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**ANTHELMINTIC EFFECTS OF TROPICAL SHRUB LEGUMES  
IN RUMINANT ANIMALS**

Thesis submitted by

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in October 2007

for the degree of Doctor of Philosophy

in the Australian Institute of Tropical Veterinary and Animal Science

School of Veterinary and Biomedical Sciences

James Cook University

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## **DECLARATION ON ETHICS**

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics, Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers A687\_01, A751\_02, A826\_03).

Keryn Cresswell

October 2007

## **CONTRIBUTIONS OF OTHERS**

Associate Professor Esala Teleni assisted with the design of the research presented in this thesis, provided editorial advice and assistance on the preparation of the thesis and is a co-author on papers arising from this thesis.

Dr Pat Pepper performed the statistical analysis of the data presented in Chapters 5 and 6 of this thesis.

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## ABSTRACT

Gastrointestinal (GI) nematode parasitism causes major economic losses and animal health problems in farmed livestock and thus poses a serious challenge to livestock production worldwide. Parasite control strategies historically have relied heavily on anthelmintic drenches, but with the emergence of anthelmintic resistance, new strategies are required. A number of forage species containing high concentrations of condensed tannins have been shown to have anthelmintic properties. The aim of the current study was to determine whether selected tropical legumes used as protein supplements for livestock, and in particular *Calliandra calothyrsus*, might also be useful for the control of GI nematode parasites.

A pilot study was undertaken initially to determine the effects of *Calliandra*, when fed to lambs as a sole diet, on burdens of *Haemonchus contortus* or *Trichostrongylus colubriformis* and on egg production by adult worms. Lucerne (*Medicago sativa*) pellets were used as a high protein control diet and Mitchell grass (*Astrebla* species) hay was used as a low protein control diet. The effects of diet on the worms were assessed by faecal egg counts and post-mortem worm counts. Worm burdens of each species and egg production by *H. contortus* were similar in lambs on all three diets. However, egg production of *T. colubriformis* was reduced by 85-90 % by feeding *Calliandra* to the host lambs and the number of eggs *in utero* of female *T. colubriformis* was significantly increased.

These results were confirmed and extended in a larger-scale feeding experiment, in which the Mitchell grass diet was omitted. A number of haematological parameters were measured to examine the nutritional status, resistance and resilience of the host lambs in response to the worm burdens. Because pH was expected to affect the ability of condensed tannins to bind to worms, the pH was recorded immediately after slaughter in each segment of the GI tract to examine the relationship between pH and faecal egg counts. Worm burdens of *H. contortus* were similar in lambs fed *Calliandra* ( $2237 \pm 395$  worms) and lucerne ( $1861 \pm 230$  worms). Worm burdens of *T. colubriformis* were also similar in lambs fed *Calliandra* ( $5718 \pm 339$  worms) and

lucerne ( $4861 \pm 452$  worms). However, egg production by both *H. contortus* and *T. colubriformis* were reduced by 64-84 % and 24-68 % respectively. There were no improvements in any of the haematological parameters measured attributable to the different diets, indicating that reduced egg counts were due to a direct toxic effect of the *Calliandra* diet and not due to improved resistance or resilience to the worms. The variation in pH in each of the GI segments was too small to identify any relationship between pH and reduced egg output by female worms.

A study was then conducted to confirm that the anthelmintic effects of *Calliandra* were due to the condensed tannins in the plant. Condensed tannins were extracted and purified from *Calliandra* leaves and incubated with *H. contortus* and *T. colubriformis* eggs and larvae in *in vitro* assays. Egg hatch and larval development rates were determined. *Calliandra* tannins delayed hatching and reduced egg hatch and larval development in both species. Development of infective larvae was almost completely inhibited at tannin concentrations above 300  $\mu\text{g/mL}$ .

*In vitro* assays were also used to screen a number of other tropical legumes for anthelmintic activity. Crude extracts of nine legume species, including *Calliandra* and lucerne pellets, and two different fractions of purified *Calliandra* were incubated with *H. contortus* and *T. colubriformis* eggs and larvae. In addition to *Calliandra*, *Leucaena leucocephala* and *Desmanthus virgatus* were identified as having possible anthelmintic activity against nematode eggs and larvae. The two *Calliandra* fractions both had similar anthelmintic activity to the *Calliandra* crude extract.

In an attempt to determine the mode of action of *Calliandra* condensed tannins against GI nematodes, a staining technique was developed to identify tannins histologically in mammalian and nematode tissues. Haematoxylin & Eosin (H&E), ferric chloride, butanol- $\text{H}_2\text{SO}_4$  and vanillin-HCl techniques were examined, initially using plant tissues, and then optimised for use in mammalian tissues. The H & E and vanillin-HCl techniques were then used to stain GI tissues and nematodes obtained from *Calliandra*-fed lambs. In the ovine GI tissue, condensed tannins were present in the lumen and in macrophages and giant cells in the lamina propria, particularly in the abomasum. Condensed tannins were observed on the outside of the cuticle of intact adult *H. contortus* and *T. colubriformis*, mainly in adherent digesta. Condensed



tannins were also observed in the pharynx and intestine of sectioned *H. contortus*, but no condensed tannins were observed in the reproductive tract.

