ANTHELMINTIC EFFECTS OF TROPICAL SHRUB LEGUMES IN RUMINANT ANIMALS

Thesis submitted by

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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).

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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 form this thesis.

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

Funds to support this project came from JCU IRA accounts maintained by Associate Professor Esala Teleni.

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Dr Yvette Cunningham provided me with statistical advice and Dr Pat Pepper performed the statistical analysis for Chapters 3 and 4. Ms Jessica Viduka assisted with proof-reading.Their assistance is gratefully acknowledged.

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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 ± 395 worms) and lucerne (1861 ± 230 worms). Worm burdens of *T. colubriformis* were also similar in lambs fed *Calliandra* (5718 ± 339 worms) and

lucerne (4861 ± 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 µg/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-H_2SO_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.

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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_{50}	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	*In vitro* organic matter digestibility
J	Jejunum
JCU	James Cook University
LAP	Leucine aminopeptidase
LD_{50}	Concentration required to prevent 50 % eggs developing to the L_3
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 tempetature medium
OM	Organic matter

Abbreviation	Definition
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