep, *Ostertagia* spp., and *Trichostrongylus* spp., larval nematode populations decreased in pastures (Larsen et al., 1998). One of the

principal challenges to effective use of fungi as a parasite-control method is identifying the best system of utilization. Some delivery systems under development include feeds that support fungal growth, feed blocks and controlled-release devices.

2.2.3. Nutrition

Nutrition plays a major role in enabling animals to overcome the negative effects of internal parasites.

- Bairden et al. (1997) showed that sheep fed increased amounts of by-pass protein (in the form of fish meal), increased their rate of weight gain compared to control animals.
- Barrell et al. (1997) also observed that in parasitized lambs a higher level of dietary phosphorus supplementation (0.28 % vs. 0.18% P in the DM) increased average daily gain (ADG) by 40% compared to the lower P level.
- Whitlock (1948) reported that sheep placed on a high plane of nutrition were able to reduce their worm burden significantly and many of them were even able to become parasite-free.

Nutritional influences on nematode parasite infestations in sheep and goats have been suspected for many years (Gibson, 1963). Given the increased demand for nutrients, caused by parasitism, sheep and goats on a higher level of nutrition and/or with a higher body condition score are better able to overcome parasite challenges. Good nutrition in early pregnancy increases fat storage and has been shown to enhance the immune

response to parasites. Ewes receiving increased protein levels during late gestation are also better able to mount an immune response to parasites. The presence of certain minerals in the diet can decrease the number of gastrointestinal parasites in livestock. Dietary Copper (Cu), Molybdenum (Mo) and Phosphorous (P) supplementation can decrease the growth rate of *Haemonchus contortus*. Copper has direct anthelmintic properties, especially against abomasal parasites (Bang et al., 1990a). There is also evidence that the outcome of a larval challenge may be influenced by dietary Mo concentration (Suttle et al., 1992).

Recent studies showed that supplying amble metabolizable protein in the diet can reduce the incidence of *H. contortus* infestation (Abbott et al., 1985; Wallace et al., 1999). The effects of by-pass protein and high dietary level of total protein on the longevity and establishment of gastrointestinal parasites have been studied for many years. Coop et al. (1998), found an effect of dietary protein on daily weight gains in lambs infected with *T. colubriformis* and fed fish meal relative to non-infected lambs. In this study, infected lambs fed protein levels of 178-180 and 110-120 g of CP/kg fresh matter decreased significantly weight gains by 18 and 27% below those of non-infected control lambs. It was concluded that an increase in dietary protein and in the general quality of the diet decrease parasite infections of *Haemonchus contortus*.

Furthermore, Suttle et al. (1992) indicated that adding Mo (0.05 mmol/kg DM) to sheep diets decreased populations of *Haemonchus contortus* and *T. vitrinus* parasites in the gastrointestinal tract by 78 and 23%, respectively. Coop and Field (1983) found that including adequate P (1.88 to 2.75 g/kg DM) in diets of lambs infected with

Trichostrongylus vitrinus decreased worm burdens and fecal egg output, while increasing rate of weight gains. Changing dietary composition as a means to prevent disease is a promising concept worthy of pursuing. One of the basic challenges is determining the quantity of a given nutrient that is beneficial in the prevention of parasitism versus the excess quantity that can cause adverse effects. Also, it is difficult to determine how to incorporate nutrient recommendations for disease prevention into existing tables of nutrient requirements.

2.2.4. Copper oxide wire particles

Copper oxide wire particles (COWP) have been used for many years to treat copper deficiency in ruminants (Judson et al. 1982, 1984; Langlands et al. 1983; Dewey et al., 1988). Copper is a necessary trace element in the animal diet. COWP administration has reduced worm burdens in growing lambs (Bang et al., 1990b; Knox, 2002) and decreased both worm burdens and fecal egg counts (FEC) in mature goats (Chartier et al., 2000).

The maximum immune response in an animal is directly dependent on adequate copper availability as indicated by depressed antibody titers in deficient animals (Salt Institute, 2002). Treating copper deficiency in grazing livestock with COWP can at the same time serve as an anthelmintic (Dewey et al. 1988). After dosing, the ingesta flows from the rumen to the abomasum where the low pH induces the release of high concentrations of soluble copper, which have an adverse effect on abomasal species of nematodes (Knox, 2002). However, this method is being monitored for possible increasing anthelmintic resistance.

The use of COWP should be combined with other worm control strategies such as use of the FAMACHA system. FAMACHA is used for classifying animals into categories based upon level of anemia. It was developed in South Africa and has been validated in the USA (Kaplan et al., 2004). Animals are assigned to anemia-level groups on a 1 to 5 scale by examining the eyelid coloration of sheep and goats. Recent work has demonstrated the possibility of using COWP to specifically target the intestinal parasite *Haemonchus contortus* for control. Studies with lambs showed that as little as ≤ 1 g and 2 g may remove a substantial part of an *H. contortus* infestation by changing the environment the nematodes are accustomed to and causing them to be ejected from the host (Chartier et al., 2000). COWP have been proved successful against *H. contortus* and constitute a very promising control method (Burke et al. 2004).

2.2.5. Selecting resistant animals

Most animal species develop natural immunity against internal parasites. However, immunity is never 100%. Some breeds show more resistance to parasitic infection than others.

- Several breeds are known to be parasite resistant, meaning that when exposed to parasites, the animal avoids establishment of the same in its body.
- The increased resistance to infection is thought to be the result of a more effective immune response to the parasites, although the exact mechanism of the resistance is not known. Even within parasite-resistant breeds there is variation in the amount of resistance shown by individual sheep.

- Individual animals that demonstrate superior resilience to parasites can be selected.
- Resilient animals can host a parasite burden without negative effect (show no signs of parasitism, and remain productive).

Resistance is defined as the ability of the host to prevent establishment of parasitic infestation and depends on its ability to modify the growth and fecundity of the parasite. By contrast, *resilience* is defined as the ability of the host to thrive in the presence of the parasite and reflects the response of the host to the parasitic infection (Gray, 1997; Gray and Gill, 1993).

Selecting lines of animals, either from within a breed or by introducing a "resistant" breed to produce crosses that have improved ability to regulate their internal parasitic populations, offers a means of increasing immunity against gastrointestinal nematodes. Sheep studies have confirmed that at least a part of the variation in an individual's ability to regulate worm infestation is genetic (Morris, 1998). Kyriazakis et al. (1994) suggested that sheep subjected to a chronic infection of *T. colubriformis*, were able to modify their diet selection to meet the increased protein requirement due to parasitism.

Currently, selection for *resistance* to infection can make use of the criteria FEC, DNA markers, host antibody (Ab) and parasite antigen assays (Gray, 1997). Windon and Dineen (1984) sought to study the response of young random-bred Merino lambs to vaccination with irradiated *T. colubriformis* larvae in order to classify them into "responders "and "non-responders". Responders were lambs that had FEC below the lower 90% confidence limit of unvaccinated control lambs.

Selection is feasible using phenotypic traits such as FEC and immunological markers such as antibodies. Genetic selection can produce more resistant animals as a sustainable means of dealing with the problem of nematode infestations. Most parasite infections affect the animal's immune system. The host possesses immune mechanisms that allow for maintenance of the host- parasite relationship in equilibrium (Amarante, 2001).

2.2.6. Vaccines

The use of vaccines is potentially an important means of internal parasite control in small ruminants. Much effort has been expended in recent years to develop functional vaccines. Progress has been made in new technologies for gene discovery and antigen identification, characterization and production. Successful vaccines have been developed for lungworms in cattle and tapeworms in sheep. The most efficient and promising vaccine against small ruminant worms is based on a "hidden gut" antigen and specifically targets *H. contortus.* Efficiency of the vaccine is based on this antigen that is derived from the gut of the worm and then administered to the animal, where the antibodies are produced. The mechanism of action starts when the worm ingests blood during feeding; it also ingests these antibodies (Kabagambe et al. 2000; Barras and Sherman, 2004). The antibodies attack the target cells in the worm gut and disrupt the worm's ability to process the nutrients necessary to maintain its growth and development. A number of vaccinations may be required to maintain sufficiently high antibody titers to combat the infection (Smith and Zarlenga, 2005; Van Wyk, 2001). Vaccines for other types of worms that do not feed on blood must focus on the use of antigens found in the worm's secretory and excretory products (Knox et al., 2003; Redmond et al., 2003).

2.2.7. Antiparasitic plants

There are several research reports suggesting that some pasture plants have anthelmintic properties, as summarized in Table 5.

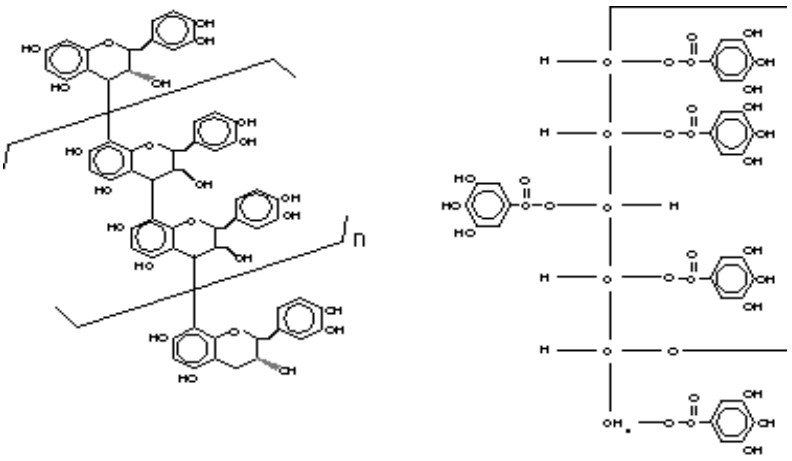
Table 5. Published results of research on the effects of various forages on internal parasites in sheep and goats.

Animals used	Treatment	Results	Notes	Reference
Spanish wether goats, grazing	15 d grazing Sericea or rye/crabgrass, switch to other forage 15 d.	Fecal egg counts (FEC) reduced, percentage of eggs developing to larvae reduced.	FEC increased after switching to rye/crabgrass; tannins seemed to have short residual effect.	Min et al., 2004
Goats, confined and fed hay	Ground hay-Sericea or bermudagrass – 4wk trial, all on bermudagrass hay for 3 wk.	Reduced fecal egg counts (FEC) for Sericea- fed goats.	FEC climbed again when animals were taken off Sericea.	Shaik et al., 2004
Goats confined and fed hay (75% diet) and grain (25%)	Ground Sericea (0,25,50 and 75%) and/or bermudagrass (75, 50, 25, and 0%) in combinations equaling 75% hay; levels testing dose of Sericea needed 6 wk.	FEC reduced for those fed Sericea at all levels, greater reduction as % Sericea increases.	Optimum level of Sericea hay appeared to be 50-75% of total diet.	Dykes et al., 2006
Goats, confined and fed hay and grain	Sericea hay or bermudagrass hay, 7wk.	FEC reduced, number of adult worms reduced, hatchability of eggs into L_3 larvae reduced.	Egg counts dropped by about 80% one wk after Sericea 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.	Shaik et al., 2006
Lambs, fed hay; natural and experimental *H. contortus* infestations	Sericea hay or bermudagrass hay, 7 wk. Bermudagrass, 2 wk.	FEC reduced for those receiving Sericea (67-98%); FEC increased after Sericea feeding stopped.	Sericea fed as hay reduced naturally infected worm burdens 67%. Reduced establishment of incoming larvae 26%.	Lange et al., 2006

The potential anthelmintic property of certain grazed forages is a topic of current interest. Among the plant compounds thought to have anthelmintic properties are the CT. In general, saponins, alkaloids, non-protein amino acids (NPAA), tannins, lignins and glycosides are considered responsible for the anthelmintic action of forages in ruminants (Guarrera, 1999). Research in this field has included *in vivo* experiments with tanniferous forages fed to sheep and goats with natural infection or experimental parasite infection with *Haemonchus contortus* and *Trichostrongylus colubriformis*. Beneficial effects on host physiology and performance under parasitic challenge have generally been found in these studies, when the consumption of bioactive plants was compared with control herbage with lower percentages of CT in the DM. Reductions in nematode numbers, worm fecundity, nematode egg excretion, and egg output have also been observed.

2.3. Plant tannins

There is a structural distinction between hydrolysable (HT) and condensed tannins (CT). This distinction is based mainly on two properties: the presence of ester-linked gallic or ellagic acids; and an interflavan C-C- linkage in HT. Condensed tannins may also contain gallic acid esterified to the 3-OH of the C ring showed in Figure 2.



Condensed tannin

Hydrolyzable tannin

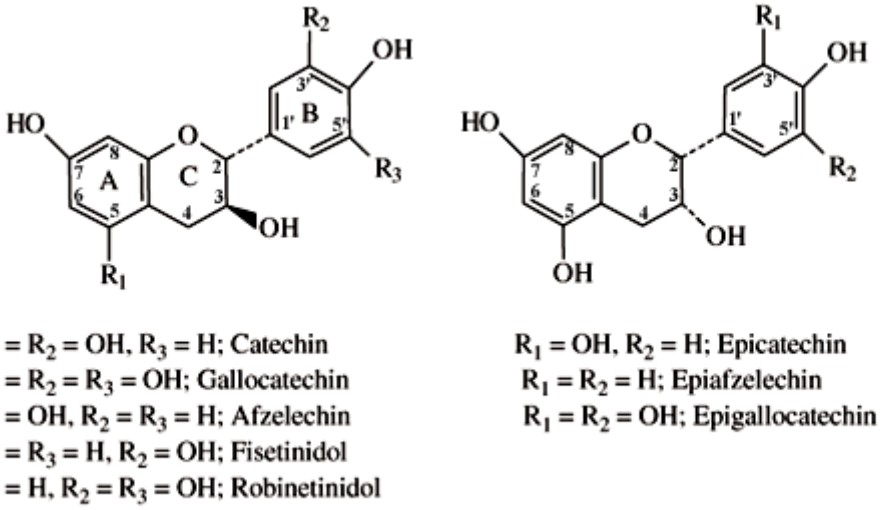


Figure 2. Structure of condensed tannins and hydrolysable tannins in plants (From Swain, 1979; Zucker, 1983).