A final experiment was conducted to determine the effects of *Calliandra* on the development, establishment and reproductive capacity of nematodes derived from eggs exposed to the legume in the host diet but with no subsequent exposure as adults. Eggs of *H. contortus* and *T. colubriformis* obtained from *Calliandra*-fed or lucerne-fed donor lambs were cultured *in vitro* to obtain infective larvae, which were used to infect groups of recipient lambs. Worm egg and larval production from the donor lambs, and adult worm egg output and worm burdens of the recipient lambs, were monitored. Although worm egg production and larval production from the donor lambs was reduced by the *Calliandra* diet, the ratio of eggs to larvae was also reduced. It was not clear whether this was due to problems with the experimental technique or to selection pressure exerted on the worms by the *Calliandra* diet. Worm burdens were higher in the recipient lambs receiving larvae from the *Calliandra*-fed donors. Egg production by the female worms derived from *Calliandra*-fed donors may have been reduced, but the results were not clear.

*Calliandra*, in addition to its value as a source of protein for tropical livestock, may be useful in reducing egg production by GI parasitic nematodes, thus reducing larval contamination of tropical pastures and infection rates of livestock. *Calliandra* therefore has potential as an anthelmintic alternative in tropical regions.

## TABLE OF CONTENTS

Statement of Access	ii
Declaration	ii
Electronic Copy of Thesis for Library Deposit	iii
Declaration	iii
Declaration on Ethics	iv
Contributions of Others	iv
Acknowledgments	v
Abstract	vi
Table of Contents	ix
List of Tables	xiii
List of Figures	xviii
List of Plates	xxiii
List of Abbreviations	xxv
<b>CHAPTER 1 General Introduction</b>	<b>1</b>
<b>CHAPTER 2 Review of the Literature</b>	<b>3</b>
<b>2.1 Gastrointestinal nematodes of importance in the tropics</b>	<b>3</b>
2.1.1 Haemonchus species	3
2.1.2 Trichostrongylus species	4
<b>2.2 Biology of nematode parasites</b>	<b>5</b>
2.2.1 Structure of the cuticle	5
2.2.2 Moulting and exsheathment	7
2.2.3 Gametogenesis	9
2.2.4 Embryogenesis	10
2.2.5 Structure and formation of eggshells	11
2.2.6 Mechanism of hatching	12
2.2.7 Excretory-secretory products	13
<b>2.3 Nutrition/Parasite Interactions</b>	<b>14</b>
2.3.1 Feed Intake	14
2.3.2 Gastrointestinal function	16
2.3.3 Energy metabolism	21
2.3.4 Minerals	22
2.3.5 Protein metabolism	24
2.3.6 Nutritional effects on immunity to parasites	27
<b>2.4 Issues in Parasite Control</b>	<b>32</b>
2.4.1 Anthelmintics	32
2.4.2 Alternative control methods	34
<b>2.5 Tropical Shrub Legumes</b>	<b>35</b>
<b>2.6 Tannins</b>	<b>36</b>
2.6.1 General properties	36
2.6.2 Protein-binding properties	38
2.6.3 Tannins and ruminant nutrition	41
2.6.4 Tannins and animal production	46
2.6.5 Potential of tanniniferous plants as animal feeds	49
<b>2.7 Tannin-Parasite Interactions</b>	<b>49</b>
2.7.1 Effects on production	49
2.7.2 Effects on nematode parasites	52
2.7.3 Factors determining efficacy	58
2.7.4 Persistence of effects	62
2.7.5 Tropical plants	62
	ix

2.8	<b>Conclusion</b>	<b>64</b>
<b>CHAPTER 3</b>	<b>Anthelmintic Effects of <i>Calliandra calothyrsus</i> in Lambs: A Pilot Study</b>	<b>66</b>
3.1	<b>Introduction</b>	<b>66</b>
3.2	<b>Materials and methods</b>	<b>67</b>
3.2.1	Experimental design	67
3.2.2	Animals, housing and management	67
3.2.3	Feeds and feeding	68
3.2.4	Samples and sampling	70
3.2.5	Laboratory analyses	71
3.2.6	Parasitology	73
3.2.7	Statistical analysis	74
3.3	<b>Results</b>	<b>75</b>
3.3.1	Parasitology	75
3.3.2	Nutritive value of diets	77
3.4	<b>Discussion</b>	<b>79</b>
3.4.1	Effects on <i>T. colubriformis</i>	79
3.4.2	Effects on <i>H. contortus</i>	80
3.4.3	Protein nutrition	81
3.5	<b>Conclusion</b>	<b>82</b>
<b>CHAPTER 4</b>	<b>Anthelmintic Effects of <i>Calliandra calothyrsus</i> in Sheep Infected with <i>H. contortus</i> or <i>T. colubriformis</i></b>	<b>83</b>
4.1	<b>Introduction</b>	<b>83</b>
4.2	<b>Materials and Methods</b>	<b>84</b>
4.2.1	Experimental design	84
4.2.2	Animal management	85
4.2.3	Feeds and feeding	86
4.2.4	Samples and sampling	86
4.2.5	Biochemical analyses	87
4.2.6	Parasitology	89
4.2.7	pH values	90
4.2.8	Statistical analysis	91
4.3	<b>Results</b>	<b>92</b>
4.3.1	Parasitology	92
4.3.2	Nutritional analysis	93
4.3.3	Blood biochemistry	98
4.3.4	pH	105
4.3.5	Total condensed tannin concentration	108
4.3.6	Gastrointestinal pathology	109
4.4	<b>Discussion</b>	<b>109</b>
4.4.1	Effects of <i>Calliandra</i> on GI nematodes	109
4.4.2	Development of immunity	111
4.4.3	Energy and protein nutrition	112
4.4.4	Effects of diet and GI parasitism on plasma constituents	113
4.4.5	Gastrointestinal pathology	119
4.4.6	pH	119
4.4.7	Total condensed tannin concentrations	122
4.5	<b>Conclusion</b>	<b>123</b>

