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

A Thesis presented in partial fulfilment of the requirements for the degree of

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Bornwell Mupeyo

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Declaration

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

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

Candidate: Bornwell Mupeyo
Signature:

Chief Supervisor: Professor Tom N. Barry
Signature:

Co-supervisor: Professor Bill Pomroy
Signature:

Abstract

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

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

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

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

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

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

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

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

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Dedication

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

NDALUMBA KAPATI

List of Abbreviations

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

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

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

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