Groups R_1, R_2, R_3 may have a significant effect on tannin reactivity. In HT R_2 is an OH group, esterified to gallic acid (as epigallocatechin gallates, a major polyphenolic ingredient of green tea). The presence of gallate esters in tannins may change their

biological properties significantly (Hagerman, 1992). Hydrolysable tannins can be divided into two types, the gallotannins and the ellagitannins. Both types have a carbohydrate core, typically glucose. Gallic acid and its derivates are products of acid hydrolysis of gallotannins.

2.3.1. Bioactivity of hydrolysable tannins

Tannins (CT and HT) are polyphenolic compounds with varying molecular weights that occur in nature and have the ability to precipitate proteins (Haslam, 1989). They range from 500 to sometimes greater than 20,000 in molecular weight. Hydrolysable tannins bind at pH 3-4 and binding decreases above pH 5 (Van Sumere et al., 1975). Tannins are usually soluble in water (Haslam, 1989), except for some high molecular weight structures that are insoluble. The hydroxyl groups of the carbohydrate proportions are partially esterified with phenolic groups like gallic acid in gallotannins or ellagic acid in ellagitannins (Waghorn and McNabb, 2003). Hydrolysable tannins are usually present in low amounts in plants (Mueller-Harvey, 2001). These tannins are found in oak (*Quercus* spp.), Acacia and a variety of other browse and tree leaves (Waghorn and McNabb, 2003). Leaves and apices of these browse plants can contain up to 200 g tannins per kg of DM and in some species they can contain total phenolic compounds exceeding 500 g per kg of DM (Reed, 1995; Lowry et al., 1996).

Hydrolysable tannins are potentially toxic to animals, but most ruminants can adjust to them (Waghorn and McNabb, 2003). Ruminants are able to adjust to these potentially toxic tannins by increasing their urinary excretion of degradation products, allowing them

to consume tannin-containing diets (Lowry et al., 1996). However, an excessive amount of tannin in the diet can result in liver and kidney lesions, as well as death (Waghorn and McNabb, 2003). Hydrolysable tannins are less soluble and less accessible to proteolytic enzymes and are stable at rumen pH (Broderick, 1974). The astringency of tannins makes them unpalatable, as they form complexes with salivary gycoproteins, resulting in a reduction in the feed intake (Fahey and Jung, 1989).

Nelson et al. (1995) reported obtaining HT degrading diplococcoid anaerobic bacteria from the ruminal contents of goats browsing on a diet of *Desmodium ovalifolium* containing 17% of CT (Table 6). *Selenomonas ruminantium* is another anaerobic bacterium with the ability to degrade tannins, isolated from the rumen of goats browsing on *Acacia* (Skene and Brooker, 1995). Mc Sweeney et al. (2001) isolated proteolytic ruminal bacteria of the genera *Clostridium spp.* and *Streptococcus spp.* from sheep and goats fed on the tannin-containing, shrub legume Calliandra, containing about 6% of CT. Most of the CT compounds have a greater effect on digestibility than HT. Microbial metabolism and gastric digestion can convert HT into compounds of lower molecular weight (Murdiati et al. 1992).

Table 6. Gastrointestinal microorganisms from domestic animals that are capable of degrading hydrolysable tannin (HT)-protein complexes

Reference	Site of origin	Microorganisms
Goel et al. (2005)	Bovine rumen	*Streptococcus* species
Ephraim et al. (2005)	Rumen of wild ruminants	Gram-negative
Skene and Brooker (1995)	Bovine rumen	*Selenomonas ruminantium*
Bhat et al. (1998)	Bovine intestine	*Aspergillus niger*
Nelson et al. (1995)	Rumen of feral goats	Anaerobic diplococcoid bacterium

Source: Goel et al. 2005

2.3.1.1 Biochemical structure of hydrolysable tannins

Tannins of the gallotannin group can interact with proteins of diverse functional types causing inhibition of the enzymatic activity (Swain, 1965; Haslam, 1979). *In vitro*, these tannins have a much reduced ability to cause protein precipitation. This provides further evidence for specificity between protein and tannin.

Ellagitannins are an example of structural proliferation that involves oxidations, reductions, and additions of tannins. The selective advantage to the plant having this type of HT is that it provides a means of achieving new specificities, directed toward proteins and other target compounds. Upon hydrolysis by acids, bases or certain enzymes, gallotannins yield glucose and gallic acids. Ellagitannins contain one or more hydroxydiphenoyl residues which are linked to glucose as a diester addition to gallic acid.

2.3.2. Bioactivity of condensed tannins

Among tannins, CT are the most widely distributed. These are water-soluble phenolic compounds that are present naturally in plants. They are mainly characterized by being able to precipitate proteins, which is the principal reason for their influence on the nutritional value of forage legumes (Haslam, 1989). High tannin concentrations in forages reduce voluntary consumption and digestibility of proteins and carbohydrates and adversely affect animal performance (Barry and Manley, 1984; Barry, 1985; Reed et al. 1990).

Condensed tannins are best known for forming complexes with proteins (Van Sumere et al. 1975); but they can also form complexes with cellulose, pectin, starch, and alkaloids (Swain, 1965; Haslam, 1979). This type of tannin can bind to proteins using the three types of bonding: hydrogen bonds, ionic bonds, and covalent bonds. The possibility of hydrophobic interactions is suggested by the observation of strong adsorptions of CT, which may also contribute to the protein-tannin complex formation.

Plants containing CT evolved over time and use these substances as a defense mechanism, for protection against pathogenic microorganisms and against being consumed by herbivorous insects or grazing animals (Swain, 1979). Condensed tannins extracted from various plants have been used to improve animal health. Extraction of CT was performed using acetone-water (Barry et al., 1999). Van Sumere et al. (1975) reported that CT bind almost independently of pH at values less than 7-8, but binding decreases above pH 8. Tannin chemistry is complex. The monomeric units are variable (Foo et al. 1996, 1997) making a nearly infinite variety of chemical structures possible, which in turn affect the biological properties of CT (Barry and McNabb, 1999).

2.3.3. Presence of tannins in temperate and tropical forages

The amount and type of CT synthesized by forages vary considerably (Table 7), depending on botanical species and cultivar, soil type (Table 8), stage of development, and environment conditions (Broadhurst and Jones, 1978; Terrill et al. 1992; Barry and Fross, 1983; Iason et al. 1995; Jackson et al. 1996). Therefore, the study of the effects of CT on animal nutrition requires identification of the type of CT present in a particular forage.

In most cases CT are present in the leaves and stems of plants whilst in some legumes, such as white clover and red clover, CT occur only in the flower petals (Barry, 1989; Iason et al. 1995). Concentrations of CT in some plants (leaf and stem parts) are presented in Table 7, where it is evident that total CT content varies widely even within a given species. The extractable fraction generally contains the highest CT concentration.

Condensed tannins found in tropical forages are thought to promote plant growth by reducing the release of leaf litter into the soil (Palm and Sanches, 1991) and reducing eggs in animal feces (Waghorn and McNabb, 2003). Many different types of foliage contain CT; for example lotus (Table 8), sainfoin (*Onobrychus viciifolia*), sulla (*Hedysarum coronarium*) and lespedeza (*Sericea lespedeza*).

Table 7. Concentrations (g/kg DM) of extractable condensed tannins (ECT), protein-bound condensed tannins (PCT), fiber-bound condensed tannins (FCT) and total condensed tannins (TCT) of leaf and stem in a range of temperate and tropical plants.

Temperate forages

Forage	ECT	PCT	FCT	TCT	Reference
Birdsfoot trefoil	7.0	13.0	1.0	21.0	1
Birdsfoot trefoil[1] (Grasslands Goldi)	35.8	8.6	1.8	46.2	2
Birdsfoot trefoil	36.1	10.9	1.2	49.2	3
Narrow leaf birdsfoot trefoil	2.0	3.0	1.0	6.0	1
Big trefoil	61.0	14.0	1.0	76.0	1
Sulla	33.0	9.0	3.0	45.0	1
Sulla	27.5	8.1	0.65	36.2	4
Sainfoin	29.0	nd	nd	nd	5
Crownvetch	16.0	13.0	2.0	31.0	1
Hairy canary clover	121.0	65.0	1.0	187.0	1
Prostate clover	100.0	23.0	3.0	126.0	1
Canary clover	83.0	54.0	6.0	143.0	1
Red clover	0.4	0.6	0.7	1.7	2
Sheep burnet	1.0	1.4	1.0	3.4	1
Yorkshire fog (wild ecotype)	1.1	0.3	0.4	1.8	1
Lucerne	0.0	0.5	0.0	0.5	2
Perennial ryegrass	0.8	0.5	0.5	1.8	2
Dock	11-23	0.0	0.0	0.0	6

Tropical tree and legume forages (leaf only)

Forage	ECT	PCT	FCT	TCT	Reference
Acacia mangium	71.8	25.1	3.5	100.4	7
Arachis pintoi	31.7	1.2	0.7	33.6	7
Senna velutina	54.0	nd	nd[2]	54.0	7
Calliandra sp	158.1	28.4	7.8	194.3	7
Desmodium ovalifolium	196.9	30.5	10.1	237.5	7
Leucaena diversifolia	75.8	13.2	3.5	95.5	7

[1]Diet selected samples. nd, not determined and nd[2], FCT non-detectable. All samples were freeze dried and ground. CT concentration determined by the Butanol-HCl method of Terrill *et al*., 1992. (1) Terrill *et al*. (1992), (2) Jackson *et al*. (1996), (3) Min *et al*. (1997), (4) Douglas *et al*. (1993), (5) Barry and Manley (1986), (6) Waghorn and Jones, (1989), (7) Jackson *et al*. (1996).

Table 8. Condensed tannin concentration (g/kg DM) of vegetative Lotus species as affected by soil fertility. (All determined by the vanillin-HCl procedure of Broadhurst and Jones, 1978).

Soil Fertility	*Lotus pedunculatus* cv Maku	*Lotus corniculatus* cv Empire	cv Maitland	Reference
High soil fertility[1]				
	20.0	3.0	15.0	John and Lancashire (1981)
	32.0	NA	NA	Barry and Fross (1983)
	45.6	NA	NA	Barry and Manley (1984)
Low soil fertility[2]				
	94.5	2.8	28.1	Lowther *et al*., (1987)
	78.0	NA	NA	Barry and Fross (1983)
	105.9	NA	NA	Barry and Manley (1984)

[1] High soil fertility pH>5.3; Olsen P > 18 g/ml; SO_4-S >12 μg/g.

[2] Low soil fertility pH <5.2; Olsen P > 8g/ml; SO_4-S > 5 μg/g. NA, Not applicable.

3.0. Use of tropical forages in ruminant feeding

Low productivity of ruminants in the tropics due to low availability and poor quality of feed in the dry season is a serious problem. Leguminous fodder trees and shrubs have a great potential as feed supplements both to increase animal productivity and combat internal parasites. Supplementation with Calliandra forage can be effective in reducing GIN infestations in goats. Plants, even those of the same species vary in CT content (Koupai-Abyasani et al. 1993; Douglas et al. 1993; Heering et al. 1996; Hedqvist et al. 2000) and composition (Foo et al. 1982) depending of the geographical region and season of growth. Profiles of CT in Calliandra (12.04%) have been carried out and correlated with DM digestibility data to better understand the role of tannins (Rakhmani et al. 2005). Calliandra is widely used as a supplemental source of nitrogen in many tropical livestock production systems (Gutteridge and Shelton, 1994). Calliandra is of low ruminal degradability, as a result of its high CT concentration. The non-legume species *Morus alba L.* (2.9% CT) possesses a high nutritional potential due to its high protein concentration and rapid degradation in the rumen. Its addition to diets of low nutritional value helps to improve efficiency and maintain suitable levels of animal production. Some herbaceous legumes with good nutritional qualities such as *Arachis pintoi* (1.9% CT), are well adapted to acidic soils, and maintain high DM digestibility during the dry season when most tropical forages decreased their quality (Hess et al. 2002). Other legumes such as *Leucaena leucocephala*, are limited in their tolerance to acidic soils, in which they show a slow growth rate. In recent years *Cratylia argentea*, a shrub legume (0.8% CT), has came into use for livestock production in tropical countries. It was

selected for use in dry-season supplementation, in regions where acid soils and extended dry season are predominant (Argel and Mass 1995; Argel and Lascano 1998; Peters et al. 2002). This legume contains only trace amounts of tannins (Lascano, 1996; Shelton, 2001), which may be either advantageous or disadvantageous depending on the intended use. However, Cratylia has an excellent regrowth capacity after cutting and can be used as soil cover. When one of the intended uses of CT-containing forages is control of GIN it is important to analyze the impact that the CT will have on the animal in terms of both rate of weight gain and internal parasitism.

3.1. Use of tannins in parasite control

The influence of tannin content of forages on protein metabolism may be either beneficial (low CT levels) or detrimental (high CT levels) (Terrill et al. 1989; Aerts et al. 1999; Barry et al. 2001). Many studies have shown that some particular forage may reduce helminthic parasites in sheep and goats and thereby increase performance of young animals due to action of the CT. Among the principal causes of lost production in parasitized animals such as lambs are the loss of endogenous protein and anorexia (Niezen et al. 1995). The possible antiparasitic role of CT is supported by *in vivo* studies that confirmed the effects on different nematode stages of crude plant extracts or CT from different origins (Athanasiadou et al. 2001; Barrau et al. 2005).

Feeding plants containing CT might reduce the effects of parasitism indirectly, by increasing the post ruminal availability of dietary protein, thus improving the nutritional status of the animal and maintaining productivity. Bioactive plants or forages with

secondary metabolites, particularly legumes with a high content of proanthocyanidins (CT), have been reported to reduce worm burdens in grazing lambs by up to 50% (Niezen et al. 1995).

Min and Hart (2003) reported a reduction in FEC in goats grazing *Sericea lespedeza* compared with non-CT grass pasture in two trials completed in Oklahoma. Other authors have reported anthelmintic effects of dried forages fed to goats (Paolini et al. 2003). Many plants with anthelmintic properties can reduce adult parasite populations in the host by decreasing parasite fecundity or parasite egg development. Niezen et al. (1998) demonstrated the effect of common New Zealand pasture plants and other feeds on the establishment of parasites in the ruminant host and parasite fecundity. Grazing trials carried out by Niezen et al. (1995) compared the performance of parasitized lambs grazing either sulla (*Hedysarum coronarium* L.) or alfalfa (*Medicago sativa)*, and demonstrated that sulla had the higher parasite reduction potency.