<b>CHAPTER 5</b>	<b>The Effect of <i>Calliandra</i> Condensed Tannins on Free-Living Stages of Ovine <i>H. contortus</i> and <i>T. circumcincta</i></b>	<b>125</b>
5.1	<b>Introduction</b>	<b>125</b>
5.2	<b>Materials and Methods</b>	<b>126</b>
5.2.1	Experiment 1: Egg hatch assays (EHA)	126
5.2.2	Experiment 2: larval development assays (LDA)	130
5.2.3	Calculations	131
5.2.4	Statistical analysis	132
5.3	<b>Results</b>	<b>134</b>
5.3.1	Experiment 1: EHA	134
5.3.2	Experiment 2: LDA	138
5.4	<b>Discussion</b>	<b>145</b>
5.4.1	Identity of the plant extract	145
5.4.2	Egg hatching	145
5.4.3	Larval development	149
5.4.4	Effects of condensed tannin concentrations	153
5.5	<b>Conclusion</b>	<b>154</b>
<b>CHAPTER 6</b>	<b>Screening of Tropical Shrub Legumes for Anthelmintic Activity Against <i>H. contortus</i> and <i>T. colubriformis</i> in Sheep</b>	<b>156</b>
6.1	<b>Introduction</b>	<b>156</b>
6.2	<b>Materials and Methods</b>	<b>157</b>
6.2.1	Experiment 1: Screening EHA	157
6.2.2	Experiment 2: Screening LDA	159
6.2.3	Calculations	160
6.2.4	Statistical analysis	160
6.3	<b>Results</b>	<b>161</b>
6.3.1	Screening EHA	161
6.3.2	Screening LDA	162
6.4	<b>Discussion</b>	<b>168</b>
6.4.1	<i>Calliandra</i> condensed tannin fractions	168
6.4.2	Extract concentrations	169
6.4.3	Effects of plant extracts on egg hatching	170
6.4.4	Effects of plant extracts on larval development	171
6.5	<b>Conclusions</b>	<b>175</b>
<b>CHAPTER 7</b>	<b>Histological Demonstration of Condensed Tannins <i>In Situ</i> in Plant and Animal Tissues Including Nematodes</b>	<b>178</b>
7.1	<b>Introduction</b>	<b>178</b>
7.2	<b>Materials and Methods</b>	<b>179</b>
7.2.1	Staining techniques examined	179
7.2.2	Preparation of tissues for staining	181
7.2.3	Development of staining techniques using plant and ovine GI tissues	186
7.2.4	Staining of experimental ovine GI tissues	189
7.2.5	Staining of nematodes	190
7.2.6	Photography	191
7.2.7	Statistical analysis	191
7.3	<b>Results</b>	<b>192</b>
7.3.1	Haematoxylin and eosin	192
7.3.2	Ferric chloride	196
7.3.3	Butanol-H <sub>2</sub> SO <sub>4</sub>	197

7.3.4	Vanillin-HCl	199
7.3.5	Demonstration of condensed tannins in experimental tissues	201
<b>7.4</b>	<b>Discussion</b>	<b>212</b>
7.4.1	Positive controls	212
7.4.2	Haematoxylin and Eosin	213
7.4.3	Ferric chloride	216
7.4.4	Butanol-H <sub>2</sub> SO <sub>4</sub>	217
7.4.5	Vanillin-HCl	217
7.4.6	Condensed tannins in GI tissue	219
7.4.7	Condensed tannins in nematodes	220
<b>7.5</b>	<b>Conclusion</b>	<b>223</b>
<b>CHAPTER 8</b>	<b>Second Generation Anthelmintic Effects of <i>Calliandra calothyrsus</i> in Lambs</b>	<b>226</b>
<b>8.1</b>	<b>Introduction</b>	<b>226</b>
<b>8.2</b>	<b>Materials and Methods</b>	<b>227</b>
8.2.1	Experimental design	227
8.2.2	Animals, housing and management	228
8.2.3	Donor lambs	229
8.2.4	Recipient lambs	232
<b>8.3</b>	<b>Results</b>	<b>234</b>
8.3.1	Animal health	234
8.3.2	Feed intake	234
8.3.3	Calculations	234
8.3.4	Parasitology	236
<b>8.4</b>	<b>Discussion</b>	<b>239</b>
8.4.1	Donor lambs	239
8.4.2	Recipient lambs	243
<b>8.5</b>	<b>Conclusion</b>	<b>245</b>
<b>CHAPTER 9</b>	<b>General Discussion</b>	<b>247</b>
<b>REFERENCES</b>		<b>253</b>

## LIST OF TABLES

Table No.	Title	Page
Table 3.1	Experimental design in which Merino ram lambs were infected with either <i>H. contortus</i> or <i>T. colubriformis</i> and fed a diet of either Mitchell grass hay (MGH), pelleted lucerne or <i>Calliandra</i> leaves.	66
Table 3.2	The anticipated dry matter (DM), crude protein, rumen-undegradable protein and rumen-degradable protein in Mitchell grass hay, lucerne and <i>Calliandra</i> diets.	67
Table 3.3	Composition of mineral licks offered to eighteen experimental sheep.	69
Table 3.4	Size of <i>Haemonchus contortus</i> and total number of eggs present <i>in utero</i> in female <i>Trichostrongylus colubriformis</i> harvested immediately after euthanasia of lambs that were infected with either of the two worm species. Values are means $\pm$ s.e. Within each row, means with different superscripts are significantly different ( $P < 0.050$ ).	76
Table 3.5	The dry matter (DM), organic matter (OM), crude protein (CP) and metabolisable energy (ME) content and digestibility (D) of the feeds offered to lambs infected with either <i>H. contortus</i> or <i>Trichostrongylus colubriformis</i> . Within each row, values with different superscripts are significantly different from one another ( $P < 0.05$ ).	77
Table 3.6	The dry matter (DM), Crude protein (CP) and digestible organic matter (DOM) intakes, estimated protein entering the duodenum (PED) and N analysis of pooled samples from lambs infected with either <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Within each row, figures with different superscripts are significantly different from one another ( $P < 0.05$ ).	77
Table 4.1	Lambs infected or not infected with either of two worm species, <i>Haemonchus contortus</i> (Hc) or <i>Trichostrongylus colubriformis</i> (Tc) and fed a diet of either <i>Calliandra</i> * or lucerne** over two observation periods.	84
Table 4.2	Total number of worms, percentage of female worms, estimated egg output and eggs present <i>in utero</i> in <i>Haemonchus contortus</i> and <i>Trichostrongylus colubriformis</i> harvested immediately after euthanasia of lambs that were infected with either of the two worm species and fed either lucerne or <i>Calliandra</i> . Values are means $\pm$ standard errors.	92

## LIST OF TABLES continued

Table No.	Title	Page
Table 4.3	The dry matter (DM), organic matter (OM) and crude protein (CP) content of the lucerne and <i>Calliandra</i> diets fed to lambs infected with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> immediately after infection and during the patent period. Values are means $\pm$ standard error.	92
Table 4.4	Live weight (LW), maintenance energy requirement (Mm), intakes of dry matter (DM), digestible organic matter (DOM) and metabolisable energy (ME), nitrogen (N) intake, excretion and retention in lambs infected with either <i>Trichostrongylus colubriformis</i> or <i>Haemonchus contortus</i> and fed either the lucerne or <i>Calliandra</i> diet during the immediate post-infection period or during the patent period. Values are means $\pm$ standard errors.	93
Table 4.5	Live weight (LW), maintenance energy requirement (Mm), intakes of dry matter (DM), digestible organic matter (DOM) and metabolisable energy (ME), nitrogen (N) intake, excretion and retention in nematode-free lambs fed either the lucerne or <i>Calliandra</i> diet. Values are means $\pm$ standard errors.	
Table 4.6	Plasma total protein, albumin, urea, glucose and inorganic phosphate concentrations and packed cell volume (PCV) in lambs fed either the lucerne or <i>Calliandra</i> diets immediately after infection (Post-infection 1) or during the patent period (Post-infection 2). Values are means $\pm$ standard errors.	95
Table 4.7	The pH of digesta in different segments of the gastrointestinal (GI) tract of uninfected lambs or lambs infected with either <i>Trichostrongylus colubriformis</i> or <i>Haemonchus contortus</i> and fed a diet of either lucerne or <i>Calliandra</i> . Values are means $\pm$ standard error. R = rumen, A= abomasum, D = duodenum, J = jejunum, I = ileum, Cae = caecum, Co = colon.	102
Table 4.8	The pH of digesta in different segments of the gastrointestinal (GI) tract of uninfected lambs or lambs infected with either <i>Trichostrongylus colubriformis</i> or <i>Haemonchus contortus</i> across both lucerne and <i>Calliandra</i> diets. Values are means $\pm$ standard error. R = rumen, A= abomasum, D = duodenum, J = jejunum, I = ileum, Cae = caecum, Co = colon.	103

## LIST OF TABLES continued

Table No.	Title	Page
Table 4.9	The pH of digesta in different segments of the gastrointestinal (GI) tract of lambs fed a diet of either lucerne or <i>Calliandra</i> and either uninfected or infected with either <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means $\pm$ standard error. R = rumen, A= abomasum, D = duodenum, J = jejunum, I = ileum, Cae = caecum, Co = colon.	104
Table 4.10	Condensed tannin (CT) concentration in different GI segments of uninfected <i>Calliandra</i> -fed lambs. Data from D. Martin, personal communication. Condensed tannin analysis was carried out using a modification of the method of Terrill <i>et al.</i> (1992b). R = rumen, A= abomasum, D = duodenum, J = jejunum, I = ileum, Cae = caecum, Co = colon.	105
Table 5.1	Design of egg hatch assays, showing diet of sheep from which eggs were obtained and worm species used in each assay. (CT = condensed tannins.)	126
Table 5.2	Design of larval development assays, showing diet of sheep from which eggs were obtained and worm species used in each assay. Two sets of plates (Day 0 and Day 1) were used (CT = condensed tannin; L = lucerne; C = <i>Calliandra</i> .)	128
Table 5.3	Dose of <i>Calliandra</i> condensed tannins required to inhibit hatching in 50 % of eggs (ED <sub>50</sub> ) of <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> from lambs fed either <i>Calliandra</i> or lucerne diets. Values are means and 95 % fiducial limits (FL). Means with different superscripts are significantly different (P < 0.050).	132
Table 5.4	Dose of <i>Calliandra</i> condensed tannins required to inhibit development to the L <sub>3</sub> of 50 % of eggs (LD <sub>50</sub> ) of <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> from lambs fed either <i>Calliandra</i> or lucerne diets and exposed to condensed tannins either before (Day 0) or after hatching (Day 1). Values are means and 95 % fiducial limits. Means with different superscripts are significantly different (P < 0.050).	141
Table 6.1	Design of larval development assays, showing the Day of tannin addition and worm species used in each assay. Two sets of plates (Day 0 and Day 1) were used.	157