The capacity of purified condensed tannins from legumes to reduce nematode larval infestations has been studied *in vivo*. *In vivo* anthelmintic effects against sheep nematodes have been observed using a single high dose of CT extracted from quebracho (Athanasiadou et al. 1999). Preliminary tests with *Sericea lespedeza* (SL), a perennial warm-season legume, have shown effects of reducing FEC and worm burden in grazing goats and in sheep and goats fed hay in confinement. In addition to its potential use in controlling worms, SL is a useful crop for limited resource producers in the southern region of the USA.

The reactivity of CT from SL may explain why this forage maintains its anthelmintic properties when fed as hay. Drying CT containing forage reduces the proportion of tannin that is extractable using an aqueous solvent and increases the proportion that is bound to protein (Terrill et al. 1992). The unbound CT is more capable of forming complexes with protein and other macromolecules than the CT that is present in bound form (Barry and Manley, 1986). This unbound CT is the active agent giving SL its anthelmintic properties. These considerations suggest that the particular type of CT in the plant is more critical for controlling GIN nematode than the total concentration of bound and unbound forms in the diet.

In the USA and Puerto Rico, the beneficial effects of tanniferous plants against internal parasites could be due to one or a combination of factors. First, tannins may form non-biodegradable complexes with protein in the rumen, which dissociate at low pH in the abomasum to release more protein for metabolism in the small intestine of the host animal. Better nutrition, improves the host's resistance and resilience to nematode parasite infections. Secondly, tannins may have a direct anthelmintic effect on resident worm populations in the animal. Tannins and other secondary metabolites may have a direct effect on the viability of the free-living stages and thus prevent development of eggs to the infective larval stages.

3.2. Larval migration inhibition assay

The larval migration inhibition (LMI) assay developed by Wagland et al. (1992) and modified by Rabel et al. (1994), has been used to determine the anthelmintic activity of purified tannins against gastrointestinal parasitic larval infestations. The LMI assay is dependent on the active migration of larvae through pore sieves (Rabel et al. 1994). Only a few *in vivo* studies have examined under controlled conditions the consequences of a tannin-rich digestive environment on the third-stage infective larvae (L_3). In a recent study, Brunet et al. (2006) examined the possible interactions of extracts from four tannin-rich plants with the L_3 larval exsheathment *in vitro*. Contact with most of the plant extracts was associated with a partial or total inhibition of the larval exsheathment. These results suggest that extracts of tannin-rich plants might interfere with host animal invasion and that the nature of the tannins is strictly related to the parasitism control process.

Molan et al. (2003) emphasized that differences in the structure of flavan-3-ols and flavan-3-ol gallates modulated the inhibitory effects on larval development of *T. colubriformis* and migration of the larvae. This could help to explain the variations in *in vivo* effects on nematode populations of various tannin-rich plants. Tannin-rich plants could have variable effects related to differences in the biochemical structure of their CT. Bioactive plants with the highest activity against gastrointestinal nematodes in ruminants are those that contained a high prodelphinidin/procyanidin ratio (Min et al. 2003; Molan et al. 2003; Hoste et al. 2006).

In vitro studies have shown that proanthocyanidins extracted from the forage legumes sulla (Molan et al. 2000; Niezen et al. 2002), sainfoin (*Onobrychis viciifolia)*, *Lotus corniculatus* and *Lotus pendunculatus* (Molan et al. 2000), can inhibit the motility of nematodes. The migration can be reduced by increasing proanthocyanidin concentrations from different sources. The impact of proanthocyanidins upon motility is indicative of an anti-parasitic potential, but a correlation between the level of parasitism in grazing animals and forage content of proanthocyanidins does not always exist (Niezen et al. 2002).

The larval exsheathment process results from the action of several enzymes, including proteinases (Rogers, 1982; Gamble et al. 1989). The chemical composition of plants can be modified by passage through the gastrointestinal tract. In the rumen, bacterial degradation and formation of protein complexes takes place (Makkar et al. 1988). The mechanisms by which tannins can inactivate parasite eggs are not known, but they may inactivate enzymes responsible for the hatching process. Tannins added to the media containing eggs and L_1 larvae could interact with the protein surface of the eggs and larvae, thus potentially affecting hatching and development. Tannins are more potent inhibitors of egg hatching and larval development than of larval motility (Molan et al. 2000).

These facts suggests that CT may be able to break the life cycle of nematodes and reduce the contamination of pastures with viable eggs. Such an effect could help to decrease the dependence on anthelmintics usage as the principal method of parasite control. To improve understanding of the interactions between CT and the parasites, further research

is needed to determine the mode of action of tannins. The variability in CT effectiveness depends on the parasite species, its stages and the tannin-containing plants species. By measuring the migration of L_3 larvae through sieves, it has been possible to demonstrate that flavan-3-ols and flavan-3-ols gallates have inhibitory effects as evidenced by their ability to immobilize larvae and prevent their passage through the sieves. Thus, it is evident that CT from certain forages can inhibit some biological process in *T. colubriformis* and *H. contortus*, blocking the nematode cycle of pasture contamination, through effects on larval development, reinfection and worm viability.

4.0. MATERIALS AND METHODS

Two experiments were conducted to determine the potential of CT-containing tropical legumes to mitigate internal parasites impacts in grazing goats. Experiment 1 was an *in vivo* feeding trial that utilized eight growing Boer goats. Experiment 2 used an *in vitro* assay procedure to determine migration inhibition, of *Haemonchus contortus* third-stage larvae, by plant extracts of CT and CT monomers.

4.1. Experiment 1 *in vivo*

4.1.1. Facilities, animals and experimental design

This work was conducted at Finca Alzamora (Small Ruminant Project) of the University of Puerto Rico, Mayagüez Campus (UPRM) from September to December 2006. The animals were randomly assigned to two feeding treatments based on the use of Calliandra and Arachis as supplemental legumes. These tropical legumes were compared as to their efficacy in mitigating internal parasite impacts and decreasing fecal egg excretion. The animals were initially adapted to conditions of management, which involved training them to use the individual cages under roof (Figure 3), and two enclosed pasture areas (931.9 and 1334.4m^2 for a total of 0.2266 ha) over a seven-day period. All animals grazed this common pair of paddocks sown to guineagrass (*Panicum maximum*), throughout the study period. Prior to initiation of test forage feeding, the animals were weighed using a hanging scale and fecal samples were taken to establish base lines for statistical comparisons in terms of body weight gain and FEC reductions. On day zero all animals were orally dewormed using the commercial anthelmintic Ivomec (2 mg/kg of BW). Body weight measurements were taken during morning hours (8:00 am) and fecal

and blood samples were collected thereafter every 21 days (Figure 7). Feces for egg counting were collected directly from the rectum and blood for determination of PCV was collected in EDTA vaccutainer tubes via jugular venipuncture.

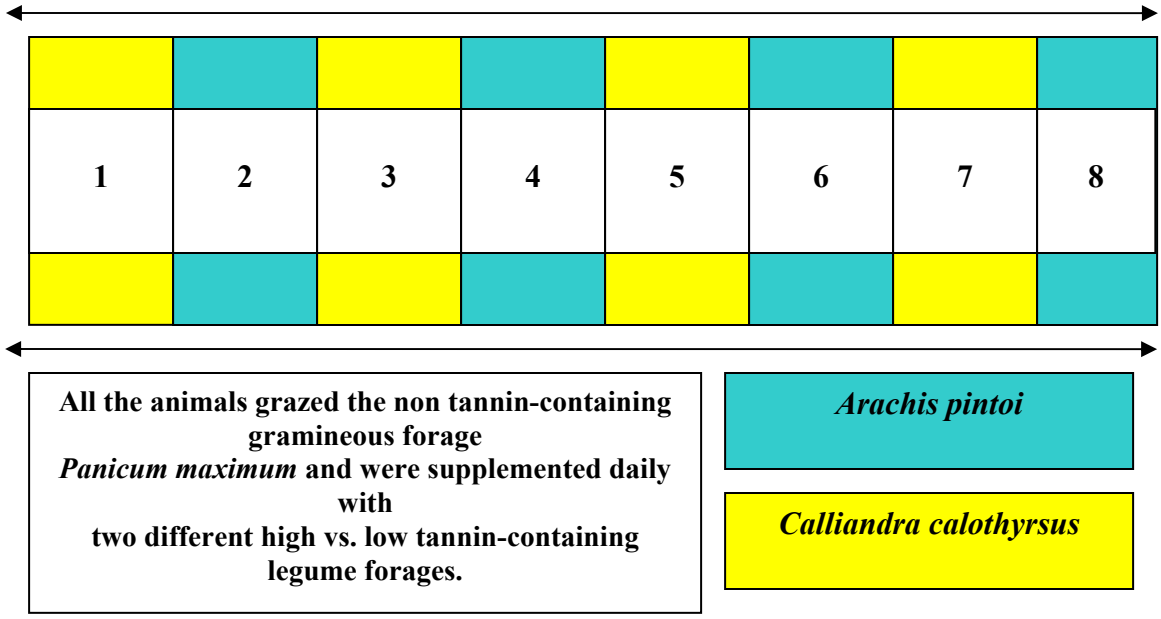


Figure 3. Diagram of the individual cages used for the *in vivo* forage supplementation experiment and photo of the structure housing them.

4.1.2. Supplemental test forages

Fresh leaves of the tropical leguminous forages, Calliandra (Figure 4) and Arachis (Figure 5), located in small plots of the Alzamora Farm forage collection were hand cut and fed each morning (8:00 to 10:30 AM) of the experimental period. All the animals were offered daily the supplemental forages in the fixed amount of 500 g or approximately 3% of mean initial BW (16.64 kg), assuming that the remainder of their nutrient requirements would be supplied by grazed forage. Forage mass of guineagrass was determined by weighing material clipped at ground level in two random areas (0.5m x 0.5m), on days 21 and 84 of the experimental period. A portion of each sample was oven dried at 60^{o}C for 48h to determine DM content. Overall, herbage availability appeared adequate, exceeding 2,000kg of DM/ha or 450 kg DM in the pastured area for

eight animals. Green herbage masses measured in the two paddocks on day 21 were equivalent to 2,035 and 2,085 kg of DM/ha. Corresponding figures for day 84, were 3,108 and 2,361 kg of DM/ha.

Figure 4. Tropical legume: *Calliandra calothyrsus.* High tannin-containing forage (12.4% of total CT).

Figure 5. Tropical legume: *Arachis pintoi*. Low tannin-containing forage (1.9% of total CT).

Initial liveweight mean of goats assigned to Calliandra (Figure 8) and Arachis (Figure 9) treatments was 14.4 ± 0.5 and 18.8 ± 0.5 kg, respectively. The animals grazed one of the two common grass (*Panicum maximum*) pastures during four hours daily from 11:00 a.m. to 3:00 p.m. (Figure 6) and were in confinement during the remain twenty hours of the 24-h cycle. They were provided with water *ad libitum*.

Figure 6. Animals grazing on *Panicum maximum* grass pasture

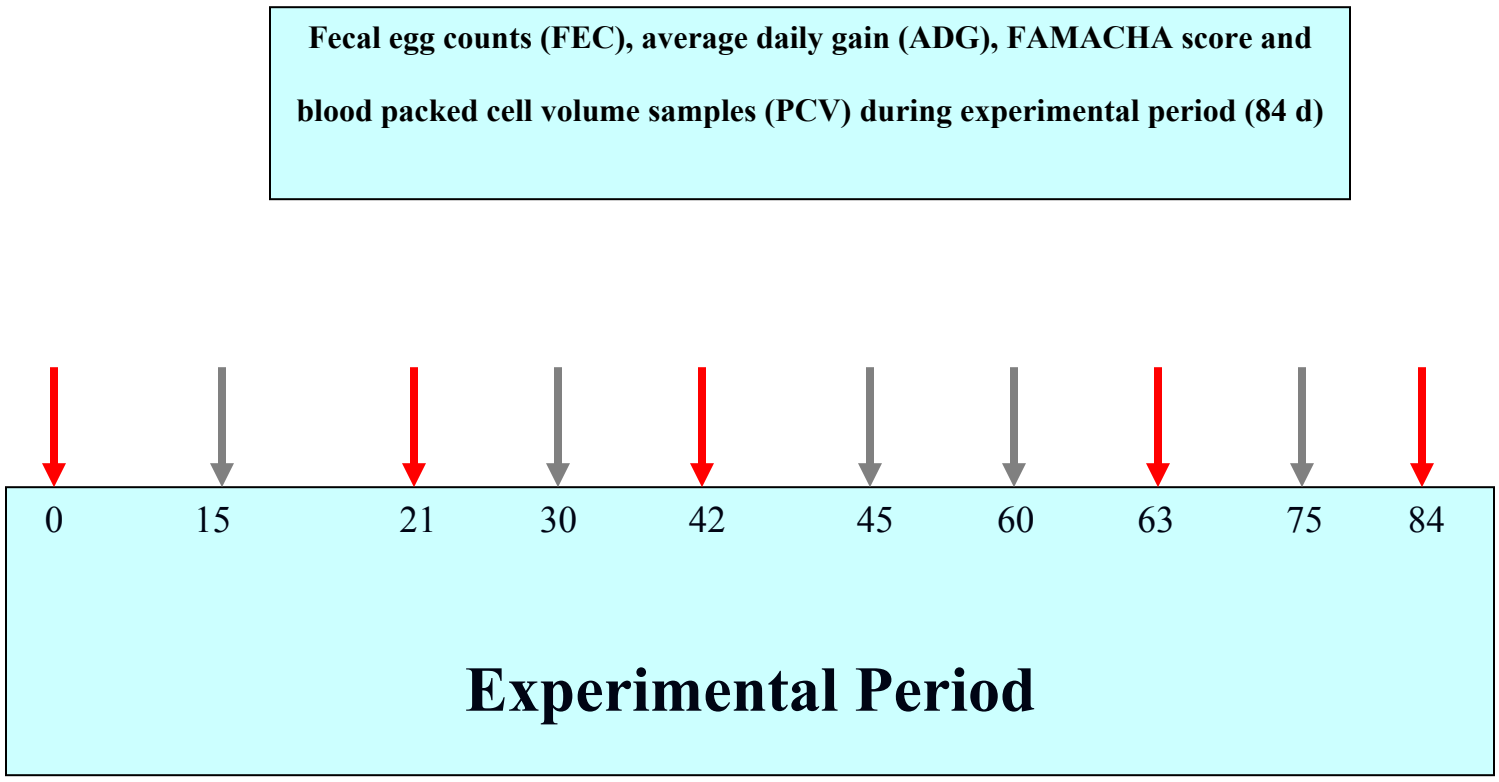


Figure 7. Treatment scheduled for the *in vivo* forage supplementation experiment

Figure 8. Animal supplemented with high tannin-containing *Calliandra calothyrsus*

Figure 9. Animal supplemented with low tanning-containing *Arachis pintoi*

4.1.3. Laboratory and diagnostic analyses

4.1.3.1. Forage chemical composition

Prior to laboratory analysis, all the forage samples were stored at -20^{o}C before being dried at 60^{o}C for 48 h and ground for chemical analysis. Nutritive value analyses included DM, organic matter (OM), CP, NDF, IVDMD, and total CT. Dry matter was determined by oven-heating at 100^{o}C for 48 h. The Kjeldahl digestion method was used for CP analysis (N*6.25) and the concentration of OM was determined by ashing in a muffle furnace at 550^{o}C for 6 h (AOAC, 1990). Concentration of NDF (Van Soest et al., 1991) and IVDMD were determined by the Filter Bag Technique of ANKOM Technology Corp (Fairport, NY). *In vitro* DM digestion was determined through 48 h of incubation using the Daisy II system (Ankom Technology Corp.) with residual DM assessed as NDF. The CT concentration in the legume forages was determined on frozen samples by the butanol-HCl colorimetric procedure of Terrill et al. (1992), using CT extracted (Sephadex LH-20, Sigma Chemical Co., St Louis, MO) from Calliandra as a standard (Jackson et al. 1996).