**LIST OF TABLES continued**

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
Table 6.2	The percentage of <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> eggs developing to the L <sub>3</sub> stage over seven days when crude plant extracts were added on Day 0 or Day 1. Values are means ± standard error. Values with different superscripts are significantly different (P < 0.05).	<b>163</b>
Table 6.3	The percentage of <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> eggs resulting in degenerating larvae over seven days when crude plant extracts were added on Day 0 or Day 1. Values are means ± standard error. Values with different superscripts are significantly different (P < 0.050).	<b>165</b>
Table 7.1	Composition of FAA solution used for fixation of fresh plant material prior to embedding and staining. From Sass (1958).	<b>179</b>
Table 7.2	Composition of Kreb's Solution used to incubate sections of GI tissue from a freshly killed ewe.	<b>180</b>
Table 7.3	Percentage of H&E-stained GI sections from lambs fed either <i>Calliandra</i> or lucerne in which condensed tannins were identified. Results for lambs infected with <i>Trichostrongylus colubriformis</i> and <i>Haemonchus contortus</i> and uninfected controls have been pooled within diet. Values are means ± standard error.	<b>193</b>
Table 7.4	Percentage of vanillin-HCl-treated GI sections from lambs, fed either <i>Calliandra</i> or lucerne, in which condensed tannins were identified. Results for <i>Trichostrongylus colubriformis</i> and <i>Haemonchus contortus</i> infected lambs were pooled within diet. Values are means ± standard error.	<b>200</b>
Table 8.1	Four donor lambs infected with either <i>H. contortus</i> or <i>Trichostrongylus colubriformis</i> larvae and fed a diet of either lucerne or <i>Calliandra</i> , supplied the four groups of recipient lambs with respective nematode species.	<b>224</b>
Table 8.2	Total worm numbers and percent female worms for four lambs infected with either <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> and fed either lucerne or <i>Calliandra</i> .	<b>233</b>
Table 8.3	Numbers of eggs and larvae cultured from the faeces of four donor sheep infected with either <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> and fed either lucerne or <i>Calliandra</i> . Values are means ± standard errors.	<b>233</b>

**LIST OF TABLES continued**

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
Table 8.4	Eggs shed per female worm, L <sub>3</sub> larvae developed per female worm and ration of eggs to larvae from larval cultures of four donor lambs infected with either <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> and fed either lucerne or <i>Calliandra</i> . Values are means ± standard errors.	<b>234</b>
Table 8.5	Number of eggs laid by female worms in the wells of cell culture plates in one hour ( <i>Haemonchus contortus</i> ) or two hours ( <i>Trichostrongylus colubriformis</i> ), and number of eggs <i>in utero</i> of 25 female <i>Trichostrongylus colubriformis</i> from lambs fed different diets Values are means ± standard errors.	<b>234</b>
Table 8.6	Faecal egg counts on Day 21 (FEC1) and Day 28 (FEC2), number of worms and percentage of female worms in recipient lambs fed lucerne and infected with either <i>T. colubriformis</i> (T) or <i>Haemonchus contortus</i> (H) larvae obtained from donor lambs infected with either of the nematode species and fed either a lucerne (L) or a <i>Calliandra</i> (C) diet. Data are means and standard errors. Means with different superscripts within rows are significantly different (P<0.050).	<b>235</b>
Table 8.7	Eggs per female worm shed on Day 21 (D21) and Day 28 (D28), number eggs produced in each well during incubation of female worms in cell culture plates and number of eggs <i>in utero</i> per female worm in recipient lambs fed lucerne and infected with either <i>Trichostrongylus colubriformis</i> (T) or <i>Haemonchus contortus</i> (H) larvae obtained from donor lambs infected with either of the nematode species and fed either a lucerne (L) or a <i>Calliandra</i> (C) diet. Data are means ± standard errors.	<b>235</b>

## LIST OF FIGURES

Figure No.	Title	Page
Figure 2.1	The generalised structure of flavan-3-ols. $R_2 = O$ -galloyl in the catechin gallates. Some examples of common flavan-3-ols are also given. From Schofield (2001).	37
Figure 2.2	The generalised structure of proanthocyanidins. $R = H$ in procyanidin or $OH$ in prodelphinidin. The $4 \rightarrow 8$ linkage is most common, but the $4 \rightarrow 6$ linkage also occurs. The number of repeating units ( $n$ ) is usually between 3 and 30. From Schofield (2001).	37
Figure 3.1	Lambs in metabolism crates set up for nitrogen balance determination in the Metabolism Unit, James Cook University.	70
Figure 3.2	Faecal egg counts (mean $\pm$ s.e.) of lambs fed Mitchell grass hay (▨), lucerne pellets (■) or <i>Calliandra</i> leaves (□) three and four weeks after infection with <i>Trichostrongylus colubriformis</i> . Within each week, treatments with different superscripts are significantly different ( $P < 0.050$ ).	75
Figure 3.3.	Faecal egg counts (mean $\pm$ s.e.) of lambs fed Mitchell grass hay (▨), lucerne pellets (■) or <i>Calliandra</i> leaves (□), three, four and five weeks after infection with <i>H. contortus</i> . There were no significant differences between dietary treatments within each week ( $P < 0.100$ ).	75
Figure 3.4	Postmortem worm counts (mean $\pm$ s.e.) of lambs fed Mitchell grass hay (▨), lucerne pellets (■) or <i>Calliandra</i> leaves (□), four or five weeks after infection with <i>Trichostrongylus colubriformis</i> or <i>Haemonchus contortus</i> respectively. Burdens of each worm species were not significantly different between diets ( $P > 0.100$ ).	76
Figure 4.1	Weekly faecal egg counts for lambs infected with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> and fed either the lucerne or <i>Calliandra</i> diets. (■ <i>Haemonchus contortus</i> , lucerne diet; □ <i>Haemonchus contortus</i> , <i>Calliandra</i> diet; ▨ <i>Trichostrongylus colubriformis</i> , lucerne diet; ▩ <i>Trichostrongylus colubriformis</i> , <i>Calliandra</i> diet). Values are means $\pm$ standard errors.	91