4.1.3.2. Fecal egg counts

The most common method of determining fecal egg counts for sheep and goats is using the McMaster technique. For this experiment, fecal egg counts were determined using the modified McMaster technique (Hansen and Perry, 1994) in which 2 g of feces were diluted in 30 ml of a saturated salt solution (flotation solution). The sample solution was then thoroughly mixed for 3 to 5 min, after which 1000µl of the solution was extracted using a pipette and placed into one half of a McMaster slide. After half of the slide's

chambers had been filled with the manure suspension in flotation solution, the eggs were counted, under a grid that defines a known volume of the suspension, using a microscope (10X). Over time (20-30 minutes) the eggs will float up to the top and adhere to the glass plate. This procedure was repeated to fill the other half of the side. Usually, the area under two grids is counted and the results averaged and multiplied by a dilution factor of 50 to estimate the total number of eggs in each sample. All the results were reported as eggs per gram of feces (epg).

4.1.3.3. Blood packed cell volume

The PCV of all the blood samples was determined the same day on which they were collected. After thorough mixing using a *vortex mixer*, the hematocrit tubes were filled to three-fourths of the blood volume, sealed with crit-o-seal and centrifuged for 5 minutes in a microhematocrit centrifuge. The PCV values were determined from the hematocrit reader.

4.1.3.4. Visual scoring level of anemia

The FAMACHA© card was used in conjunction with eye examination to identify anemic animals every 21 days over 4 months. This procedure diagnoses indirectly the presence of internal parasites such as *H. contortus* (Bath and Van Wyk, 2001). Scoring on the 1 to 5 scale is as follows: Index one, the eye mucus membrane is a normal red; Indeces two, three and four, correspond to progressively decreasing pink colors and, less brightness; Index five, the eye mucus membrane shows a white color. Animals scored as index one and two are considered to be in a normal condition; those showing index three are

starting to enter an anemic condition; while animals judged index four and five show an anemic and severe anemic state, respectively. In this experiment only the animals observed to have pale eyelids (categories 4 and 5), were treated with an anthelmintic, and vitamin complex (1.5 cc/kg animal BW).

4.2. Experiment 2 *in vitro*

The *in vitro* experiment was performed in the Nutrition Laboratory, Texas Agriculture Experimental Station at Vernon, from January to April 2007. This study was designed to measure *Haemonchus contortus* larval-inhibitory activity of extracted tannins from three tropical legumes, *Acacia angustissima*, Calliandra, and *Sericea lespedeza*, containing considerable levels of CT. The criterion of effectiveness used was the *in vitro* larval migration inhibition (LMI) rate of infective third-stage (L_3) of *Haemonchus contortus* larvae.

4.2.1. Preparation of condensed tannin extracts

Condensed tannins were extracted from the leaves of *Calliandra calothyrsus*, *Acacia angustissima*, and *Sericea lespedeza* by the procedures described by Terrill et al. (1992) and Min et al. (2005), as follows: leaves were homogenized in 70% aqueous acetone (Hagerman 1988) containing 0.1% ascorbic acid, and subjected to three rounds of diethyl ether extraction to remove chlorophyll and lipids. The aqueous defatted crude extracts were freeze dried and approximately 25 g of the material was redissolved in 150 ml of 1:1 methanol/water (v/v). This material was purified on a Sephadex LH-20 column (Pharmacia, Uppsala, Sweden), containing 200 ml of gel, then washed with 2000 ml of

1:1 methanol/water before eluting the CT with 200 ml of acetone: water. The tannin extracts were stored in the dark at 4°C.

4.2.2. Assay procedure for larval migration inhibition (% LMI)

The larval migration inhibition (LMI) bioassay procedure developed by Wagland et al. (1992) and modified by Rabel et al. (1994) was used to determine the inhibiting effect of purified CT against *Haemonchus contortus*. Third-stage larvae (L_3) were obtained from donor sheep infected with pure strains (collected from the same infected sheep and held at 4°C) of *Haemonchus contortus*. The same batch of 2 mon-old larvae was used in all assays. The procedures involved preparation of test solutions of CT and of L_3 larvae which were combined and incubated in the wells of tissue culture plates containing both negative controls (no CT) and a range of CT concentrations from each of three plant species (Costar, Cambridge, MA). The freeze dried CT extracts were dissolved in phosphate buffered saline (PBS; 0.1 M phosphate, 0.05 M NaCl; pH 7.2) and serially diluted with PBS immediately prior to incubation. Concentrations of CT in the incubations were 0, 2, and 4 mg/ml. One hundred microliters of larval solution (~150) were added to wells containing the CT extracts from each forage species. In order to demonstrate that CT were responsible for the anthelmintic activity, a series of incubations was undertaken using commercially available tannin monomers (ellagitannins, gallotannins, and catechins). The concentrations of CT monomers were 1, 2, and 4 mg/ml.

All the incubations were carried out in 48-well tissue plates for 12 h at 37^{o}C, after which solutions were transferred to sieves (7 mm ID with 20 μm mesh at one end) and left overnight (16-18 h) at room temperature to enable the active larvae to migrate through the sieves for counting. The 20 μm mesh size was selected in order to ensure that active migration of the larvae through the sieve was determined. The cross-diameter of L_3 larvae is 25 μm (Rabel et al., 1994), which is slightly larger than the mesh and would thus prevent the larvae "falling" through the sieve. Two replicate samples were run for each concentration of each CT as well as negative controls.

4.3. Calculation of data and statistical analyses

Data from the *in vivo* grazing experiment were analyzed as a random design applying repeated-measures analysis using the MIXED procedure of SAS (1992) with the model including treatment and days. Results are presented including standard errors of the mean (SEM). Data for FEC were log transformed prior to statistical analysis. Means of FEC were back-transformed from log-based means. For Exp. 2 data for LMI were analyzed as well as a random design with factorial arrangement treatment for a 3x3 factorial (CT monomers) and 3x2 factorial (tropical legumes) using the MIXED procedure of SAS. Linear and quadratic analyses were developed to compare any dose level response between CT concentrations.

The repeated measures analysis, in terms of sampling time (days), treatment (Calliandra vs. Arachis), and the interaction of treatment by sampling time is described by the following model (Lymon and Longnecker, 2001):

$$Y_{ijk} = \mu + \alpha_i + \delta_{k(i)} + T_j + \alpha T_{ij} + \varepsilon_{(k)ij}$$

Where:

Y_{ij} = Evaluated variable (FEC, PCV, and ADG)

μ = General mean estimated

i = Treatment effect (Calliandra vs. Arachis)

j = Sampling time effect (21, 42, 63 and 84 d)

αT_{ij} = Effect of the interaction treatment * sampling time

$\varepsilon_{(k)\,ij}$ = Experimental error associated with both treatments

Hypothesis:

1) Ho: $\alpha 1=\alpha 2=0$
   Ha: The effect of one of the treatments is different.

2) Ho: $T_1=T_2=T_3=T_4=0$
   Ha: The effect of one of the sampling times is different.

3) Ho: $\alpha T_{ij}=0$
   Ha: Interaction for treatment * sampling time.

A significance level of $P = 0.05$ was used for all dependent variables.

For the larval migration inhibition assay, the numbers of larvae which had migrated through the sieves were counted using 10 x magnification, and the rate (%) of LMI was determined according to Rabel et al. (1994), using the following equation and the general lineal model (SAS, 1992):

$$\% \text{ LMI} = \frac{A-B}{A} \times 100$$

Where:

A = number of larvae migrating through sieves in negative control wells (containing no CT)

B = number of larvae migrating through sieves in treatment wells (containing CT)

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ij}$$

Where:

Y_{ij} = Evaluated variable (% LMI)

μ = General mean estimated

α_i = Purified CT extracts from tropical legumes effect and CT monomers effect

β_j = Dose response effect (2, 4 mg/ml or 1, 2, 4 mg/ml)

$(\alpha \times \beta)_{ij}$ = Effect of the interaction purified CT * dose response

ε_{ij} = Experimental error associated with each CT extract or CT monomer

5.0. Results

5.1. Experiment 1 *in vivo*

5.1.1. Forage chemical composition

Calliandra showed a much lower mean value ($p < 0.001$) than Arachis for IVDMD (30.9 vs. 61.9 %), whereas it had higher values for DM, OM, CP, NDF and especially CT (12.04 vs. 1.9 %) (Table 9). The low IVDMD values for Calliandra are likely the result of its high CT concentration and possibly the fact that the rumen fluid used for this analysis was collected from steers maintained on bermudagrass (*Cynodon dactylon*) and not adapted to tannin-containing forages.

Table 9. Chemical composition on dry basis and *in vitro* dry matter digestibility (IVDMD) of tropical forages used in the experiment.

Item	Arachis	Calliandra	SEM	P-value
Dry matter (%)	95.59	96.67	8.57	0.17
Fresh forage (%)	44.10	30.30	4.20	0.001
Organic matter (%)	75.57	76.69	4.60	0.11
Crude protein (%)	14.9	18.4	0.29	0.001
Neutral detergent fiber (%)	58.8	61.1	6.90	0.82
IVDMD (%)	61.9	30.9	2.27	0.001
Total condensed tannins (%)	1.9	12.04	0.316	0.001
Panicum maximum				
Dry matter (%)	29.4			
Crude Protein (%)	5.43			
Neutral detergent fiber (%)	77.4			

Dry matter basis

5.1.2. Liveweight gain, fecal egg counts, FAMACHA score and blood packed cell volume

One mortality occurred on day 72 of the experiment, that of animal 128 of the Calliandra treatment (Appendix 1) from severe anemia. Significant differences were found between supplementation treatments for the dependent variables FEC and FAMACHA score, but not for PCV values (Figure 12). As shown in Table 10, mean values for all of these criteria indicated better results with Calliandra than with Arachis: FEC (5262 vs. 7644 eggs/g; $P < 0.001$); mean log FEC (4.22 vs. 3.74 eggs/g; $P < 0.001$), FAMACHA score (2.5 vs. 2.9; $P < 0.02$); and PCV (22.3 vs. 20.5; $P = 0.13$). Cumulative daily logarithmic FEC (Figure 10) was lower for Calliandra and continuously differed from day 42 ($P = 0.07$) to day 84 ($P < 0.001$), because more tannin consumption was showed for *Calliandra calothyrsus* (52.36 g tannin/d) than for *Arachis pintoi* (6.25 g tannin/d). The lack of differentiation between the two supplemental forage treatments prior to day 42 fits well with the life cycle of *H. contortus*. Furthermore, at least through 84 d of feeding Calliandra there was no observed rebound response in *H. contortus* (Figure 11), which would have indicted that this parasite species was adapting to the CT challenge. The positive overall change in body weight obtained with Calliandra feeding (Appendix 1), even though Calliandra had a lower IVDMD than Arachis, points to a possible effect of decreased internal parasitism and associated anemia due to the CT supplied by Calliandra.

Table 10. Fecal egg count (FEC), packed cell volume (PCV), FAMACHA score and tannin consumption in grazing goats supplemented with two tropical forages.

Item	Arachis supplement	Calliandra supplement	SEM	P-value
n	4	4		
FEC (Egg/g)	7644	5262	790.35	0.001
Logarithmic FEC	4.22	3.74	0.09	0.001
PCV (%)	20.5	22.3	1.32	0.13
FAMACHA score	2.9	2.5	0.19	0.02
Tannin consumption (g/d)				
	6.25	52.36		

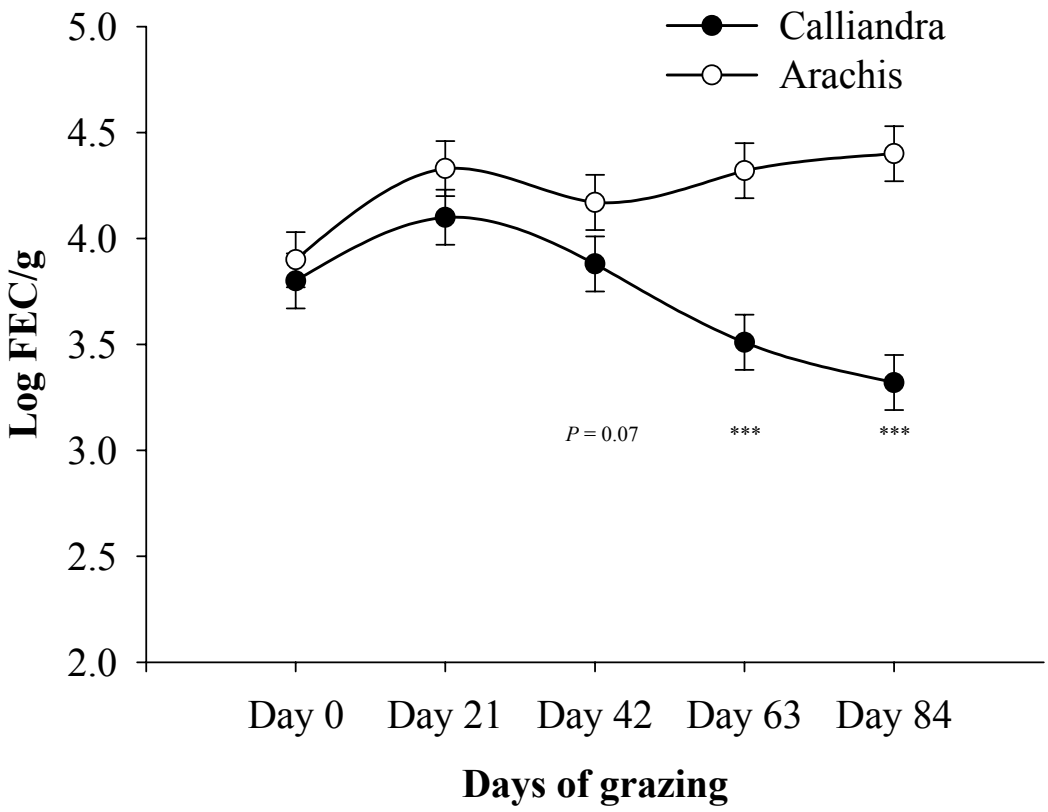


Figure 10. Log means of fecal egg counts (FEC/g) of growing Boer goats and variations. Results are the mean of four determinations (n = 4), and error bars represent standard error of the mean (SEM). *** $P < 0.001$

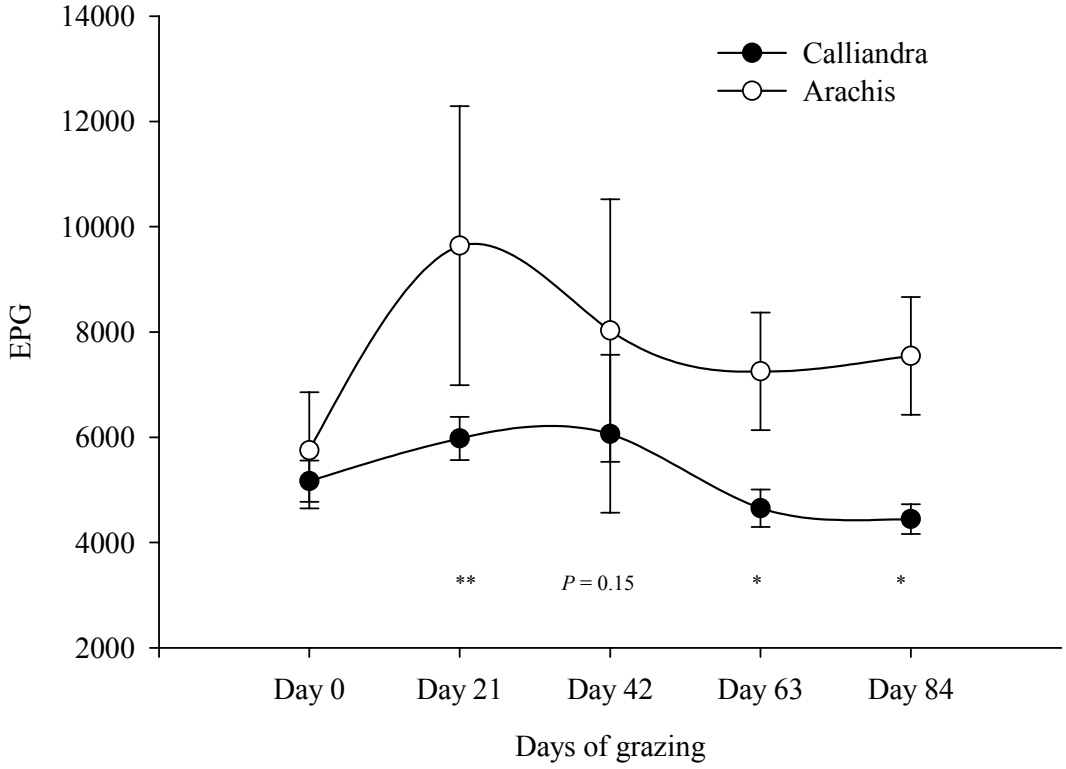


Figure 11. Means of fecal egg count per gram (EPG) of growing Boer goats and variations. Results are the mean of four determinations (n = 4), and error bars represent standard error of the mean (SEM). * $P < 0.05$; ** $P < 0.01$.

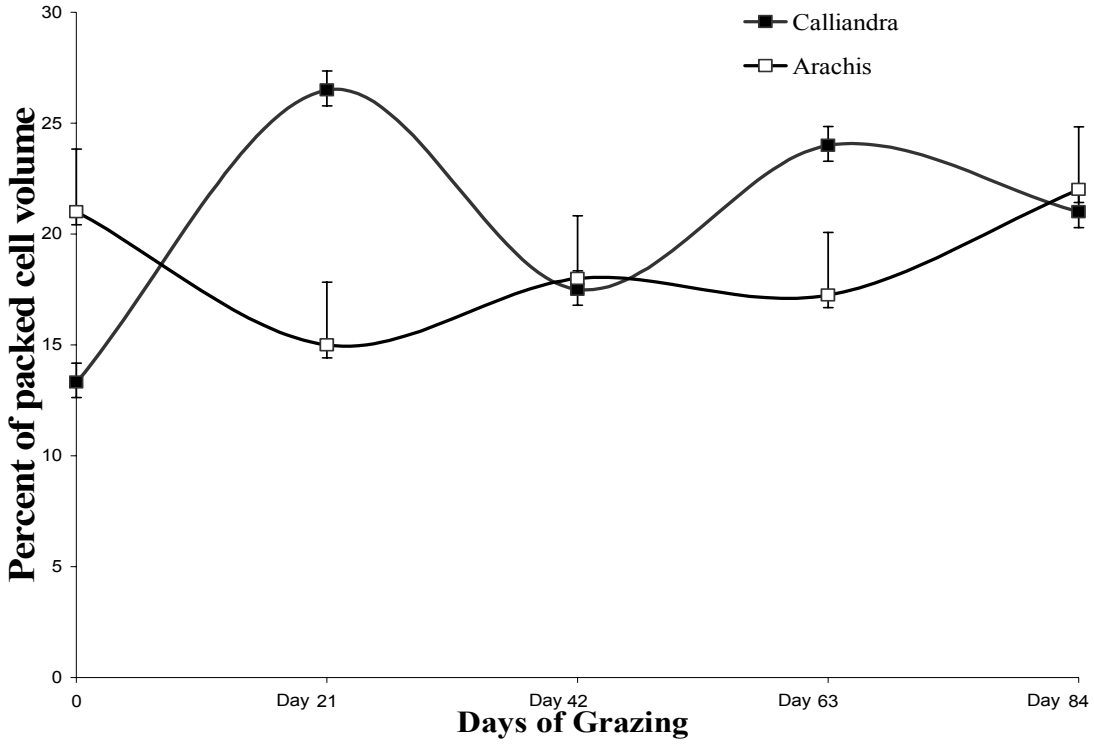


Figure 12**.** Percent blood packed cell volume of growing Boer goats with natural infestations of *Haemonchus contortus* supplemented with *Calliandra calothyrsus* and *Arachis pintoi*. Overall difference between groups was not significant ($P = 0.13$).

5.2. Experiment 2 *in vitro*- Larval migration inhibition (LMI) assay

The results of the LMI assay using CT extracted from three different tropical forages and three commercially purified CT monomers are presented in Table 11.

Table 11. Effect of concentration of purified CT (mg/mL) from three tropical legumes on the larval migration inhibition rates (% LMI) of infective third-stage (L_3) larvae of *Haemonchus contortus in vitro*.

Items	**% LMI**	**SEM**
Tropical legumes CT		
Acacia angustissima	41.65[a]	5.19
Sericea lespedeza	28.65[b]	10.89
Calliandra calothyrsus	47.7[a]	8.78
CT concentration (mg/mL)		
2	37.3[a]	4.25
4	41.4[a]	3.18
	***P*-value**	
Tannins	0.05	
Dose	0.001	
Tannins x dose	0.33	

Table 12. Effect of concentration of CT monomers (mg/mL) on the larval migration inhibition rates (% LMI) of infective third-stage (L_3) larvae of *Haemonchus contortus in vitro*.

Items	**% LMI**	**SEM**
CT monomers		
Catechin	24.06[b]	11.21
Ellagic acid	37.3[a]	3.86
Gallic acid	25.2[b]	4.02
CT concentration (mg/mL)		
1	19.2[c]	8.33
2	30.4[b]	2.44
4	37.0[a]	4.55
	***P*-value**	
Tannins	0.07	
Dose	0.001	
Tannins x dose	0.45	

In the presence of the lower and higher concentrations of purified tannin (2 and 4 mg/ml), LMI rates of *H. contortus* were greater at the higher concentration for *Acacia angustissima;* (41.65 mg/mL); ($p < 0.01$) and Calliandra; (47.7 mg/mL); ($p < 0.001$) extracts. CT monomers were used in an attempt to determine which tannin components are active in the three CT-containing forages extracts. Both, tropical legumes and CT monomers depends on tannin concentrations; (mg/mL). Ellagic acid showed the greater response for the larval migration inhibition rates in comparison with catechins and gallic acid (Table 12). Otherwise, a greater dose increased the response with two of the hydrolysable tannin monomers; ellagic acid ($P < 0.001$) and gallic acid ($P < 0.01$). No significant linear dose response was found for catechin ($P = 0.21$) and no interaction was found for tropical legumes ($P = 0.33$) and CT monomers ($P = 0.45$).

LMI of *Haemonchus contortus* by *Acacia angustissima* extract exhibited a negative linear dose dependent ($P < 0.01$) effect at 2 and 4 mg/mL. Ranking monomers as to their inhibitory activity was ellagitannins > gallotannins > catechins (Table 12), showing higher tannin concentration increases larval migration inhibition as well.

6.0. Discussion

The current study was carried out to investigate the effects of supplementation with two tropical leguminous forages that contain different levels of CT on weight gains, FEC dynamics and anemia status in goats grazing tropical grass; and secondly to quantify *in vitro* larval activity inhibition of *Haemonchus contortus* by tannin extracts and tannin monomers. The most significant result of Exp.1 was a decrease in FEC (5262 vs. 7644 epg) and a concurrent increase in ADG (11.1 vs. -34.7 g) observed in the group supplemented with Calliandra compared to Arachis. However, the weight gain results are based on small body weight changes in few animals per treatment and require further verification. In this and other studies Calliandra feeding has led to reductions in the fecal egg output in goats parasitized naturally with *Haemonchus contortus* and other classes of nematodes such as *Trichostrongylus colubriformis*. Acero (2007) also demonstrated the beneficial supplementation effect of Calliandra versus guineagrass in terms of fecal egg reductions in Boer goats. Animals supplemented with Calliandra showed a lower FEC of 638.16 epg compared with those animals supplemented with guineagrass (982.13 epg). This reduction in fecal egg excretion could result in reduced pasture GIN contamination.

Calliandra (12.04% CT), showed sufficient anthelmintic potential to adequately control internal parasites in Boer goats under the tropical grazing conditions of this study. Conversely, Arachis has insufficient CT content (1.9%) to be useful as an anthelmintic under the same conditions. Otherwise, Arachis is considered a better leguminous forage in terms of nutritive value compared to Calliandra. In spite of this fact, animals supplemented with Arachis showed a net loss in BW, while those fed Calliandra

registered a modest ADG. Over the 84-day experimental period animals supplemented with Arachis rejected or left uneaten on average 170.8 g daily of the 500 g of fresh forage offered, compared to the lesser amount of rejection in those supplemented with Calliandra (65.06 g) (Appendix 3). Thus, possibly, *Haemonchus contortus* larval infestations increased in animals supplemented with Arachis, because they were only consuming around 6.25 g daily of tannins from the legume compared to animals supplemented with high tannin-containing Calliandra (52.36g of CT).

It is of interest to screen common tropical forages used as supplements to determine which are effective in both parasite control and improving animal performance. Condensed tannin-containing forages have recently been proposed as an alternative for internal parasite control (Min and Hart, 2003; Min et al. 2003). Studies in which *Sericea lespedeza* hay was fed *ad libitum* to confined sheep and goats with natural and artificial infestations of internal parasites, have demonstrated the inhibitory effect of CT on FEC and worm burdens, principally through reduced worm fecundity (Shaik et al. 2004; Lange et al. 2005). Likewise, in the present study Boer goats grazing *Panicum maximum* pasture and fed the CT-containing tropical legume Calliandra had reduced FEC compared to those fed the low-CT legume Arachis. The *in vivo* results of Exp. 1, which showed that CT-containing Calliandra was more efficacious than low-CT Arachis in decreasing FEC are supported by the *in vitro* LMI results of Exp. 2, in which the presence of 2 and 4 mg/ml of purified tannin extracted from Calliandra increased inhibition rates of *H. contortus* larval activity. However, the reasons for the effect of CT in forages on gastrointestinal parasites are not clear. *Sericea lespedeza* (15.2 % CT) and *Hedysarum*

coronarium (Sulla: 12.5% CT) are the most common forages used in experiments to investigate this effect contained relatively high levels of CT (55 g of CT/kg of DM) (Terrill et al. 1989; Kahiya et al. 2003).