## LIST OF FIGURES continued

Figure No.	Title	Page
Figure 4.2	Concentration of total protein in plasma of lambs fed the lucerne (—●—) or <i>Calliandra</i> (···○···) diets pre- and post-infection with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means $\pm$ standard errors.	96
Figure 4.3	Concentration of albumin in plasma of lambs fed the lucerne (—●—) or <i>Calliandra</i> (···○···) diets pre- and post-infection with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means $\pm$ standard errors.	97
Figure 4.4	Concentration of albumin in plasma of lambs infected with <i>Haemonchus contortus</i> (—●—) or <i>Trichostrongylus colubriformis</i> (···○···) and fed either the <i>Calliandra</i> or lucerne diets pre- and post-infection. Data are pooled for diet. Values are means $\pm$ standard errors.	97
Figure 4.5	Concentration of urea in plasma of lambs fed the lucerne (—●—) or <i>Calliandra</i> (···○···) diets pre- and post-infection with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means $\pm$ standard errors.	98
Figure 4.6	Concentration of glucose in plasma of lambs infected with <i>Haemonchus contortus</i> (—●—) or <i>Trichostrongylus colubriformis</i> (···○···) and fed either the <i>Calliandra</i> or lucerne diets pre- and post-infection. Data are pooled for diet. Values are means $\pm$ standard errors.	99
Figure 4.7	Concentration of inorganic phosphate in plasma of lambs fed the lucerne (—●—) or <i>Calliandra</i> (···○···) diets pre- and post-infection with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means $\pm$ standard errors.	100
Figure 4.8	Concentration of inorganic phosphate in plasma of lambs infected with <i>Haemonchus contortus</i> (—●—) or <i>Trichostrongylus colubriformis</i> (···○···) and fed either the <i>Calliandra</i> or lucerne diets pre- and post-infection. Data are pooled for diet. Values are means $\pm$ standard errors.	100

**LIST OF FIGURES continued**

<b>Figure No.</b>	<b>Title</b>	<b>Page</b>
Figure 4.9	Packed cell volume (PCV) of blood of lambs fed the lucerne (—●—) or <i>Calliandra</i> (···○···) diets pre- and post-infection with <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means ± standard errors.	<b>101</b>
Figure 4.10	Packed cell volume (PCV) blood of lambs infected with <i>Haemonchus contortus</i> (—●—) or <i>Trichostrongylus colubriformis</i> (···○···) and fed either the <i>Calliandra</i> or lucerne diets pre- and post-infection. Data are pooled for diet. Values are means ± standard errors.	<b>102</b>
Figure 4.11	The pH in the digestive tract of uninfected lambs (▨) and lambs infected with either <i>Haemonchus contortus</i> (■) or <i>Trichostrongylus colubriformis</i> (□) across both lucerne and <i>Calliandra</i> diets. Values are means ± standard errors. Within each GI segment, columns with different superscripts are significantly different (P < 0.050). R = rumen, A= abomasum, D = duodenum, J = jejunum, I = ileum, Cae = caecum, Co = colon.	<b>103</b>
Figure 4.12	The pH in the digestive tract of lambs fed either the lucerne (■) or <i>Calliandra</i> (□) diet and either uninfected or infected with either <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> . Data are pooled for worm species. Values are means ± standard errors. Within each GI segment, columns with different superscripts are significantly different (P < 0.050). R = rumen, A= abomasum, D = duodenum, J = jejunum, I = ileum, Cae = caecum, Co = colon.	<b>104</b>
Figure 5.1	Effects of <i>Calliandra</i> condensed tannins on hatchability of eggs of <i>Haemonchus contortus</i> or <i>Trichostrongylus colubriformis</i> from lambs fed <i>Calliandra</i> or lucerne diets. (—*— <i>H. contortus</i> , lucerne diet; —△— <i>Haemonchus contortus</i> , <i>Calliandra</i> diet; —□— <i>Trichostrongylus colubriformis</i> , lucerne diet; —◇— <i>Trichostrongylus colubriformis</i> , <i>Calliandra</i> diet).	<b>131</b>
Figure 5.2	Logit model of the effects of <i>Calliandra</i> condensed tannins on hatchability of eggs of <i>H. contortus</i> or <i>Trichostrongylus colubriformis</i> from lambs fed <i>Calliandra</i> or lucerne diets. (—×— <i>H. contortus</i> , lucerne diet; —●— <i>Haemonchus contortus</i> , <i>Calliandra</i> diet; —★— <i>Trichostrongylus colubriformis</i> , lucerne diet; —▲— <i>Trichostrongylus colubriformis</i> , <i>Calliandra</i> diet).	<b>132</b>