Some authors have suggested that inhibition of the establishment and fecundity of nematode parasites depends on the efficiency of the forage tannin in binding dietary protein in the gastro-intestinal tract of the host animal (Molan et al., 1999, 2000). The ability of tannins to bind protein in the rumen and release it post-ruminally is believed to cause a reduction in worm egg counts and enhance the expression of immunity in parasitized animals (Bown et al. 1986; Coop and Holmes, 1996; Min et al. 2005). Tannins that remain unbound to protein may have important antinutritional effects by causing changes in the intestinal micro-structure and inhibiting nutrient digestion and absorption in the small intestine. The immunological status of the host may also influence the course of nematode infections.

The inhibitory effects of CT extracted from three legumes and CT monomers on *Haemonchus contortus* was determined using the LMI assay. By measuring the migration of L_3 larvae through 20 μm pore-size sieves, it was possible to demonstrate that commercially available CT monomers have inhibitory effects on the migration of these larvae, as evidenced by prevention of their passage through the sieves. In the LMI assay, ellagic and gallic acids were more effective than the catechin monomer. Molan et al., (2003) reported that the flavan-3-ol gallates were more effective than the flavan-3-ols at immobilizing the infective stage of larvae of *Trichostrongylus colubrifomis*. These

results may indicate that antiparasitic activities of CT are not significantly related to their isomeric structure (2, 3-*cis* or 2, 3-*trans*), but rather to the number of hydroxyl groups in the B-ring (prodelphnidin: procyanidin polymers ratio). The present results showed that the CT-containing forage Calliandra reduced FEC and exhibited anti-larval activity *in vitro*. This forage fed as a supplement could result in a reduction of pasture contamination due to obstructed larval development or a possible direct effect on L_3 larval activity in the gastrointestinal tract. Further studies are required to determine how CT-containing forages can be utilized in a grazing system to take full advantage of their antiparasitic properties while also permitting reduced anthelmintic usage without decreasing animal production.

The available evidence indicates that feeding small ruminants with Calliandra can effectively decrease the nematode parasite infection levels in the gastrointestinal tract. A direct anthelmintic effect can be claimed when there is evidence of extreme reduction in parasite egg viability and larval development in the feces. In the present case, this anthelmintic activity of forage-CT was demonstrated by feeding the forage in Exp.1 and by the *in vitro* inhibition of larval migration caused by Calliandra CT extract in Exp. 2.

Kaplan et al. (2004) reported that the FAMACHA method is a very useful tool for identifying anemic sheep and goats in the Southern region of the USA. These authors considered animals with FAMACHA eye score values of 3, 4, and 5, and PCV values of ≤ 19 to be anemic. In the present study, both FAMACHA and PCV data in goats supplemented with Arachis indicated a condition close to anemic (2.9 and 20.5, respectively), whereas better scores were obtained with Calliandra supplementation. The

present study constituted a validation of FAMACHA for use with growing Boer goats in Puerto Rico. The FAMACHA data obtained lend support to the effectiveness of Calliandra in preventing anemia development. However, it is important that the FAMACHA method be tested in other regions before its use is broadly recommended.

7.0. Conclusions

- Growing Boer goats grazing grass pasture and supplemented daily with 500 g of CT-containing (12.04%) Calliandra had lower FEC than those supplemented with a like amount of low-CT (1.9%) Arachis, thus Arachis is not a good supplement for combating parasitism, whereas Calliandra has an anthelmintic potential to control internal parasites in small ruminants.

- Supplementation with high CT-containing Calliandra resulted in normal FAMACHA scores and PCV values and thus was useful for prevention of anemia in naturally parasite-infested goats.

- The larval activity inhibition seen *in vitro* indicates that the anthelmintic activity of forage-CT tannin may be attributable to a direct effect on L_3 larval activity in the gastrointestinal tract.

8.0 Implications

- The use of certain tropical forages as supplements or possibly as primary grazing forages is a promising strategy in tropical livestock systems to reduce the impact of internal parasites in small ruminants, resulting in better animal productivity and efficiency, and thus should be promoted.

- Feeding forages rich in CT and applying the FAMACHA technique to monitor parasite infection are valuable components of integrated internal parasite management in small ruminants and should be made use of by local producers.

- More studies are needed to describe how other tropical forages can reduce internal parasitism and to determine the role of high tannin-containing forages in the scheme of control.

9.0. Literature Cited

Abbott, E.M., Parkins, J.J., and Holmes, P.H., 1985. Influence of dietary protein on parasite establishment and pathogenesis in Finn Dorset and Scottish Blackface lambs given a single moderate infection of *Haemonchus contortus*. Res. Vet. Sci. 38, 6-3.

Acero, A., 2007. Evaluación de dos estrategias de alimentación en ganado caprino: Vigorización energética ("Flushing") en hembras reproductoras y uso de *Calliandra calothyrsus* en cabros destetados. Tesis de Maestría; Universidad de Puerto Rico, Recinto Universitario de Mayagüez.

Aerts, R.J., Barry, T.N., and McNabb, W.C., 1999. Polyophenols and agriculture: effects of proanthocyanidins in forages. Agri. Eco and Environ. 75, 1-12.

Amarante, A. F.T., 2001. Controle de endoparasitoses de ovinos. Reunião Anual da Sociedade Brasileira de Zootecnia. 39, 461-471.

Anderson, M.S., Peters, M., Schultze-Kraft, R., Franco, L.H., and Lascano, C.E., 2006. Phenolical, agronomic and forage quality diversity among germoplasm accessions of the tropical legume shrub *Cratylia argentea*. J. Agric. Sci. 144, 237-248.

Argel, P.J., and Lascano, C.E., 1998. *Cratylia argente*a (Desveaux) O. Kuntze: Una nueva leguminosa arbustiva para suelos ácidos en zonas subhúmedas tropicales. Pasturas Tropicales. 20, 37-43.

Argel, P.J., and Villarreal, M., 1998. Cultivar porvenir: Nuevo maní forrajero perenne (Arachis pintoi Krap. y Greg. nom.nud., CIAT 18744): Leguminosa herbácea para alimentación animal, el mejoramiento y conservación del suelo y el

embellecimiento del paisaje. Centro Internacional de Agricultura Tropical (CIAT), Consorcio Tropileche, Cali, CO. 23.

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. 15ed. Arlington, VA.

Athanasiadou, S., Kyriazakis, I., Coop, R.L., and Jackson, F., 1999. Evidence for direct anthelmintic effect of condensed tannins. In: Proceedings of the WAAVP Conference, Copenhagen, f.5.04.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, and R.L., 2000. Effects of short term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. Vet. Rec. 146, 728-732.

Athanasiadou, S., Kyriazakis, I., Jackson, F., and Coop, R.L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. J. Vet. Parasitol. 99, 205-219.

Bairden, K., Stear, M.J., Bishop, S.C., Gettinby, G., McKellar, Q.A., Park, M., Strain, S., and Wallace, D.S., 1997. The process influencing the distribution of parasitic nematodes among naturally infected lambs. J. Agric. Sci. 117, 165-171.

Bang, K.S., Familton, A.S., and Sykes, A.R., 1990a. Effect of copper wire particle treatment on establishment of major gastrointestinal nematodes in lambs. Vet. Sci. 49, 132-137.

Bang, K.S., Familton, A.S., and Sykes, A.R., 1990b. Effect of ostertagiaisis on copper status: a study involving the use of copper oxide wire particles. Vet. Sci. 49, 306-314.

Barger, I., Siale, K., Banks, D., and LeJambre, L., 1994. Rotational grazing for control of gastrointestinal nematode of goats in a wet tropical environment. J. Vet. Parasitol. 53 (1), 109-116.

Barras, A.E., and Sherman, D.E., 2004. Efficacy of a laser device for hazing Canada geese from urban areas of northeast Ohio. Ohio J. Sci. 104(3), 38-42.

Barrau, E., Fabre, N., Fouraste, I., and Hoste, H., 2005. Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia* scop) on the in vitro larval migration of *Haemonchus contortus*: a role of tannins and flavonol glycosides. Parasitol. 131, 531-538.

Barrell, N., Amouroux, I., Aprelon, R., and Samut, T., 1997. Résistance des strongles gastro intestinaux aux anthelminthinques dans les élevages caprins en Guadalupe (Antilles Frančaises). Revue d' Elevage et de Médecine Vétérinaire des Pays Tropicaux. 50, 105-110.

Barron, G.L., 1977. Observations on predatory fungi. Can. J. Botany. 57, 187-193.

Barry, T.N., 1985. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. Bri. J. Nutr. 54, 211 217.

Barry, T.N., 1989. Condensed tannins. Their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In The Roles of Protozoa and Fungi in Ruminant Digestion. JV Nolan, RA Leng and DI Demeyer, (eds). Penambul Books Armidale, NSW Australia. pp. 153-169.

Barry, T.N., and Blaney, B.J., 1987. Secondary compounds of forages. In: Hacker, J.B. and Ternouth, J.H. (eds). *Nutrition of Herbivores*. Academic Press, Sydney, Australia, pp 91-119.

Barry, T.N., and Fross, D.A., 1983. The condensed tannin content of vegetative *Lotus pedunculatus*, its regulation by fertilizer application and effect upon protein solubility. J. Sci. Food Agric. 34, 1047-1056.

Barry, T.N., and Manley, T.R., 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. Br. J. Nutr. 51: 493.

Barry, T.N., and Manley, T.R., 1986. Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus* sp. and their possible consequences in ruminant nutrition. J. Sci. Food Agric. 37, 248-254.

Barry, T.N., and McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. (Review article). Bri. J. Nutri. 81, 263-272.

Barry, T.N., McNeill, D.M., and McNabb, W.C., 2001. Plant secondary compounds: their impact on forage nutritive value and upon animal production. In: Gomide, J.A., Matos, W.R.S., da Silva, S.C. (Eds), Proceedings of XIX International Grasslands Congress, 2001/2002, Sao Paulo, Brazil, pp. 445-452.

Bath, G.F., and Van Wik, J.A., 2001. Using the FAMACHA© system on commercial sheep farms in South Africa, *Proceedings of the Fifth International Sheep Veterinary Congress*, Stellenbosch, South Africa.

Baudena, M.A., Chapman, M.R., Larsen, M., and Klei, T.R., 1998. Efficacy of the nematophagous fungus *Diddingtonia flagrans* in reducing equine cyathostome larvae on pasture in south Louisiana. J. Vet. Parasitol. 89, 219-230.

Bhat, T.K., Singh, B., and Sharma, O.P., 1998. Microbial degradation of tannins- a current prospective. Biodegradation 9, 343-357.

Bown, M.D., Poppi, D.P., and Sykes, A.R., 1986. The effect of post-ruminal infusion of protein or energy on the pathology of *Trichostrongylus colubriformis* infection and body composition in lambs. Proc. N.Z. Soc. Anim. Prod. 46, 27-30.

Broadhurst, R.B., and Jones, W.T., 1978. Analysis of condensed tannins using acidified vanillin. J. Sci. Food Agric., 29, 788-794.

Broderick, G.A., 1974. *In vitro* procedures for estimating rate of ruminal protein degradation and proportions of protein escaping the rumen undegraded. J. Nutr., 108, 181-190.

Brooker, J.D., O' Donovan, L.A., Skene, I., Clarke, K., Blackall, L., and Muslera, P., 1995. Streptococcus caprinus sp. nov., a tannin-resistant ruminal bacterium from feral goats. Lett. Appl. Microbiol., 18, 313-318.

Brunet, S., Aufrere, J., El Babili, F., Fouraste, I., and Hoste, H., 2006. The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of tannin rich plant extract (sainfoin) both in vitro and in vivo. J. Agric. Sci. 134, 1253 1262.

Burke, J.M., Miller, J.E., Olcott, D.D., Olcott, B.M., and Terrill, T.H., 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs. J. Vet. Parasitol. 123, 235-243.

Cabaret, J., 2000. Anthelmintic resistance in goats. From Fictions to Facts. 7th International Conference on Goats. 2, 793-794.

Castells, D., and Bonino, J., 2001. Evaluación del Moxidectin como dosificación estratégica del preparto en ovinos. J. Vet. Parasitol. 36, 17-22.

Chartier, C., Etter, E., Hetse, H., Pors, I., Koch, C., and Dellac, B., 2000. Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. Vet. Res. Commun. 24, 389-399.

Coop, R.L., and Field, A.C., 1983. Effect of phosphorus intake on growth rate, food intake, and quality of the skeleton on growing lambs infected with the intestinal nematode *Trichostrongylus colubriformis*. Vet. Sci. 35, 175-181.

Coop, R.L., and Holmes, P.H., 1996. Nutrition and parasite interaction. Int. J. Parasitol. 26, 951-962.

Coop, R.L., Richardson, K., Jackson, E., Bartley, D., and Jackson, F., 1998. Effects of amino acid supplementation on resilience of lambs infected with either abomasal or intestinal nematodes (Abstract). Novel approaches to the control of helminth parasites of livestock, Second Int. Conf., Baton Rouge, LA, March 1989.

Craig, T.M., 1988. Impact of internal parasites on beef cattle. J. Anim. Sci. 55, 1565 1569.

Dewey, P.J.S., Kimambo, A.E., and MacRae, J.C., 1988. The effect of daily challenge with *Trichostrongylus colubriformis* larvae on the nutrition and performance of immunological – resistant sheep. J. Vet. Parasitol. 28, 205-212.

D'Mello J.P.F., and Devendra, C., 1995. Tropical Legumes in Animal Nutrition. CAB International: Wallingford, Oxon., U.K.

Douglas, G.B., Wang, Y., Waghorn, G.C., Barry, T.N., Purchas, R.W., Foote, A.G., and Wilson, G.F., 1993. Liveweight gain and wool production of sheep grazing *Lotus corniculatus* and Lucerne (*Medicago sativa*). NZ. J. Agric. Res. 38, 95-104.

Duval, J., 1997. Accessed 07/26/07 at http://www.eap.mcgill.ca/publications/eap70.htm.

Dykes, G.S., Terrill, T.H., Shaik, S.A., Miller, J.E., Kouakou, B., Kannan, G., Burke, J.M., Kaplan, R.M., and Mosjidis, J.A., 2006. Effect of *Sericea lespedeza* hay on gastrointestinal nematode infection in goats. Proc. Am. Forage Grassl Counc. 15, 245-249.

Elard, L., Cabaret, J., and Humbert, J.F., 1999. PCR diagnosis of benzimidazole resistance in natural populations of the small ruminant parasite, *Teladorsagia circumcincta.* J. Vet. Parasitol. 80, 231-237.

Ephraim, E., Odenyo, A., and Ashenafi, M., 2005. Isolation and characterization of tannin degrading bacteria from fecal samples of some wild ruminants in Ethiopia. Anim. Feed Sci Technol. 118, 243-253.

Fahey, G.C., and Jung, H.G., 1989. Phenolic compounds in forages and fibrous feedstuffs. In: P.R. Cheeke (ed). Toxicants of Plant Origin. 4, 123-190.

Foo, L.Y., Jones, W.T., Porter L.J., and Williams V.M., 1982. Proanthocyanidin polymers of fodder legumes. Phytochemistry, 21, 933-935.

Foo, L.Y., Lu, Y., McNabb, W.C., Waghorn, G.C., and Ulyatt, M.J., 1997. Proanthocyanidins of *Lotus corniculatus*. Phytochemistry. 45, 1689-1696

Foo, L.Y., Newman, R., Waghorn, G.C., McNabb, W.C., and Ulyatt, M.J., 1996. The proanthocyanidins of *Lotus corniculatus*. Phytochemistry. 41, 617-624.

.

Fross, D.A., and Barry, T.N., 1984. The condensed tannin content of vegetative *Lotus pedunculatus*, its regulation by fertilizer application and effect upon protein solubility. J. Sci. Food Agric. 34, 1047-1056.

Gamble, H.R., Lichtenfels, R., and Purcell, J.P., 1989. Light and scanning microscopy of the ecdysis of *Haemonchus contortus* infective larvae. J. Parasitol. 75, 303-307.

Gibson, T.E., 1963. Experiments on the epidemiology of nematodiariasis. Res. Vet Sci. 4, 258-268.

Githori, J.B., Athanasiadou, S., and Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminthes in livestock with emphasis on small ruminants. J. Vet. Parasitol. 139, 308-320.

Githori, J.B., Höglund, J., and Waller, P.J., 2005. Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. Anim. Health Res. Rev. 6, 91-103.

Goel, G., Puniya, A.K., and Singh, K., 2005. Tannic acid resistance in ruminal streptococcal isolate. J. Basic Microbiol. 45, 243-245.

Gray, G.D., 1997. The use of genetically resistant sheep to control nematode parasitism. J. Vet. Parasitol. 72, 345-366.

Gray, G.D., and Gill, H.S., 1993. Host genes, parasites and parasitic infections. Inter J. Parasitol. 23, 485-494.

Guarrera, P.M., 1999. Traditional anthelmintic, antiparasitic and repellet uses of plants in central Italy. J. Ethnopharmacol. 68, 183-192.

Gutteridge, R.C., and Shelton, H.M., 1994. The role of forage tree legumes in cropping and grazing systems. In: Gutteridge, R.C., Shelton, H.M. (Eds.), Forage Tree Legumes in Tropical Agriculture. CAB International, UK, pp. 3-11.

Hagerman, A.E., 1988. Extraction of tannin from fresh and preserved leaves. J. Chem. Ecol. 14, 453-462.

Hagerman, A.E., 1992. Tannin-protein interactions. In: Ho, C-T., Lee, C.Y., Huang, M. T. (Eds). Phenolic Compounds in Food and their Effects on Health. 1. Analysis, Occurrence and Chemistry. American Chemical Society, Washington DC. 19, 236-247.

Hansen, J., and Perry, B., 1994. The epidemiology, diagnosis and control of helminth parasites of ruminants: A handbook. International Livestock Research Institute, Nairobi, Kenya.

Haslam, E., 1979. Vegetable tannins. Recent Adv. Phytochem. 12, 475-524.

Haslam, E., 1989. Plant Polyphenols- Vegetable Tannins. Cambridge University Press, UK.

Haslam, E., and Lilley, T.H., 1988. Natural astringency in foodstuffs - a molecular interpretation. CRC Crit. Rev. Food Sci. Nutr. 27, 1-40.

Hedqvist, H., Harvey Mueller, I., Reed, J.D., Krueger, G.C., and Murphy, M., 2000. Characterization of tannins and in vitro protein digestibility of several *Lotus corniculatus* varieties. Anim. Feed. Sci Tech. 87, 41-56.

Herring, H., Reed, J.D., and Hanson, J., 1996. Differences in *Sesbania sesban* accessions in relation to their phenolic concentration and high performance finger prints. J. Sci Food Agric. 71, 92-98.

Hess, H.D., Tiemann, T.T., Noto, F., Carulla, J.E., and Kreuzer, M., 2002. Strategic use of tannins as means to limit methane emission from ruminant livestock. International Congress Series. 1293, 164-167.

Holmes, P.H., 1985. Pathogenesis of trichostrongylosis. J. Vet. Parasitol. 18, 89-101.

Hoste, H., Gaillard, L., and Le Frileux, Y., 2005. Consequences of the regular distribution of sainfoin hay on gastrointestinal parasitism with nematodes and milk production in dairy goats. Small Ruminant Res. 59, 265-271.

Hoste, H., Jackson, F., Athanasiadaou, S., Thamborg, S., and Hoskin, S., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends in Vet Parasitol. 20, 1471-4922.

Iason, G.R., Hodgson, J., and Barry, T.N., 1995. Variation in condensed tannin concentration of a temperate grass (*Holcus lanatus*) in relation to season and reproductive development. J. Chem. Ecol. 21, 1103-1112.

IVRI Annual reports (1986-1998). Ind. Vet. Res. Inst. Izatnajar (U.P.).

Jackson, F.S., McNabb, W.C., Barry, T.N., Foo, Y.L., and Peters, J.S., 1996. The condensed tannin content of a range of subtropical and temperate forages and the reactivity of condensed tannin with ribulose-1,5-biphosphate carboxylase (Rubisco) protein. J. Sci. Food Agric. 72, 483-492.

Jaffe, B.A., Santos, P.F., and Muldoon, A.E., 1997. Supression of nematophagous fungi by enchytraeid worms: a field exclosure experiment. *Oecology*. 112, 412-423.

James, L.F., Nielsen, D.B., and Panter, K.E., 1989. Impact of poisonous plants on the livestock industry. J. Range Manage. 45, 3-8.

John, A., and Lancashire, J.A., 1981. Aspects of feeding and nutritive value of Lotus species. Proc. NZ Grassl. Assoc. 42, 152.

Jones, V., 1993. Grazing to control worms. The New Farm, 15 (7): 52-53.

Judson, G.J., Brown, T.H., Gray, D., Dewey, D.W., Edwards, J.B., and McFarlane, J.D., 1982. Oxidized copper wire particles for copper therapy in sheep. Aust. J. Agric. Res. 33, 1073-1083.

Judson, G.J., Trengore, C.L., Langman, M.W., and Vandergraaff, R., 1984. Copper supplementation of sheep. J. Aust. Vet. 61, 40-43.

Kabagambe, E.K., Barras, S.R., Li, Y., Pena, M.T., Smith, W.D., and Miller, J.E., 2000. Attempts to control haemonchosis in grazing ewes by vaccination with gut membrane proteins of the parasite. Vet. Parasitol. 92, 15-23.

Kahiya, C., Mukaratirwa, S., and Thamsborg, S.M., 2003. Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. Vet. Parasitol. 15, 265-274.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends in Vet. Parasitol. 20, 477-481.

Kaplan, R.M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williams, L.H., Larsen, M., and Vatta, A.F., 2004. Validation of FAMACHA eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol. 123, 105-120.

Kaplan, R.M., and Miller, J.E., 2004. FAMACHA information guide. Southern Consortium for small ruminant parasite control. www.scsrpc.org.

Kerry, N.L., and Abbey, M., 1997. Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation *in vitro*. Atherosclerosis. 135, 93-102.

Knox, M.R., 2002. Effectiveness of copper oxide wire particles for *Haemonchus contortus* control in sheep. Aust. Vet. J. 80, 224-227.

Knox, D.P., Redmond, D.L., and Newlands, G.F., 2003a. The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. Int. J. Parasitol. 33, 1129-1137.

Knox, P., Redmond, D., Newlands, G., Suke, P., Pettit, D., and Smith, W., 2003b. The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. Int. J. Parasitol. 33, 1129-1137.

Koupai-Abyazani, M.R., McCallum, J., Muir, A.D., Bohm, B.A., Towers, G.H.N., and Gruber, M.Y., 1993. Developmental changes in the composition of proanthocyanidins from leaves of sainfoin (*Onobrychis viciifolia* Scop.) as determined by HPLC analysis. J. Agric. Food Chem. 41, 1066-1070.

Kyriazakis, I., Oldham, J.D., Coop, R.L., and Jackson, F., 1994. The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. Bri. J. Nutri. 72, 665-677.

Lange, K., Olcott, D., Miller, J.E., Mosjidis, J.A., Terrill, T.H., and Burke, J.M., 2005. Effect of the condensed tannin-containing forage, sericea lespedeza, fed as hay, on natural and experimental challenge infection in lambs. Southern Sec. ASAS Mtg., Little Rock, AR, pp. 15-16. (Abst.)

Lange, K., Olcott, D.D., Miller, J.E., Mosjidis, J.A., Terrill, T.H. Burke, J.M., and Kearney, M.T., 2006. Effect of sericea lespedeza (*Lespedeza cuneata*) fed as hay, on natural and experimental *Haemonchus contortus* infections in lambs. J. Vet. Parasitol. 141, 273-278.

Langlands, J.P., Bowles, J.E., Donald, G.E., Smith, A.J., and Paull, D.R., 1983. Copper oxide particles for grazing sheep. Aust. J. Agric. Res. 34, 751-765.

Larsen, M., Faedo, M., Waller, P.J., and Hennessy, D.R., 1998. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: studies with *Duddingtonia flagrans*. J. Vet. Parasitol. 76, 121-128.

Larsen, M., Nansen, P., Grønvold, J., Wolstrup, J., and Henriksen, S.A., 1997. Biological control of gastrointestinal nematodes- facts, future or fiction? J. Vet. Parasitol. 72, 479-492.

Lascano, C.E., 1996. Calidad nutritiva y utilización de *Cratylia argentea*. In Potencial del Género Cratylia como Leguminosa Forrajera. E.A. Pizarro & L. Coradin (eds). pp 83-97.

Lichtenfels, J.R., Hoberg, E.P., and Zarlenga, D.S., 1997. Systematics of gastrointestinal nematodes of domestic ruminants: advances between 1992 and 1995 and proposals for future research. Vet. Parasitol. 72, 225-238.

Lopez, J., Tejada, I., Vasquez, C., Garza, J.D., and Shimada, A., 2004. Condensed tannins in humid tropical fodder crops and their *in vitro* biological activity. Part 1. J. Sci. Food Agric. 84, 291-294.

Lowry, J.B., McSweeney, C.S., and Palmer, B., 1996. Changing perceptions of the effect of plant phenolics on nutrient supply in the ruminant. Aust. J. Agric. Res. 47, 829-842.

Lowther, W.L., Manley, T.R., and Barry, T.N., 1987. Condensed tannin concentrations in *Lotus corniculatus* and *L. pedunculatus* cultivars grown under low soil fertility conditions. NZ J. Agric. Res. 30, 23-25.

Lymon, R. and Longnecker, M. 2001. An introduction to statistical methods and data analysis. Fifth edition. Texas A & M University, Duxbury Thomson Learning.

Machen, R., 1993. A *Haemonchus contortus* management plant for sheep and goats in Texas. Texas Agricultural Extension Service. The Texas A&M University System. Accessed 07/26/07 at www.boergoats.com/clean/article.

Makkar, H.P.S., Singh, B., and Dawra, R., 1988. Effect and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49, 241-256.

McSweeney, C.S., Palmer, B., McNeill, D.M., and Krause, D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci. Technol. 91, 81-93.

Min, B.R., Attwood, G.T., Reilly, K., Sun, W., Peters, J.S., Barry, T.N., and McNabb, W.C., 2002. *Lotus corniculatus* condensed tannins decrease in vivo populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. Can. J. Microbiol. 48, 911-921.

Min, B.R., Barry, T.N., Attwood, G.T., and McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: review. Anim. Feed Sci. Technol. 106, 3-19.

Min, B.R., Barry, T.N., Wilsom, P.R., and Kemp, P.D., 1997. The effect of grazing chicoryand birdsfoot trefoil on venison and velvet production by young red and hydride deer. NZ. J. Agric. Res. 40, 325-347.

Min, B.R., and Hart, S.P., 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81, E102-E109.

Min, B.R., Hart, S.P., Miller, D., Tomita, G.M., Loetz, E., and Sahlu, T., 2005. The effect of grazing forage containing condensed tannins on gastrointestinal parasite infection and milk composition in Angora goats. J. Vet. Parasitol. 130, 105-113.

Min, B.R., McNabb, W.C., Barry, T.N., and Peters, J.S., 2000. Solubilisation and degradation of ribulose-1,5-biphosphate carboxylase/oxygynase (EC 4.1.1.39; Rubisco) protein from white clover (*Trifolium reprense*) and *Lotus corniculatus* by rumen microorganisms and the effect of condensed tannins on these processes. J. Agri. Sci. Camb. 134, 305-317.

Min, B.R., Pinchak, W.E., Anderson, R.C., Fulford, J.D., and Puchala, R., 2006. Effects of condensed tannins supplementation level on weight gain and *in vitro* bloat precursors in steers grazing winter wheat. J. Anim. Sci. 84, 2546-2554.

Min, B.R., Pomroy, W.E., Hart, S.P., and Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. Small Rum. Res. 51, 279 283.

Molan, A.L., Hoskin, S.O., Barry, T.N., and McNabb, W.C., 2000. Effect of condensed tannins extracted from four forages on the viability of the larvae of deer lungworms and gastrointestinal nematodes. Vet. Rec. 146, 44-48.

Molan, A.L., Meagher, L.P., Spencer, P.A., and Sivakumaran, S., 2003. Effect of flavan 3ols on in vitro egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. Int. J. Parasitol. 33, 1691-1798.

Molan, A.L., Waghorn, G.C., and McNabb, W.C., 1999. Condensed tannins and gastrointestinal parasites in sheep. Proc. NZ. Grassland Assoc. 61, 57-61.