**LIST OF FIGURES continued**

Figure No.	Title	Page
Figure 5.3	<p>Hatch rates of <i>Haemonchus contortus</i> (H) or <i>Trichostrongylus colubriformis</i> (T) eggs after seven days incubation with increasing concentrations of <i>Calliandra</i> condensed tannins. Eggs were obtained from lambs fed either a <i>Calliandra</i> (C) or lucerne (L) diet and tannin was added either before (0) or after the eggs hatched (1).</p> <p>HL0 —*—; HL1 —+—; TL0 —□—; TL1 —○—; HC0 —△—; HC1 —x—; TC0 —◇—; TC1 —☆— .</p>	135
Figure 5.4	<p>Percentage of eggs of <i>Haemonchus contortus</i> (H) or <i>Trichostrongylus colubriformis</i> (T) developing to the L<sub>1</sub> after seven days incubation with increasing concentrations of <i>Calliandra</i> condensed tannins. Eggs were obtained from lambs fed either a <i>Calliandra</i> (C) or lucerne (L) diet and tannins were added either before (0) or after the eggs hatched (1).</p> <p>HL0 —*—; HL1 —+—; TL0 —□—; TL1 —○—; HC0 —△—; HC1 —x—; TC0 —◇—; TC1 —☆— .</p>	138
Figure 5.5	<p>Percentage of eggs of <i>Haemonchus contortus</i> (H) or <i>Trichostrongylus colubriformis</i> (T) developing to the L<sub>2</sub> after seven days incubation with increasing concentrations of <i>Calliandra</i> condensed tannins. Eggs were obtained from lambs fed either a <i>Calliandra</i> (C) or lucerne (L) diet and tannins were added either before (0) or after the eggs hatched (1).</p> <p>HL0 —*—; HL1 —+—; TL0 —□—; TL1 —○—; HC0 —△—; HC1 —x—; TC0 —◇—; TC1 —☆— .</p>	139
Figure 5.6	<p>Percentage of eggs of <i>Haemonchus contortus</i> (H) or <i>Trichostrongylus colubriformis</i> (T) developing to the L<sub>3</sub> after seven days incubation with increasing concentrations of <i>Calliandra</i> condensed tannins. Eggs were obtained from lambs fed either a <i>Calliandra</i> (C) or lucerne (L) diet and tannins were added either before (0) or after the eggs hatched (1).</p> <p>HL0 —*—; HL1 —+—; TL0 —□—; TL1 —○—; HC0 —△—; HC1 —x—; TC0 —◇—; TC1 —☆— .</p>	140
Figure 5.7	<p>Percentage of eggs of <i>Haemonchus contortus</i> (H) or <i>Trichostrongylus colubriformis</i> (T) resulting in degenerating larvae after seven days incubation with increasing concentrations of <i>Calliandra</i> condensed tannins. Eggs were obtained from lambs fed either a <i>Calliandra</i> (C) or lucerne (L) diet and tannins were added either before (0) or after the eggs hatched (1).</p> <p>HL0 —*—; HL1 —+—; TL0 —□—; TL1 —○—; HC0 —△—; HC1 —x—; TC0 —◇—; TC1 —☆— .</p>	141

## LIST OF FIGURES continued

Figure No.	Title	Page
Figure 6.1	Percentage of <i>H. contortus</i> (■) or <i>T. colubriformis</i> (□) eggs hatching when incubated for 26 hours with 990 µg/ml of acetone extracts from a variety of tropical plant species. There was no difference between the extracts ( $P > 0.100$ ). Data for <i>H. contortus</i> exposed to the <i>Sesbania</i> extract and <i>T. colubriformis</i> exposed to the <i>Albizia</i> extract are missing.	158
Figure 6.2	Percentage of <i>H. contortus</i> (■) or <i>T. colubriformis</i> (□) eggs hatching when incubated for seven days with 990 µg/ml of crude acetone extracts from a variety of tropical plant species. <i>T. colubriformis</i> eggs had higher hatch rates than <i>H. contortus</i> eggs ( $P < 0.050$ ).	160
Figure 6.3	Percentage of nematode eggs hatching when incubated for seven days with 990 µg/ml of crude acetone extracts from a variety of tropical plant species. Extracts were added either on Day 0 (■) or Day 1 (□). Hatch rates were higher on Day 1 than on Day 0 ( $P < 0.050$ ).	160
Figure 6.4	Hatch rates of nematode eggs exposed to 9.09 µg/mL of crude acetone extracts from a number of tropical plant species. Columns with different letters are significantly different ( $P < 0.05$ ).	161
Figure 6.5	The effect of exposure to different plant extracts for seven days on the percentage of nematode eggs that developed to the L <sub>1</sub> stage. Development to the L <sub>1</sub> was not affected by extract ( $P > 0.100$ ).	162
Figure 6.6	The effect of exposure to different plant extracts for seven days on the percentage of nematode eggs that developed to the L <sub>2</sub> stage. Columns with different letters are significantly different ( $P < 0.05$ ).	163
Figure 6.7	The effect of exposure to different plant extracts for seven days on the percentage of nematode eggs that developed to the L <sub>3</sub> stage. Columns with different letters are significantly different ( $P < 0.05$ ).	164
Figure 6.8	The effect of exposure to different plant extracts for seven days on the percentage of nematode eggs that resulted in degenerating larvae. Columns with different letters are significantly different ( $P < 0.05$ ).	165
Figure 7.1	Procedures for the preparation of plant tissues for staining.	179