Morris, C.A., 1998. Responses for selection to disease resistance in sheep and cattle in New Zealand and Australia. In: Proc. Sixth World Congress on Genetics Applied to Livestock Production. 27, 295-302.

Mueller-Harvey I. 2001. Analysis of hydrolysable tannins. Anim. Feed Sci. Technol., 91, 3-16.

Murdati, T.B., McSweeney, C.S., and Lowry, J. B., 1992. Metabolism in sheep of gallic acid, tannic acid and hydrolysable tannin from *Terminalia oblongata*. Aust. J. Agric. Res. 43, 1307-1319.

Nelson, K. E., Pell, A.N., Schofield, P., and Zinder, S., 1995. Isolation and characterization of an anaerobic ruminal bacterium capable of degrading hydrolysable tannin. Appl. Environ. Microbiol. 61, 3293-3298.

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Whaghorn, G.C., and Green, R., 2002a. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. J. Vet. Parasitol. 105, 229-245.

Niezen, J.H., Waghorn, G.C., and Charleston, W.A.C., 1998. Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (*Lotus pedunculatus*) or perennial ryegrass (*Lolium perenne*). J. Vet. Parasitol.78, 13-21.

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G., and Waghorn, G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agric. Sci. Camb. 125, 281-289.

Niezen, J.H., Waghorn, G.C., and Graham, T., 2002b. The effect of diet fed to lambs on subsequent development of *Trichostrongylus colubriformis* larvae *in vitro* and on pasture. J. Vet. Parasitol. 105, 269-283.

Ortega, S., Bennet, J.M., and Cornell, J.A., 1992. Rhizome characteristics and canopy light interception of grazed rhizome peanut pastures. J. Agron. 84, 804-809.

Overend, D.J. and Bowen, F.L., 1995. Resistance of *Fasciola hepatica* to triclabendazole. J. Aust. Vet. 72, 275-276.

Palm, C.A., and Sanches, P.A., 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biol. Biochem. 23, 83-88.

Paolini, V., Bergeaud, J.P., and Grisez, C., 2003. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. J. Vet. Parasitol. 113, 253 261.

Parkins, J.J., and Holmes, P., 1989. Effects of gastrointestinal helminth parasites on ruminant nutrition. Nutri. Res. Rev. 2, 227-246.

Peters, M., Schultze-Kraft, R., Anderson, M.S., Franco, L.H., and Lascano, C.E., 2002. Phenolical, agronomic and forage quality diversity among germoplasn accessions of the tropical legume shrub *Cratylia argentea*. J. Agric. Sci. Camb. 144, 237 248.

Prichard, R., 1994. Anthelmintic resistance. J. Vet. Parasitol. 54, 259-268.

Prine, G.M., Dunavin, L.S., Moore, J.E., and Roush, R.D., 1981. Florigraze rhizome peanut,a perennial forage legume. Fla. Agric. Exp. Sta. Circ. S-275. Univ of Florida, Gainesville.

Rabel, B., McGregor, R., and Douch, P.G.C., 1994. Improved bioassay for estimation of inhibitory effects of ovine gastrointestinal mucus and anthelmintics on nematode larval migration. Int. J. Parasitol. 24, 671-676.

Rakhmani, S.I., Brooker, J.D., Jones, G.P., and Palmer, B., 2005. Composition of condensed tannins from *Calliandra calothyrsus* and correlation with sacco digestibility. Anim. Feed Sci. Technol. 121, 109-124.

Redmond, D.L., Newlands, G.F., Knox, D.P., Skuce, P.J., Pettit, D., and Smith, W.D., 2003. The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. Int. J. Parasitol. 11, 1129-1137.

Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73, 1516-1528.

Reed, J.D., Soller, H., and Woodward, A., 1990. Fodder tree and straw diets for sheep: Intake, growth, digestibility and the effects of phenolics and nitrogen utilization. Anim Feed. Sci. Technol. 30:39.

Robertson, A.P., Bjorn, H.E., and Martin, R.J., 1999. Resistance to levamisole resolved at the single-channel level. FASEB Journal. 13, 749-760.

Robertson, H.A., Niezen, J.H., Waghorn, G.C., Charleston, W.A.C., and Jinlong, M., 1995. The effect of six herbages on liveweight gain, wool growth and faecal egg count of parasitized ewe lambs. Proc. NZ Soc. Anim. Prod. 55, 199-201.

Rogers, W.P., 1982. Enzymes in the exsheathing fluid of nematodes and their biological significance. Int. J. Parasitol. 12, 495-502.

Salt Institute Annual Meeting. 2002. Key Biscayne, Florida.

Sani, R.A., Chong, D.T., Halim, R.A., Chandrawathni, P., and Rajamanickam, C., 1995. Control of gastrointestinal Strongylosis by grazing management. International Conference "Novel Approaches to the Control of Helminth Parasites of Livestock" (Abstracts). University of New England. Armidale. NSW. Aust. 61.

SAS Institute, 1992. SAS/SAT Software: Changes and Enhancements. Release 6.07, SAS Technical Report, SAS Institute Cary, NC.

Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., and Kallu, R.K., 2004. Effects of feeding *Sericea lespedeza* hay to goats infected with *Haemonchus contortus*. South African J. Anim. Sci. 34, Supplement 1.

Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., Kaplan, R.M., Burke, J.M., and Mosjidis, J., 2006. Sericea lespedeza hay as a natural deworming agent

against gastrointestinal nematode infection in goats. J. Vet. Parasitol. 139, 150 157.

Shelton, H.M., 2001. Advances in forage legumes: Shrub legumes. XIX International Grassland Congress, São Pedro, São Paulo, Brazil. pp. 549 556.

Skene, I.K., and Brooker, J.D., 1995. Characterization for tannin acyl hydrolase activity in ruminal bacterium, *Selenomonas ruminantium*. Anaerobe, 1, 321-327.

Smith, W.D.S., and Zarlenga, D.S., 2005. Developments and hurdles in generating vaccines for controlling helminth parasites of grazing ruminants. J. Vet. Parasitol. 16, 231-241.

Stuedemann, J.A., Kaplan, R.M., Miller, J.E., and Seman, D.H., 2005. Importance of nematode parasites in cattle grazing research. Proc. 59th Southern Pasture and Forage Crop Improvement Conference, Philadelphia, MS.

Suttle, N.F., Knox, D.P., Jackson, F., Coop, R. L., and Angus, K.W., 1981. Effects of the dietary molybdenum on nematode and host during *Trichostrongylus vitrinus* infection in lambs. Res Vet. Sci. 52, 224-229.

Suttle, N.F., Knox, D.P., Jackson, F., Coop, R.L., and Angus, K.W., 1992. Effects of dietary molybdenum on nematode and host during *Haemonchus contortus* infection in lambs. Res. Vet. Sci. 52, 230-235.

Swain, T., 1965. The tannins. In Plant Biochemistry, Academic Press, New York. pp. 552-580.

Swain, A., 1979. In G.A. Rosenthal and D.H. Danzen (ed), Hervibores; Their interaction with secondary plant metabolites. Academic Press. London and New York, pp. 657-682.

Sykes, A.R., 1983. Effects of parasitism on metabolism in the sheep. In: Hanesya W. (ed). Sheep Production. Butterworths, London. pp: 317-334.

Terrill, T.H., Rowan, A.M., Douglas, G.B., and Barry, T.N., 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food. Agric. 58, 321-329.

Terrill, T.H., Windham, W.R., Hoveland, C.S., and Amos, H.E., 1989. Influence of forage preservation method on tannin concentration, intake and digestibility of *Sericealespedeza* by sheep. J. Agron. 81, 435-439.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Sumere, C.F., Albrecht, J., Dedonder, A., DePooter, H., and Pé, I., 1975. Plant proteins and phenolics. In J.B. Harborne and C.F. Van Sumere, (eds). Annu. Proc. Phytochem. Soc. 11, 211-264.

Van Wyk, J.A., 2001. Refugia: overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort J. Vet. Res. 68, 55-67.

Wagland, B.M., Jones, W.O., Hribar, L., Bendixen, T., and Emery, D.L., 1992. A new simplified assay for larval migration inhibition. Int. J. Parasitol. 22, 1183-1185.

Waghorn, G.C., and Jones, W.T., 1989. Bloat in cattle. Potential of dock (*Rumex obtusifolius*) as an anti-bloat agent for cattle. N.Z. J. Agric. Res. 32, 227–235.

Waghorn, G.C., and McNabb, W.C., 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. Proc. Nutrition Society. 62, 383-392.

Waghorn, G.C., Ulyatt, M.J., John, A., and Fisher, M.T., 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed *Lotus corniculatus* L. Bri. J. Nutri. 57, 115-126.

Walton, J.P., Waghorn, G.C., Plaizier, J.C., Birtles, M., and Mcbride, B.W., 2001. Influence of condensed tannins on gut morphology in sheep fed *Lotus pedunculatus*. Can. J.Anim. Sci. 81, 605-607.

Wallace, D.S., Bairden, K., Duncan, J.L., Eckersall, P.D., Fishwick, G., Holmes, P.H., McKellar, Q.A., Mitchell, S., Parkins, J.J., and Stear, M.J., 1999. The influence of increased feeding on the susceptibility of sheep to infection with *Haemonchus contortus*. J. Anim. Sci. 69, 457-463.

Wang, Y., Waghorn, G.C., Barry, T.N., and Shelton, I.D., 1994. The effect of condensed tannins in *Lotus corniculatus* upon plasma metabolism of methionine, cysteine and inorganic sulphate by sheep. Bri. J. Nutri. 72, 923-935.

Wells, R.D., Hicks, B.J., Bannon, H.J., Dyer, J and Teal, P., 2000. Impacts of grass carp on the biota of Churchill East drain. Unpublished *NIWA Client report DOC00231*. National Institute of Water and Atmosperic Research, Hamilton, N.Z. 53.

Williams, M., Hammond, J.A.C., Kunkle, W.E., and Spreen, T.H., 1991. Stocker performance on continuously stocked grazed mixed grass-rhizoma peanut and bahiagrass pastures. J. Prod. Agric. 4, 19-24.

Windon, R.G., and Dineen, J.K., 1984. Parasitological and immunological competence of lambs selected for high and low responsiveness to vaccination with irradiated *Trichostrongylus colubriformis* larvae. In: Dinnen, J.K., Outteridge, P.M. (Eds.),

Immunogenetic Approaches to Control of Endoparasites. CSIRO, Melbourne, pp. 13-28.

Witlock, H.V., 1948. Some modifications of the McMaster helminth egg-counting technique apparatus. J. Counc. Sci. Ind. Res. 21, 177-180.

Zucker, W.V., 1983. Tannins: does structure determine function? An ecological perspective. Am. Nat. 121, 335-365.

10.0 Appendices

Appendix 1. Initial and final liveweight (LW) of eight animals used in Experiment 1

Animal ID	Type of Supplementation (0.5 kg fresh/day)	Initial liveweight (kg)	Final liveweight (kg)
134	*Calliandra calothyrsus*	13.63	14.04
132	*Calliandra calothyrsus*	13.63	15.00
133	*Calliandra calothyrsus*	15.0	15.91
128	*Calliandra calothyrsus*	15.45	.
130	*Arachis pintoi*	18.18	15.91
135	*Arachis pintoi*	19.09	15.45
126	*Arachis pintoi*	17.73	17.27
136	*Arachis pintoi*	20.45	15.45
Average	*Calliandra calothyrsus*	**14.43** n = 4	**15.00** n = 3
Average	*Arachis pintoi*	**18.86**	**16.02**

Final liveweight for animal #128 is missing as this animal died before the experiment ended.

Appendix 2. Means of FAMACHA score and PCV value of eight animals used in Experiment 1.

Animal ID	Famacha Score	PCV value	Type of supplementation
134	3	26.50	*Calliandra calothyrsus*
132	2	17.50	*Calliandra calothyrsus*
133	2	24.00	*Calliandra calothyrsus*
128	2	21.00	*Calliandra calothyrsus*
130	2	15.00	*Arachis pintoi*
135	3	18.00	*Arachis pintoi*
126	3	17.25	*Arachis pintoi*
136	3	22.00	*Arachis pintoi*

Appendix 3. Mean amounts of supplemental forage rejected and consumed daily during the 84-day experimental period

Calliandra calothyrsus (12.04% CT) supplementation at 500 g/day

Animal ID	Feed rejected	Feed consumption
134	95.19 g	404.81 g
132	21.77 g	478.23 g
133	74.69 g	425.31 g
128	68.61 g	431.38 g
Average	**65.06 g**	**434.93 g**

Arachis pintoi (1.9% CT) supplementation at 500 g/day

Animal ID	Feed rejected	Feed consumption
130	145.62 g	354.38 g
135	209.10 g	290.90 g
126	184.33 g	315.67 g
136	144.53 g	355.47 g
Average	**170.80 g**	**329.10 g**

Calliandra calothyrsus: (434.93 g/d) * (0.1204 tannin) = 52.36 g tannin/d

Arachis pintoi: (329.10 g/d) * (0.019 tannin) = 6.25 g tannin/d

Appendix 4. Larval migration inhibition (%LMI) datasheet of commercial CT monomers and CT extracted from three legumes

CT monomers	Control	Dose	Replicates	% LMI
Catechin	104 94	-	1	22.22
Average	99			
		1	2	16.16
		2	1	13.13
		2	2	27.27
		4	1	16.16
		4	2	49.49
Ellagic acid	99	1	1	21.21
		1	2	20.20
		2	1	52.52
		2	2	39.39
		4	1	41.41
		4	2	49.49
Gallic acid	99	1	1	13.13
		1	2	22.22
		2	1	26.26
		2	2	24.24
		4	1	39.39
		4	**2**	26.26

CT extracted	Control	Dose	Replicates	% LMI
Acacia	70 45 48	2	1	4.23
Average	**54.33**	2	2	13.44
		2	1	37.38
		4	2	28.17
		4	1	39.22
		4	2	33.70
Lespedeza		2	1	11.60
		2	2	2.39
		2	1	0.55
		4	2	26.33
		4	1	44.75
		4	2	24.49
Calliandra		2	1	37.38
		2	2	22.65
		2	1	33.70
		4	2	2.39
		4	1	31.86
		4	2	41.06