## LIST OF PLATES

Plate No.	Title	Page
Plate 5.1	Results of egg hatch assays using strongyle eggs after 26 hours incubation at 27°C. <b>A.</b> Normal <i>Trichostrongylus colubriformis</i> larva, TL control. <b>B.</b> Unhatched <i>T. colubriformis</i> egg, TC control. <b>C.</b> Degenerating egg with contents extruding, HC control. <b>D.</b> Larva half hatched, TL 400 µg/ml. <b>E.</b> Egg with degenerating contents, TL 400 µg/ml. <b>F.</b> Enlarged egg with fully developed larva, TL 400 µg/ml.	134
Plate 5.2	<b>A.</b> <i>H. contortus</i> L1 larvae from <i>Calliandra</i> -fed sheep after 26 hours incubation of eggs at 27°C in tap water without added tannins. While the larva on the left looks normal, the one on the right has a shrivelled appearance and has a loop of reproductive tract extruding from the vulval opening. Three undeveloped eggs and an empty shell are present in the background. <b>B.</b> Enlarged <i>H. contortus</i> eggs with degenerating contents after seven days incubation in larval development assay. HC 900µg/ml. <b>C.</b> <i>T. colubriformis</i> egg with degenerating contents. TC 900µg/ml <b>D.</b> Degenerating larvae from <i>Calliandra</i> -fed lamb in larval development assay. HC control. <b>E.</b> Degenerating larvae from <i>Calliandra</i> -fed lamb in larval development assay. TC 900µg/ml.	137
Plate 7.1	Abomasal sections from a <i>Calliandra</i> -fed lamb (H & E). <b>A.</b> Condensed tannins are present in a layer across the luminal surface. <b>B.</b> Macrophages containing condensed tannins in the lamina propria.	191
Plate 7.2	Gastrointestinal sections from <i>Calliandra</i> -fed lambs (H & E). <b>A.</b> Giant cell containing condensed tannins in the lamina propria of the abomasum. A smaller macrophage containing tannin is also present (arrow). <b>B.</b> Coccidial tissue-associated stages in the ileum. Eosinophils are abundant (arrows).	192
Plate 7.3	Gastrointestinal sections from <i>Calliandra</i> -fed lambs (Vanillin-HCl). <b>A.</b> Red-staining digesta in the rumen. <b>B.</b> Condensed tannins on the luminal surface of the abomasum.	199
Plate 7.4	Intact <i>H. contortus</i> stained with vanillin-HCl. <b>A.</b> Bursa of male worm showing adherent red-staining digesta. <b>B.</b> Vulval flap of female showing red staining of the cuticle.	201
Plate 7.5	Intact worms adult worms stained with vanillin-HCl. <b>A.</b> Cuticle of female <i>H. contortus</i> showing red staining following the striae of the cuticle. <b>B.</b> Ovipositor of Female <i>Trichostrongylus colubriformis</i> containing larvated eggs. Arrows define the ends of the ovipositor.	202



**LIST OF PLATES continued**

<b>Plate No.</b>	<b>Title</b>	<b>Page</b>
Plate 7.6	Larvated eggs in the uterus of a female <i>T.colubriformis</i> from a <i>Calliandra</i> -fed lamb (Vanillin-HCl). <b>A.</b> Eggs in the ovijector. <b>B.</b> Eggs in the uterus just anterior to the ovijector.	<b>203</b>
Plate 7.7	Condensed tannins in OCT sections of adult <i>Haemonchus contortus</i> (Vanillin-HCl). <b>A.</b> Red-stained digesta overlying a transverse section through a worm. <b>B.</b> Condensed tannins (possibly in digesta) adhering to the cuticle of a worm.	<b>205</b>
Plate 7.8	Condensed tannins in OCT sections of adult <i>H. contortus</i> (Vanillin-HCl). <b>A.</b> In the centre of an egg in the uterus of a female worm. <b>B.</b> In the cells lining the oesophagus.	<b>206</b>
Plate 7.9	Condensed tannins in an OCT section from an adult <i>Haemonchus contortus</i> (Vanillin-HCl). <b>A.</b> Tannins in cells lining a hollow organ (probably the microvilli of the gut) at the level of the vulval flap. <b>B.</b> Higher power view of the same section.	<b>207</b>
Plate 7.10	Condensed tannins in OCT sections from adult <i>Haemonchus contortus</i> (Vanillin-HCl). <b>A.</b> Condensed tannins in cells lining the oesophagus, longitudinal section. <b>B.</b> Condensed tannins in cells lining the oesophagus, oblique section.	<b>208</b>

## LIST OF ABBREVIATIONS

Abbreviation	Definition
A	Abomasum
ACh	Acetyl choline
BNF	Buffered neutral formalin
Cae	Caecum
CCK	Cholecystokinin
Co	Colon
CP	Crude protein
D	Duodenum
DM	Dry matter
DOM	Digestible organic matter
DOMI	Digestible organic matter intake
DPX	Distrene, Plasticiser, Xylene (tissue mounting medium)
DW	Dry weight
EAA	Essential amino acids
ECL	Enterochromaffin-like cell
ECT	Extractable condensed tannins
ED <sub>50</sub>	Concentration required to prevent 50 % of eggs from hatching
EHA	Egg hatch assay
EHI	Egg hatch inhibition
epg	Eggs per gram
ES	Excretory/secretory
FAA	Formalin-Alcohol-Acetic acid (plant fixative)
FCT	Fibre-bound condensed tannins
FW	Fresh weight
GI	Gastrointestinal
H & E	Haematoxylin and Eosin
I	Ileum
IgG	Immunoglobulin G
IVOMD	<i>In vitro</i> organic matter digestibility
J	Jejunum
JCU	James Cook University
LAP	Leucine aminopeptidase
LD <sub>50</sub>	Concentration required to prevent 50 % eggs developing to the L <sub>3</sub>
LDA	Larval development assay
LDI	Larval development inhibition
ME	Metabolisable energy
MGH	Mitchell grass hay
N	Nitrogen
NAN	Non-ammonia nitrogen
NEAA	Non-essential amino acids
NPM	Nutritional Physiology and Metabolism
NPY	Neuropeptide Y
OCT	Optimum cutting temperature medium
OM	Organic matter

## LIST OF ABBREVIATIONS continued

<b>Abbreviation</b>	<b>Definition</b>
PCT	Protein-bound condensed tannins
PCV	Packed cell volume
PED	Protein entering the duodenum
PEG	Polyethylene glycol
pgp	P-glycoprotein
PLE	Protein-losing enteropathy
PPR	Periparturient rise in faecal egg counts
R	Rumen
s	Seconds
SVBS	School of Veterinary and Biomedical Sciences
TCT	Total condensed tannins
VFA	Volatile fatty acids